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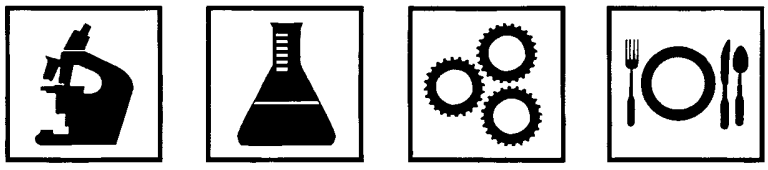

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ABSTRACTS

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IVAN PARKIN LECTURE ABSTRACT

Where Do You Put Your Chopsticks?

Gary R. Acuff
Professor
Texas A&M University
College Station, Texas

We in the field of food safety have seen some substantial change over the last few decades, and the International Association for Food Protection has accompanied us through it all. Growth of IAFP over the last few years has been nothing short of phenomenal, and while many of us have been here long enough to have personally witnessed the changes and growth, there is a large percentage of our membership that knows IAFP only by its current state. We would all no doubt agree that IAFP is important to our careers, and it is great that we can now enjoy the success we have had; however, we need to consider what has made us successful and assure that we preserve this benefit for future food safety professionals.

Experience may be one of our most important resources — how can we assure that it is not wasted? There are probably many reasons for IAFP's success, but there is likely little disagreement that the members and their ability to mentor and network have had a major impact. In this year's Ivan Parkin Lecture, we will take a journey through history with past "food safety heroes" and talk about the impact of mentoring on our careers in food safety. We will talk about lessons learned and how we can impact the future health and sustained growth of our Association.

And we'll talk about chopsticks.

JOHN H. SILLIKER LECTURE ABSTRACT

Heroes Past and Future

Ann Marie McNamara
Vice President, Food and Essentials
Safety and Quality Assurance
Target Corporation
Minneapolis, Minnesota

This year marks the 25th anniversary of the Jack in the Box *E. coli* O157:H7 outbreak — an event that changed food safety more than any other in recent memory. This crisis resulted in changes in regulation, innovations in industry practices, new research methods and tools for detection, and a changed public awareness of the importance of food safety. It led to a decade of unprecedented innovation, research and reform in food safety. Every IAFP Annual Meeting since still has dozens of papers and presentations that point to this crisis and name it as a pivotal event for change.

Anniversaries are important opportunities to focus on both lessons learned and how to do better in the future. Some of the heroes of this crisis are well known — many belong to IAFP — but many will be a surprise, even though they made important contributions. The heroes of this crisis include government and industry scientists, academicians, and test kit developers who contributed to the basic knowledge of this deadly bacterium and its detection and control; physicians; public health officials; epidemiologists and veterinarians who contributed to understanding the transmission, treatment and reservoirs of this disease; engineers, entrepreneurs and industry experts who contributed interventions in both food processing and retail settings; and regulators, lawyers and parents of the victims who contributed to regulatory reform and increased public awareness.

My background as a government scientist and regulator during the crisis, as a scientific leader at Silliker (now Mérieux NutriSciences) after this event, and as Dave Theno's successor at Jack in the Box uniquely qualify me to recognize the many heroes who have contributed to improving food safety in the wake of this crisis, and to look at how the current generation of IAFP Members can help address future problems in food safety.

This presentation will use the lessons learned from this past crisis to look toward the future and challenge current IAFP Members to use their knowledge, skills and abilities to confront current and emerging foodborne threats. What will be the next crisis? Who will be our next food safety heroes? Will it be you?

Symposium Series on Food Microbiology

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

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

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

S64 Closing in on the Research Gaps with *Listeria monocytogenes*, *Salmonella*, and Viruses in Low-moisture Foods

Low-moisture foods continue to be implicated in foodborne illness outbreaks and pathogen related recalls. Increasingly, public health officials are finding low-moisture ingredients as the source of contamination in compound foods. Most research on prevalence and the behavior of pathogens in foods has been conducted with mid- to high-water activity foods, but the data is significantly limited for low-moisture foods. Managing microbial risks in these diverse commodities remains a challenge. Importantly, some pathogens exhibit a tolerance to desiccation and interventions, such as heat, typically lethal in products of higher water activity. In the last 5–10 years, some meaningful research has been conducted on select commodities, such as spices and nuts, allowing scientists to gain insights into microbial mitigation for these foods. Yet, a vast array of low-moisture foods and associated pathogens has not yet been studied. Some of the more significant research gaps relate to *Listeria monocytogenes*, *Salmonella*, and select foodborne viruses' survival during storage, potential changes in virulence with time and temperature variations, and the overall effectiveness of various mitigation strategies when applied to low-moisture foods. Some of these gaps are being addressed using a variety of bacteria and viruses, including potential surrogates. This symposium brings researchers together to share new insights from their continuing work that begins to fill in some of the gaps in our knowledge on pathogen behavior in previously unstudied low-moisture foods, including pistachios, chocolate (both chocolate liquor and cocoa powder), corn flake cereal, dried apples, raisins, dried strawberries, skim milk powder, and almond flour/meal. Specifically, the role of water activity and the food matrix will be explored to better understand inactivation kinetics and overall survival.

Survival and the Potential for Genome Changes during the Storage of *Listeria monocytogenes* in Model Low-moisture Foods

JEFFREY FARBER, *University of Guelph, CRIFS, Department of Food Science, Guelph, ON, Canada*

Survival and Pathogenicity of Foodborne Viruses on Low-moisture Foods

SABAH BIDAWID, *Health Canada, Ottawa, ON, Canada*

Survival and Virulence of *Salmonella* in Model Low-moisture Foods

SOPHIA KATHARIOU, *North Carolina State University, Raleigh, NC*

Listeria monocytogenes Thermal Resistance: Role of Water Activity in Cocoa Powder, Skim Milk Powder, and Almond Flour/Meal

RT4 How Much of a Mystery Remains with Whole Genome Sequencing?

Whole genome sequencing (WGS) is transforming the field of food safety microbiology. The technology has already cemented its place as a tool for the investigation of foodborne illness outbreaks and as a prospective surveillance tool for the public health authorities and regulators. In the private sector, there is also a growing appreciation of the benefits of using WGS in source tracking of microorganisms and the wider potential of WGS to improve food safety. Advances in the sequencing technologies and the bioinformatics analytical tools are happening at a breakneck pace, leading to significant changes even within a short span of time.

When a new, complex and rapidly changing technology such as WGS is being implemented, there are always concerns about the reliability of the technology. It is important to understand how reliable and reproducible results can be generated using a technology which is constantly evolving. It is also important to understand what is changing and how that affects the interpretation and potential use of the technology. Clarification on these aspects will facilitate widespread use of WGS in industry.

This roundtable panel, comprised of leading scientists from the government, academia, and industry will discuss how the industry can cope with the rapid technological developments to apply WGS as routine. The panel will tackle questions that need to be answered to transition WGS from research to routine application. Cornerstones to obtain reproducible analytical results such as benchmarking, validation, harmonization, standardization and verification of WGS – both current status and future needs will be addressed by the panel. The panel will discuss how these concepts work when applied to WGS compared to traditional microbial analytics. Finally, identification and implementation of fit-for-purpose tools that meet industry needs will be discussed.

Roundtable Panelists:

ERROL STRAIN, *U.S. Food and Drug Administration – Center for Food Safety and Applied Nutrition, College Park, MD, USA*

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

MARTIN WIEDMANN, *Cornell University, Ithaca, NY, USA*

KATHIE GRANT, *Public Health England, Glasgow, United Kingdom*

PAMELA WILGER, *Cargill, Inc., Wayzata, MN, USA*

Symposium Abstracts

S1 2018 Foodborne Outbreak Updates

HUGO FRAGOSO SANCHEZ: *SENASICA, Mexico, Mexico, Mexico*
BROOKE WHITNEY: *FDA Coordinated Outbreak Response and Evaluation Network, College Park, MD, USA*
MONIQUE FOSTER: *CDC, Atlanta, GA, USA*
MONIQUE SALTER: *U.S. Food and Drug Administration, College Park, MD, USA*
SHEILA MERRIWEATHER: *FDA-CORE, College Park, MD, USA*
BARBARA HERWALDT: *CDC, Atlanta, GA, USA*
ALICE GREEN: *USDA/FSIS/OPHS/AES, Minneapolis, MN, USA*
PETER BEN EMBAREK: *World Health Organization/INFOSAN Network, Geneva, Switzerland, Switzerland*

This session will summarize the recent outbreaks associated with eight different *Salmonella* serotypes associated with papayas imported from different farms in Mexico and the efforts employed by SENESICA to work with growers to prevent future outbreaks. The 2016 outbreak of hepatitis A associated with the consumption of raw scallops in Hawaii will be presented along with the implications for ingestion of other raw seafood commodities. The investigation of and recommendations regarding a highly unusual botulism outbreak linked to nacho cheese dispensed at a service station will also be reviewed. The USA is currently experiencing an outbreak with more than 1,000 cases of cyclosporiasis in 40 states; results of the investigation will be presented. Contributing factors associated with food service-related STEC and RTE product-related Salmonella investigations for FSIS-regulated products will be presented. Aspects of the investigation into the international *Salmonella* Agona outbreak will also be presented.

SS1 Lessons Learned and Global Implications of *Listeria monocytogenes*

LUCIA ANELICH: *Anelich Consulting, Pretoria, South Africa, South Africa*
PETER BEN EMBAREK: *World Health Organization/INFOSAN Network, Geneva, Switzerland, Switzerland*
ARUN BHUNIA: *Purdue University, West Lafayette, IN, USA*
DEON MAHONEY: *Dairy Food Safety Victoria, Melbourne, Australia, Australia*
PETER TAORMINA: *Etna Consulting Group, Cincinnati, OH, USA*
CATHERINE DONNELLY: *University of Vermont, Burlington, VT, USA*

In the past year, high profile listeriosis outbreaks associated with deli meat (polony) and melons (cantaloupes) have occurred, including to date the world's most severe outbreak in South Africa. *Listeria monocytogenes* has also been associated with dairy products, including ice cream and soft cheeses. These contaminated food commodities have affected the most vulnerable populations (pregnant women, neonates and the elderly). As these outbreaks illustrate, more attention is required to comprehend how *L. monocytogenes* is disseminated through the food production environment. The fatalities and cases of illness from these outbreaks are reminders that listeriosis does not recognize any international border. In this symposium we hope to address specific food production factors and describe epidemiological investigations involved in previous outbreaks as well as in more recent ones, as presented by those directly involved in outbreak investigation response. The global burden of listeriosis and the state of epidemiological tools and data used to assess this burden will also be addressed by international experts. Underlying these outbreaks are unique attributes of *L. monocytogenes*. Specifcally, recently elucidated molecular mechanisms which *L. monocytogenes* uses to cause virulence and evade the human immunological response will be described by investigators directly involved in this research and included in this critical conversation. Real world control measures, including specific sanitation regimes and the use of *Listeria* spp. as an indicator for *L. monocytogenes* in food processing plants to limit and minimize *L. monocytogenes* contamination will also be discussed. The impact of future regulatory approaches and research topics, and the implications that these have on food processors for all commodities and of all sizes in any country will be addressed in light of these serious listeriosis outbreaks.

S2 Global Food Protection Issues: Contemporary Chemical Challenges

HYUN JUNG LEE: *University of Idaho, Moscow, ID, USA*
BRENT KOBIELUSH: *Cargill, Inc., Minneapolis, MN, USA*
ANDREW PEARSON: *Ministry of Primary Industries, Wellington, New Zealand, New Zealand*
LUÍSA CAMACHO: *FDA National Center for Toxicological Research, Jefferson, AR, USA*
CATHERINE SMITH: *Health Canada Bureau of Chemical Safety, Ottawa, ON, Canada, Canada*
ANDREW PEARSON: *Ministry for Primary Industries, Wellington, New Zealand, New Zealand*

"All things are poison and nothing without poison; only the dose makes a thing not a poison." So says Paracelsus (ca. 1493–1534), 'the father of toxicology.' Inspired by this Swiss physician, regulatory and commercial food safety programs have long been concerned with the presence in foods of residues of veterinary medicines and agricultural compounds, toxic elements, and natural toxins such as aflatoxin. Today, many consumers are raising concerns over a new range of chemical contaminants in food, even more than expressing worries about microbiological challenges. Chemical adulteration, unintentional and intentional, is an ever-expanding issue, and the need to develop related analytical methods with increasing sensitivity is challenging food protection toxicologists. Necessary thermal processing and heat treatment of foods may be accompanied by the formation of toxic compounds such as heterocyclic amines, furans, acrylamide, and 3-MCPD esters.

The 2011 Fukushima Dai-ichi Nuclear Power Plant accident in Japan released radioactive material into the atmosphere and ocean. As a result, consumer fears about radionuclide contamination of food skyrocketed. Moreover, the composition of food packaging has raised concerns that chemicals, such as Bisphenol-A, could migrate into the food contained within. Most recently, fear of the unknown has raised concerns with the presence of nanoparticles in foods, particularly in infant formula, with other emerging dietary contaminants of concern coming to light.

This symposium will summarize the latest information on the potential risk to human health, prevention, and control methods for these chemical contaminants, and present an update on international initiatives to provide harmonized approaches to risk management for detection of unexpected, low-level chemical contaminants in foods. These initiatives characterize the likely toxicity threshold of a chemical based on its structure, thereby allowing countries to undertake rapid risk assessment when chemicals are detected in food and to minimize disruption to trade.

S3 Rapid Testing Methods for Safety and Spoilage in the Dairy Industry – What is Needed, What Works and What Does Not

ALEJANDRO MAZZOTTA: *Chobani, New York, NY, USA*
DAVID BLOMQUIST: *EAS Consulting Group, Hastings, MN, USA*
MAYA ACHEN: *Abbott Nutrition, Columbus, OH, USA*

The dairy industry has been a leader in sanitation and hygiene for more than a century. As food safety becomes more critical for all industries, methods to determine the quality of dairy products rapidly becomes more critical. While many rapid methods exist for pathogens of concern, rapid methods for spoilage organisms have not received as much attention. For example, a standard plate count takes 48 hours and a Moseley test used in fluid milk takes nine days. This session will focus on different technologies that have potential to determine low levels of spoilage organisms in a variety of dairy products and will discuss the strengths and weakness of each.

S4 Building a Strategic Alliance for Sustainable Food Safety Risk Analysis Capacity Building in the Americas

SIMONE RASZL: *PAHO, Rio de Janeiro, Brazil, Brazil*
BING WANG: *University of Nebraska-Lincoln, Lincoln, NE, USA*
FERNANDO SAMPEDRO: *University of Minnesota, College of Veterinary Medicine, St. Paul, MN, USA*

Significant progress on strengthening food safety systems in the Latin American and Caribbean (LAC) region has been the result of a series of initiatives conducted by a wide array of institutions. International Organizations, regional entities, and local governments, together with academic institutions and private industry associations, have been able to increase awareness of food safety control measures and management programs while contributing to the reduction and prevention of foodborne illness. However, unacceptable rates of food-associated cases of illness remain, and emerging hazards continue to enter the food supply. A key alternative to assess potential links between hazards in the food chain and actual food safety risks to human health is the application of the Food Safety Risk Analysis (FSRA) Framework. Countries that have built risk analysis capacities to support the establishment of food standards and other food control measures have been able to secure market access, facilitate trade, and contribute to national public health objectives. Risk analysis fosters comprehensive scientific evaluation; wide stakeholder participation; transparency of the standard-setting process; consistent treatment of different hazards; and systematic decision-making by risk managers. The main goal of this symposium is to outline the establishment of the food safety risk analysis consortium, a coordinated effort for capacity building on FSRA in the Americas that brings together international organizations, food industry associations, and academia. The long-term objectives of the consortium are to: a) build trust, strengthen communication, and build synergies among organizations, local governments, and entities that work on risk analysis in the region; b) provide the foundation for coordinated, consistent, and effective approaches to capacity building and curriculum development for FSRA adoption; and c) facilitate the implementation of the risk analysis framework with strategic stakeholders within the region. The session will present a summary of current initiatives driven by the consortium founding institutions, and will provide opportunities to launch new coordinated initiatives that will maximize the use of resources and optimize the adoption of the FSRA approach at different levels. Discussion on successful examples of risk analysis implementation in the region (from academia and government sectors), the challenges experienced on implementing risk analysis, and the construction of a Capacity Building Roadmap will be proposed.

S5 Food Safety in Aisle 8: Science-based Messages for Consumer Food Safety Education Campaigns at Retail

MICHAEL ROBERSON: *Publix Super Markets, Inc., Lakeland, FL, USA*
SHELLEY FEIST: *Partnership for Food Safety Education, Arlington, VA, USA*
SANDRIA GODWIN: *Tennessee State University, Nashville, TN, USA*

Shelley Feist, Sandria Godwin and Michael Roberson will collectively present two food safety education campaigns from concept through the research process. Implementation of the campaigns by retailers will be discussed including how to effectively communicate food safety messages to customers based on research. Shelley, Sandria and Michael will provide research results and fact based information as well as practical information on how to communicate effectively from concept to final product via multiple outlets including traditional and social media while basing everything on science based messaging.

Retailers serve their communities on a daily basis. In addition to providing the products and services needed by customers, they also have the responsibility of providing essential information. Some of this information is required by regulations, yet some is voluntary and at the discretion of the retailer. Providing food safety information at retail can help build customer trust. Those who work in food safety know the challenge of proving information that will be understood and acted on by the intended audience. This symposium will focus on two projects that provide science-based food safety messaging for consumers to be utilized by retailers for in-store outreach programs. Research, campaigns, and outreach programs will be presented for two campaigns: *The Story of Your Dinner* and *Don't Wing It*. Ongoing metrics will be presented on a campaign basis and, when possible, from a retail perspective.

S6 Developing a Risk-based Food Safety Plan for Fresh Produce in Retail Food Establishments

HALEY OLIVER: *Purdue University, West Lafayette, IN, USA*
KARL MATTHEWS: *Rutgers University, New Brunswick, NJ, USA*
JILL HOLLINGSWORTH: *Chemstar Corp, Lithia Springs, GA, USA*

Retail food establishments want to ensure they are using best practices for handling fresh produce. Understanding the risks and the options for preventive controls can help with the development of a retail food safety plan. Because there is no-kill step for fresh produce, other controls and technologies need to be considered. Presenting both scientific data and practical applications, this session will provide retailers useful information in assessing their own risks and controls.

S7 Rock On! Interdisciplinary Teams Protecting Nachos at a Concert Near You

BILLY LANGENSTEIN: *U.S. Bank Stadium, Minneapolis, MN, USA*
DANIEL HUFF: *Minneapolis Department of Health, Minneapolis, MN, USA*
FRED STEPHENS: *Federal Bureau of Investigation, Brooklyn Park, MN, USA*

Go team! Rock On! Each year, sporting events and large entertainment events generate $40 billion. Additionally, thousands of celebrations and festivals are held in our hometowns. These events may serve as an attractive target due to the large crowds and iconic venue or event visibility. Food is often a

signature aspect of these events and something event goers look forward to. To protect the food supply from intentional adulteration in our entertainment venues, we need to ensure we are collectively prepared and trained across disciplines. This symposium will present challenges from large venue managers, how public health incorporates food defense and safety into their preparedness, where and how law enforcement gets involved, and share lessons from exercising food adulteration in large venues.

S8 From Cow to Curd: Defining Microbiomes in the Dairy Industry

JOELLE K. SALAZAR: *U.S. Food and Drug Administration, Bedford Park, IL, USA*
ZHENGYAO (ZEYA) XUE: *University of California - Davis, Davis, CA, USA*
ANGELA ANANDAPPA: *University of Nebraska-Lincoln, Lincoln, NE, USA*

Cheese fermentations are impacted by a diverse microbiota that originate from the raw ingredients, adjunct starter cultures, processing environment as well as post-process contamination. High throughput metagenomic sequencing technology has revolutionized the ecological study of food products and processing facilities. Metagenomics employs sequencing of a short amplicon of a conserved segment of a hypervariable region of the rRNA gene to identify a diverse array of microbial species within a particular sample. This powerful tool provides significant insight into how microorganisms influence the quality and safety of foods. This symposium will begin by presenting the effect of a diverse microbiota on the growth and survivability of foodborne pathogens such as *Listeria monocytogenes* and *Escherichia coli* O157:H7 in a raw milk Gouda-like cheese using metagenomics. A transcriptomic approach to examine the metabolic activity of the pathogens during the manufacture and aging of the raw milk cheese will also be presented. Secondly, this symposium will identify and evaluate the microbiota in dairy processing facilities, encompassing all processing steps for cheese production, and investigate the role these organisms play in causing spoilage and quality defects in cheese. Furthermore, the precipitous implementation of high throughput sequencing tools prompts this symposium to address the demand for the development of standardized metataxonomic methods for use with milk and dairy products. Lastly, key indicators in how well a sanitation program is executed can be gleaned from the ecosystem of the whole and parts of the processing facility through metagenomics. Therefore, the use of metagenomics to determine the effectiveness of a processing facility's sanitation plan will also be discussed. Overall, the goal of this symposium is to illustrate the use of metagenomics as a high throughput sequencing strategy to assess the safety and quality of dairy products by defining the associated microbiomes.

S9 Non-NGS Methods for Foodborne Pathogen Identifications

RODNEY MOXLEY: *University of Nebraska-Lincoln, Lincoln, NE, USA*
PAMELA WILGER: *Cargill, Inc., Wayzata, MN, USA*
JIANFA BAI: *Kansas State University, Manhattan, KS, USA*

Although there has been much advancement with next generation sequencing (NGS) technology and its expansion to food analytical laboratories, the complexity in NGS process and data analysis, higher costs, and longer turnaround time for results can limit its application as a routine protocol. In the near future, centralized NGS facilities may provide the majority of NGS services, as Sanger sequencing facilities presently do. There are, however, other technologies that can provide the food analytical laboratories with the necessary means to determine the microbial contaminations to food products. In this symposium, speakers will provide an overview or address specific non-NGS technology that has proven its efficacy and advantages for pathogen detection in foods and other matrices. Various forms of PCR-based technologies remain the common technologies used in many food safety laboratories for pathogen identification. The advantages and limitations of these PCR-based methods will be highlighted as alternatives to NGS. The attraction of digital PCR and highly multiplexed systems like the Luminex system that processes a few dozen targets in a single reaction will be discussed. Microarray gene chips, notably one designed based on 368 *E. coli* and *Shigella* sequence sets to identify 55,918 annotated open reading frames, have significant potential to be used as a stand-alone diagnostic tool or complement other methods to characterize the isolate, i.e., virulence gene profiling, serotyping in a single array hybridization experiment. Current methods used in the food industry will be discussed as well.

S10 Non-thermal In-package Pasteurization of Food

TONY JIN: *U.S. Department of Agriculture - ARS, Eastern Regional Research Center, Wyndmoor, PA, USA*
XUETONG FAN: *U.S. Department of Agriculture - ARS, Eastern Regional Research Center, Wyndmoor, PA, USA*
BRENDAN A. NIEMIRA: *U.S. Department of Agriculture - ARS, Wyndmoor, PA, USA*

Foodborne illness outbreaks and food waste are two major challenges for consumers, food industry and scientists, and are both linked to the microbiological conditions of the commodity. Packaging plays a critical role in ensuring food safety and extending shelf-life by preventing microbial contaminations. However, conventional packaging cannot inactivate microorganisms that already exist in food before packaging or those that are contaminated during packaging (via package materials and package machines, etc.). These microorganisms could survive or even grow during post-packaging storage and transportation, which raises the safety concern, causes quality deterioration, and shorten shelf-life. "In-package Pasteurization" is a process that inactivates pathogenic and spoilage microorganisms after the product is packaged. Thermal methods such as retort or microwave heating have been used for In-package Pasteurization of foods. However, thermal methods degrade sensory and nutrition values and are not particularly suitable for fresh products, such as fresh and fresh-cut fruits and vegetables. Clearly, non-thermal In-package Pasteurization is urgently needed. This symposium will demonstrate several different approaches to apply non-thermal In-package Pasteurization, including antimicrobial packaging materials for In-package Pasteurization; In-package aerosolization of antimicrobials, application of cold plasma for In-Package Pasteurization, etc. that effectively reduce pathogenic and spoilage microorganisms in various types of food. The advantages and disadvantages for each approach will be discussed, and challenges and considerations for commercial application of In-Package Pasteurization will also be addressed.

S11 The Challenge of Challenge Studies

CARRIE FERSTL: *Covance Food Solutions, Livermore, CA, USA*
ELIZABETH GRASSO-KELLEY: *Illinois Institute of Technology, Bedford Park, IL, USA*
NATHAN ANDERSON: *U.S. Food and Drug Administration, Bedford Park, IL, USA*

Historically, physical and chemical barriers, e.g., pH, salt, and water activity, have been relied upon to inhibit the growth of pathogens. The systematic reduction of these barriers in some products has raised safety concerns among regulators and demonstrated the need for processors to conduct challenge study. As recognized, it is impossible for the processors to conduct a challenge study on every single product that they manufactured. Therefore, a formula risk assessment plays an important role not only to help categorizing the products and evaluating how challenge study should be designed to effectively address the safety of each formula within the product category, but also help assessing the level of pathogen reduction is needed to make product safe. The purpose of this session is to provide a few case studies on framework development for conducting a formula risk assessment and designing a microbial challenge study using published tools such as, NACMCF Parameters for Inoculated Pack/Challenge Study Protocols, mathematical modeling tools, FDA decision tree on Time/Temperature Control for Safety. Before developing a framework for the formula risk assessment (including ingredient assessment)

and microbial challenge study, it is essential to understand if there are any mathematical models that can be applied and suitable to substitute for the challenge study to provide sufficient scientific information on product safety. Once it is determined a challenge study is needed, the session speakers will address some of the nuances, specific considerations and precise details to design and perform a rigorous challenge study. When developing the study framework, we should consider the most current advances in methodologies, current thinking on pathogens or spoilage organisms of concern, and an understanding of the product preparation, variability, shelf life, and storage conditions.

S12 Challenges for HACCP and Food Safety Systems in Multi-jurisdiction Food Facilities

SALLY KLINECT: *Nestlé, Solon, OH, USA*
BALASUBRAHMANYAM KOTTAPALLI: *Conagra Brands, Omaha, NE, USA*
LORALYN LEDENBACH: *Kraft Heinz Company, Glenview, IL, USA*
MICHAEL ROBACH: *Cargill, Minneapolis, MN, USA*
ADAM BORGER: *University of Wisconsin-Madison, Madison, WI, USA*
MAILE HERMIDA: *Hogan Lovells US LLP, Washington, DC, USA*

Many food manufacturers produce products in single facilities that fall under the review of different regulatory jurisdictions. Examples can include: USDA, FDA Preventive Controls for Human and Animal Food, FDA Juice HACCP, FDA Seafood HACCP, FDA Pasteurized Milk Ordinance (PMO), FDA Low-Acid Canned Foods (LACF), CFIA, and Codex HACCP. These situations present multiple challenges for those food companies that must manage HACCP systems for more than one of these jurisdictions. This symposium will present several of these challenges and provide examples of how a company may manage these situations. Discussion topics will include: documentation challenges – how to manage CCPs; preventive controls; pre-requisites; managing inspectors from different regulatory agencies and different countries; conducting training to achieve the appropriate certification for USDA, PCQI, LACF, Juice HACCP, Seafood HACCP, PMO, etc.; legal challenges and interactions of the various rules; managing differing guidance documents for hazard analysis; and auditing challenges between audit schemes and various regulatory requirements. Industry and regulatory professionals will benefit from hearing about challenges and potential solutions and will be able to participate in open discussions on this difficult aspect of food safety system and HACCP management.

S13 Agricultural Water Quality Standards: Striving for Safety with Incomplete Science because Doing Nothing Was Not an Option

CHELSEA DAVIDSON: *U.S. Food and Drug Administration, College Park, MD, USA*
CHANNAH ROCK: *University of Arizona, Maricopa, AZ, USA*
DANIEL WELLER: *Cornell University, Ithaca, NY, USA*
ELIZABETH BIHN: *Cornell University, Geneva, NY, USA*
JOHN RAVENSCROFT: *U.S. Environmental Protection Agency, Washington, DC, USA*
TREVOR SUSLOW: *University of California-Davis, Davis, CA, USA*

On September 13, 2017, the FDA extended the compliance dates for agricultural water requirements in the Produce Rule to allow additional time to consider ways to simplify the microbial quality and testing requirements. The FDA acknowledged feedback that some of the current standards may be too complex to understand and implement, and committed to striking a balance between minimizing regulatory burdens and protecting public health. In response to outcomes from the CPS Agricultural Water Colloquium (April 6–7, 2017), the FDA published a list of methods it determined are equivalent to the method (1603; modified mTEC) cited in the rule (September 11, 2017). The FDA participated in a Collaborative Forum with the Pew Charitable Trusts to discuss near-term solutions for addressing problems implementing current standards, as well as longer-term actions to improve understanding of agricultural water quality and risk reduction (October 3, 2017). The FDA also committed to an Ag Water Summit with the Produce Safety Alliance in February 2018 to develop further stakeholder input.

This symposium will begin with a recap of the issues and concerns that caused the FDA to revisit the Ag water requirements in the rule and provide information about FDA stakeholder engagements and other activities to date. Two speakers will discuss the current state of science for a variety of water testing methods and sampling strategies. The second half of the symposium will begin with a summary of key stakeholder inputs to the FDA, including findings and recommendations from the Ag Water Summit. The final two speakers will introduce opposing perspectives on the role of water testing in the larger picture of water quality management, followed by audience interaction with all panelists. The moderated discussion will focus on areas where data are available and areas where data can be bolstered to support risk-based Ag water standards that are both practical and protective.

S14 Pathogenic *E. coli* in Low-moisture Food Systems, Contamination, Survival, and Risks

KENT JULIOT: *Ardent Mills, Denver, CO, USA*
PABLO ALVAREZ: *Novolyze Inc., Cambridge, MA, USA*
SUSANNE KELLER: *U.S. Food and Drug Administration, Summit-Argo, IL, USA*

Previous risk analyses of low-moisture foods have routinely focused on *Salmonella*, due to its persistence in low-moisture environments, recalls and food related outbreaks. However, recently new pathogen-food combinations are on the rise. In particular, several outbreaks and recalls involving Shiga toxin-producing *Escherichia coli* (STEC) have recently occurred in low-moisture products, specifically soy nut butter and flour. There is little information about the reservoirs of STEC in the processing environments, and the routes of contamination in these low-moisture products are not clear. Since some of the low-moisture products are ready-to-eat (nut butters), and others reach consumers without a kill-step (flour) and have a long shelf life, it is important to know how processing affects the survival of *E. coli* in these products. There is data indicating that *E. coli* exhibits increased resistance to heat in low-moisture environments. However, there is limited knowledge with respect to the overall survival or resistance of *E. coli* to various types of processing in dry environments and products. Although some studies indicate lesser resistance of STEC as compared to *Salmonella*, some studies present mixed results. This symposium will focus on current data with respect to STEC in low-moisture foods and will explore its survival and resistance in these systems.

S15 Heat-resistant *E. coli* – Some Like It Hot

LYNN MCMULLEN: *University of Alberta, Edmonton, AB, Canada, Canada*
JOERG HUMMERJOHANN: *Agroscope, Food Microbial Systems, Bern, Switzerland, Switzerland*
NORMAN NEUMANN: *University of Alberta School of Public Health, Edmonton, AB, Canada, Canada*

Heat treatments are widely used to reduce bacteria and decontaminate surfaces, while cooking has long been considered a final lethality step for pathogenic bacteria. Heat is one method recommended for adequate decontamination and storage of drinking water. However, this may no longer be the case. Recently, *E. coli* were identified that are extremely heat resistant (XHR) and capable of withstanding exposure to 60C (140F) for one hour or more. The genetic determinants that provide this protection to *E. coli* are being identified, as are the mechanisms behind it. A chromosomally located Locus of Heat Resistance

(LHR) has been identified, as has a transferrable plasmid-borne LHR. XHR *E. coli* have been isolated from a meat processing plant, and raw milk cheese indicating that environmental reservoirs likely exist. Further, the LHR has been found in human clinical isolates of extended-spectrum beta-lactamase (ESBL) producing *E. coli*, suggesting it may influence their survival in a hospital setting. Additional characterization studies are finding evidence that XHR *E. coli* have developed a diverse armature against multiple treatment conditions (high-pressure processing, chlorine, UV-light, peroxide and ozone), allowing them to adaptively navigate around stepwise treatment processes used in food processing and water treatment. With the potential transmission of XHR *E. coli* and the LHR through the environment and waterways, their greatest impact may be felt on drinking water supplies since water treatment is recognized as the single greatest public health intervention in infectious disease control in all of human history.

In this session, world-renowned scientists involved with the initial discoveries and characterizations of these *E. coli* will share their research and address questions surrounding the problem that XHR *E. coli* may pose to food safety and public health.

S16 The Meaning of "Clean" – Fit for Purpose Water for Field, Factory and Food Preparation

ELISABETTA LAMBERTINI: *RTI International, Rockville, MD, USA*
PATRICK SMEETS: *KWR Watercycle Research Institute, Nieuwegein, Netherlands, Netherlands*
SUCHART CHAVEN: *PepsiCo, Dubai, United Arab Emirates, United Arab Emirates*

Responding to the increasing challenge of access to clean water for food production, processing and preparation require our urgent attention and a strong focus on how to safely use available water sources along the food chain.

With increasing pressure on water resources, the food production sector is more often facing a situation where it needs to simply use whatever water is available, regardless of quality, and minimize wasting water through re-use. But is there enough science and guidance available to ensure that these changing patterns of water use will present a minimal risk to food safety? This requires both an assessment of the risks from these changing water sources and uses/reuse as well as a more systematic application of some of the classical food safety risk management approaches such as HACCP. Adapting such a tool has been a key aspect of the revision and updating of the global guidance on safe water use led by WHO. However, such tools are not yet optimally used.

This symposium will present some of the key international guidance and tools pertinent to safe water use in food production systems. Use of risk assessment to support a definition of water safety and suitability along all points in food supply chains and especially in geographies that feel the mounting pressure of climate change and other challenges on water safety and security of available water resources will be shared, as well as experiences from the front line.

S17 How to Show "Done" is Done: Designing Cooking Procedures for RTE Foods

SUSAN HAMMONS: *U.S. Department of Agriculture – FSIS, Washington , D.C., USA*
KATHLEEN GLASS: *University of Wisconsin-Madison, Madison, WI, USA*
JOHN MARCY: *University of Arkansas, Fayetteville, AR, USA*

Establishments and restaurants produce a wide variety of food products that are cooked to achieve food safety. FSIS recently updated its recommendations for lethality, and the FDA and the food industry have also recently developed guidance for establishments to meet FSMA standards. However, outbreaks and illnesses continue to occur due to undercooked products. Current research focuses on these varied processes with possible new alternatives for pathogen reduction in products outside the scope of current cooking guidance This symposium will address federal government policy and recent research that can be used by establishments and restaurants to better design cooking processes to prevent foodborne illness.

S18 Using "Big Data" to Predict Critical Food Safety Violations

THOMAS FORD: *Ecolab Inc., Greensboro, NC, USA*
BENJAMIN CHAPMAN: *North Carolina State University, Raleigh, NC, USA*
CORY HEDMAN: *Meijer Inc., Grandville, MI, USA*

This session will provide research results from a joint study conducted by North Carolina State University and industry using data from third party food safety assessments of retail stores to predict food safety violations. The data spans six years of grocery store food safety inspections from eight different chains in North America using more than 72,000 unique assessment visits. Patterns were analyzed by violation, location and time/date. By using these data points, a predictive model was developed. Historically, lagging indicators have been used to identify and address food safety issues and root cause. A better approach is to use leading indicators to identify locations and actions that will be sources of food safety failures and to address those before a failure can occur. This session will show how the model was designed, how it is being used in the retail world, and share early findings on its success.

S19 No Nodding Off: Creative Ways to Make Food Safety Fun

RONALD SCHMIDT: *University of Florida (Retired), Gainesville, FL, USA*
CARL WINTER: *University of California-Davis, Davis, CA, USA*
DAVID BAUMLER: *University of Minnesota, St. Paul, MN, USA*

Do you ever catch your audiences dozing off during your presentations or lectures covering important food safety topics? Do you yourself admit that you sometimes find it hard to stay awake during dull, boring, tedious presentations about sanitation and hygiene, allergen control, foodborne pathogens, and other often mundane food safety issues? If you answered "yes" to either of these questions, this is a symposium you won't want to miss.

It's a constant struggle for food safety professionals to find effective ways of communicating food safety messages to people of all ages and walks of life, including students, food industry and food service employees, and consumers. Program design and modeling are important for the success of food safety messaging. But all the information sharing in the world is of no avail if no one pays attention.

Several communications issues will be addressed during this symposium. How can you grab people's attention and hold it? How do you make learning science-based food safety information fun? How do you creatively motivate others to embrace sound food safety practices in order to minimize the risks of foodborne illnesses? This landmark symposium promises to provide inspiration about how you can use your talents to liven up your food safety presentations and keep any audience awake, attentive, and more apt to retain important messages – not to mention more apt to jump to their feet, applauding for more!

Speakers will address how key training tips can be covered festively, including with the Twelve Days of Christmas; how educational programs relative to toxins, allergens, and pathogens can be improved upon by incorporating music into food safety curricula; and how some bubbles and an invisible trumpet solo helped a university professor engage students and win a $40,000 piece of equipment for his lab by entering a YouTube video.

S20 How Well Do We Understand Microorganisms in a Food-handling Environment?

JEFFREY KORNACKI: *Kornacki Microbiology Solutions, Inc., Madison, WI, USA*
HENK DEN BAKKER: *Center for Food Safety, University of Georgia, Griffin, GA, USA*
TIMOTHY JACKSON: *Driscoll's, Watsonville, CA, USA*
HALEY OLIVER: *Purdue University, West Lafayette, IN, USA*
SHAWN STEVENS: *Food Industry Counsel, LLC, Random Lake, WI, USA*
DON ZINK: *IEH Laboratories & Consulting Group, Taylors, SC, USA*

In a processing or food service facility, it is important to understand the microorganisms that exist. The introduction of new food products, new or modified processes, construction, and facility changes may result in the introduction of a new organism. Newer technologies increasingly in use today can point to this emerging or newly identified flora. The ability to differentiate between resident and transient organisms, to know whether a new organism has emerged, and how to manage the resulting scenario are all keys to effective food safety programs. Understanding the commodity and process can aid in understanding the risk posed by the new profiles of organisms in the food-handling environment. This symposium will discuss how to understand a persistent vs. transient strain; how to quantify when the changes are occurring; how to respond; and the legal and regulatory implications for proactively understanding the microbial profile of a food-handling environment.

S21 Biological Variability in Thermal Processing: Impact for Process Control and Validation – What You Need to Know about Microbiological Variability for Food Quality and Safety Control

HEIDY DEN BESTEN: *Wageningen University, Wageningen, Netherlands, Netherlands*
MARIEM ELLOUZE: *Nestlé, Lausanne, Switzerland, Switzerland*
JENNY SCOTT: *U.S. Food and Drug Administration – CFSAN, College Park, MD, USA*

Microbial limits are used to evaluate and validate processes that aim to control food safety and stability. However, biology is highly variable; microorganisms, raw materials, and humans are variable and diverse. And this variability is challenging the 'line in the sand' of our microbial limits. When we would control safety and spoilage for the average case, it would go wrong half the time. When we aim to control at the 99 percentile all our process parameters, we might over-process. Understanding, quantifying, and determining the impact of variability is needed to control spoilage and food safety. In this symposium, we will present experimental modelling and risk assessment aspects, including the points of view of academia, government, and industry.

S22 International Experiences with Systems for Hazard Monitoring and Rapid Risk Assessment

MICHELLE CATLIN: *U.S. Department of Agriculture–FSIS, Washington, D.C., USA*
LEON GORRIS: *Unilever R&D Vlaardingen, Vlaardingen, Netherlands, Netherlands*
JEFFREY LEJEUNE: *Food and Agriculture Organization of the United Nations, Rome, Italy, Italy*

In today's world, foods and food ingredients can be sourced from virtually any country and producer to distributor worldwide. As food supply chains have become increasingly longer and more complex, the challenge for governments and industries is to be warned as early as possible of the presence of known or emerging hazards in food supply chains or in markets.

A number of organizations have developed systems for early warning that help in the timely detection and response to food safety events, and are necessary to minimize the negative effects of these events on human health and welfare, as well as the effects on global trade, income, employment, and food security. Such early warning systems consist of tools, processes, resources, and networks that in concert identify and verify early warning signals, analyze relevant data/information, and communicate appropriate alerts to relevant stakeholders in order to inform risk management actions and decision-making. Importantly, timely risk assessment approaches are required to determine the potential impact of potential hazards and the need and urgency to communicate and/or to mitigate. In this symposium, three organizations will share their approaches and experiences with early warning systems.

S23 Integrated Approaches to Measure and Impact Consumer Food-handling Behaviors

SHERYL CATES: *RTI International, Research Triangle Park, NC, USA*
CHRIS BERNSTEIN: *U.S. Department of Agriculture – FSIS, Washington, DC, USA*
CHRIS BERNSTEIN: *U.S. Department of Agriculture – FSIS, Washington, DC, USA*
ELLEN THOMAS: *RTI International, Research Triangle Park, NC, USA*
CHRIS WALDROP: *U.S. Food and Drug Administration, College Park, MD, USA*
ROY BETTS: *Campden BRI, Gloucestershire, United Kingdom, United Kingdom*
BENJAMIN CHAPMAN: *North Carolina State University, Raleigh, NC, USA*

Myriad scientific techniques, from surveys to microbiological testing, are increasingly employed to assess safe food handling-practices and develop guidance for consumers worldwide. Self-reported behaviors, observed practices, and tracking the movement of pathogens have all been used to evaluate the effectiveness of consumer-focused food safety messaging and what actually happens during food preparation. Studies that integrate both psychological and microbiological methods are rarer, but represent the gold standard of consumer food handling. While there are various food safety-focused messages for consumers, it is less common to have a theoretical framework to support approaches, combined with integrated evaluation methods. If food safety professionals are to aid in changing consumer behaviors to reduce food safety risks, it is necessary to not only understand and characterize the public health burden of specific consumer practices, but to also have a deep qualitative and quantitative understanding of the mechanics of those practices and the motivation of individuals. This session will explore how an integrated approach using multiple assessment techniques can be used not only to develop food safety messages but also to evaluate and improve their effectiveness. An international perspective on cultural antecedents will provide a contrasting lens to consumer behaviors and message development. Significant findings of a current integrated project, lessons learned, future recommendations, study limitations, and knowledge gaps in the literature will be discussed by presenters in the context of their specific projects and goals. This panel will illustrate how to structure and implement an integrated approach to food safety messaging across all levels of message development.

S24 Pathogens in Soil: A Focus on *Salmonella* and STEC Survival in Biological Soil Amendments of Animal Origin

DAVID INGRAM: *U.S. Food and Drug Administration – CFSAN, College Park, MD, USA*
MICHELE JAY-RUSSELL: *Western Center for Food Safety, University of California, Davis, CA, USA*
LAURA STRAWN: *Virginia Tech - Eastern Shore AREC, Painter, VA, USA*

Researchers have extensively studied the survival of enteric pathogens such as *Salmonella* and Shiga toxin-producing *E. coli* (STEC) in manure, manure-amendments, and soil. While manure-amendments, also known as biological soil amendments of animal origin (BSAAO), are utilized for fertilizing produce fields and sustainability measures, BSAAO presents a food safety risk, and determining the prevalence and survival of enteric pathogens in BSAAO is a primary focus for several research laboratories in the U.S. The goal of this symposium is to provide academia, industry, and government with the latest information on the factors that can influence the prevalence and survival of *Salmonella* and STEC in BSAAO. Presenters will discuss recent research on pathogen prevalence, concentration, and survival in soils, as well as the process of integrating datasets into risk assessments to develop science-based regulations.

The first presenter will discuss the FDA's approach to the integration of BSAAO research into the development of feasible legislation. Additionally, efforts to integrate datasets into risk assessments to quantify human illness associated with the consumption of contaminated produce grown with untreated BSAAO will be discussed. The second presenter will present manure pathogen survey data from different regions in the U.S. While critical for risk assessments, this research could also lead to uncovering the geographic and environmental factors that influence survival across the U.S. The third presenter will provide insights on the conditions that aid in the survival of pathogens under controlled laboratory conditions, which will help inform risk assessments and further develop knowledge on pathogen survival in soils.

Although there are recommendations available based on the National Organic Program, there is not a current regulation on the harvest interval between manure application and crop harvest for fresh produce. Therefore, a continued discussion on the factors that influence survival and the meaning of previous research findings is warranted.

S25 What Do Genomics Tell Us about Controlling *Campylobacter* in Poultry and the Risk of Poultry-associated Illness?

MUSTAFA SIMMONS: *USDA-FSIS-OPHS-EALS, Athens, GA, USA*
BEN PASCOE: *The Milner Centre for Evolution – University of Bath, Bath, United Kingdom, United Kingdom*
EDUARDO TABOADA: *Public Health Agency of Canada, Lethbridge, AB, Canada, Canada*

Campylobacter is one of the leading causes of bacterial foodborne gastroenteritis. A major species, *C. jejuni*, is highly prevalent in raw poultry. Although most cases are self-limiting, post-infection complications, such as Guillain-Barré syndrome, can be life-threatening. *Campylobacter* ecology and epidemiology is complicated, and most human cases appear to be sporadic using traditional typing methods. *C. jejuni* lineages appear to have different host range and prevalence in human infection. Next generation sequencing technologies are providing new insights into *Campylobacter* ecology, epidemiology, and the distribution of traits impacting foodborne illness including survival, resistance, and virulence, which can inform the development of strategies for controlling this important foodborne pathogen. This session will provide an update on studies to associate *C. jejuni* genomes and genes with *Campylobacter* traits of public health importance.

S27 Edible Insects: Food Safety Considerations for a Food Security Solution

ROBERT WILLIAMS: *Virginia Tech, Blacksburg, VA, USA*
DOUGLAS MARSHALL: *Eurofins Scientific Inc., Fort Collins, CO, USA*
ODETE MENDES: *Product Safety Labs, Cranbury, NJ, USA*

Consumption of insects has been a part of human dietary practice since prehistory. Today, many peoples include insects as part of their ordinary diet, while many others disregard insects as a food source. Human population growth estimates, as well as economic and environmental concerns, have generated considerable concern about the future availability of enough protein in the diet of many people around the globe. Insects may provide a sustainable solution to this dietary problem. Historically, insects were harvested from their natural environments for consumption. However, insect farming for production of human food is growing. Although whole insects may be consumed, insect food products developed through further processing are increasing as well. Edible insect-based foods have a native microbiota that is somewhat different from other sources of animal foods. This microbiota is largely determined by the nature of the insect and conditions found in production and processing environments. Human foodborne pathogen prevalence in insects is generally believed to be small, but prudent producers and processors must implement effective controls to minimize food safety risks. This symposium will address the current status of the edible insect industry and identify common insects used for food. Foodborne hazards and risks associated with edible insects and associated products, as well as the regulatory status of insect foods will be presented.

S28 Cleaning Validations – Approaches in Retail Food and Food Manufacturing Facilities

ANNA STAROBIN: *Ecolab Inc., Greensboro, NC, USA*
DUANE GRASSMANN: *Nestlé USA, Solon, OH, USA*
VANESSA CRANFORD: *US. Food and Drug Administration-CFSAN, Division of Produce Safety, Office of Food Safety, College Park, MD, USA*

Cleaning validations are not consistently executed in the food industry. This lack of consistency is generally because the "what," the "how," and the "why" have not been clearly defined prior to validations. Additionally, validations historically have focused on the result of the cleaning as opposed to the procedure that delivered the result. The successful criteria of a robust cleaning procedure must link to risks that can be controlled through cleaning. Criteria typically begin with the subjective method of visually clean, which is prone to variability in interpretation. Visually clean is often followed by objective criteria that include numerous methods, the most popular being ATP testing. Unfortunately, objective approaches often have pass/fail limits that are not easily understood. Unlike in manufacturing, the use of ATP is not common in Food Retail and Food Service due to limitations, including challenges associated with lack of agreed upon acceptance criteria, equipment variations in multiple locations, and logistic challenges such as equipment cost, etc. In recent years, some retail and food service chains are attempting to use and validate cleaning quality using ATP. Finally, cleaning validations are one of many sanitation programs considered as preventive controls under the Food Safety Modernization Act (FSMA). The food industry has significant interest in sanitation as a preventive control within FSMA.

This symposium will have three presenters. The first speaker will discuss ATP testing approaches in food retail establishments, challenges, and solutions for such testing. Another speaker will present on best practices for performing cleaning validations in manufacturing settings. A final speaker will present on sanitation and cleaning as a preventive control.

S29 Multi-level Approach to Combating Antimicrobial Resistance

SARAH CAHILL: *Food and Agriculture Organization of the United Nations, Rome, Italy, Italy*
ISSMAT KASSEM: *American University of Beirut, Beirut, Lebanon, Lebanon*
ERIC BRUM: *FAO, Dhaka, Bangladesh, Bangladesh*

Microbes do not respect international borders. Travel and trade in food products, in response to economic development and consumer appetite for non-local food products, has resulted in the global transmission of foodborne microorganisms, pathogens, spoilage organisms, and commensal contaminants alike. Thus, the factors contributing to the presence of antimicrobial-resistant (AMR) bacteria in foods and animal feeds in one region of the world can have impact on food safety wherever that product is consumed – in the community of its production, within the country of origin, in regional trading partners, and across the world. To most effectively combat AMR, nested interventions must be applied at multiple levels – on the global level and more specifically on a regional level, in which individual countries are embedded. Within each country there are numerous individual farms. This symposium will explore examples of how activities related to governance, awareness, surveillance, and good practices can be applied at various geographical scales to combat the emergence and transmission of antimicrobial resistance in food.

S30 Soil Contamination with Foodborne Bacteria

MANAN SHARMA: *U.S. Department of Agriculture – ARS, Environmental Microbial and Food Safety Laboratory, Beltsville, MD, USA*
PATRICIA MILLNER: *U.S. Department of Agriculture – ARS, Environmental Microbial and Food Safety Laboratory, Beltsville, MD, USA*
JOSHUA GURTLER: *U.S. Department of Agriculture-ARS, Eastern Regional Research Center, Wyndmoor, PA, USA*
ELIZABETH BIHN: *Cornell University, Geneva, NY, USA*

The demand and output of fresh produce continues to increase. With advances in the sensitivity of pathogen testing and epidemiological tracebacks, the need to mitigate pre-harvest bacterial contamination of fresh produce soils will become paramount. This symposium will address means of decontaminating soil. The majority of research in the published involves inactivation of phytopathogens in soil, i.e., those pathogens harmful to fruit and vegetable production and ornamental plants. Relatively little data has been published regarding the inactivation of foodborne human pathogens in crop soil. Implementation of these mitigation and decontamination techniques should be realistic for farmers and growers to undertake on their farms. This means considering costs (e.g., time, inputs including chemicals, fuel), infrastructure (e.g., specialized machinery needed to implement the practices), and environmental impacts. Growers can then make decisions on mitigation strategies based on how well that strategy integrates with many other goals including yield; quality; produce safety; environmental and economic impacts; market demand; and consumer expectation. It is crucial to understand how enteric foodborne pathogens survive in soils or in manure-amended soils, and how this survival can affect routes of contamination to edible crops. An understanding of these factors and routes may allow more targeted interventions, or a "hurdle" approach to apply several different techniques in concert to reduce pathogen levels in soils. This would include an understanding of how biological soil amendments and soil management practices affect survival of pathogens in soils, and how enteric pathogen survival trends are influenced by the soil microbiome.

S31 Pathogen Detection and Food Microbiome Characterization Using a Metagenomics Approach

XIANGYU DENG: *University of Georgia, Center for Food Safety, Griffin, GA, USA*
SUSAN LEONARD: *U.S. Food and Drug Administration – CFSAN, Laurel, MD, USA*
ROBERT SANDERSON: *Jonathan Sprouts Inc., Marion, MA, USA*

Recent advances in high-throughput DNA sequencing provide opportunities to profile commodity-associated microbiomes either through amplicon sequencing of the 16S rRNA gene or whole-genome shotgun metagenomic sequencing. The utility of metagenomic sequencing for bacterial community profiling has been largely recognized in studies on the human microbiome, but applications specific to food safety are becoming more notable, especially in food production environments involving fermentation. Metagenomic sequencing provides accurate data about the microbial community including organisms that cannot be cultured using traditional methods. These sequencing methods can also be used to characterize shifts in microbiota composition induced by the culture methods employed to detect foodborne pathogens, such as those employed by the FDA in the Bacteriological Analytical Methods (BAM). BAM culture methods for *Salmonella*, *Escherichia coli*, and *Listeria* detection can take up to one week to identify presumptive positive colonies, which then require further testing for species confirmation and subtyping. Metagenomics is particularly suitable for characterizing these pathogens far earlier than with traditional methods. Further, the application of metagenomics may also detect competitive members of the microbial community that may hamper detection of pathogens in contaminated foods such as commensal and pathogenic *E. coli,* as well as bacteria associated with food spoilage. This symposium will provide an overview of how metagenomic methods are being employed to characterize the food microbiomes from a variety of commodities associated with foodborne outbreaks and provide insights about the changes induced by current culture methods employed for pathogen detection.

S32 Controlling Chemical Hazards in International Supply Chains – New Challenges with FSMA

CLAUDIO GALLOTTINI: *Euroservizi Impresa Srl, Torgiano (Pg), Italy, Italy*
LAUREN JACKSON: *U.S. Food and Drug Administration, Bedford Park, IL, USA*
CAROLYN MEDUSKI: *Nestlé USA, Solon, OH, USA*

Food manufacturers are facing increasing complexity when dealing with chemical hazard assessment throughout the supply chain. These challenges are especially apparent when dealing with differences in regulations between different countries in terms of both sourcing ingredients and distributing products. In a global marketplace where products are manufactured all year, issues like seasonality force manufacturers to explore sourcing from different countries, which can complicate supply chains even further. This session seeks to educate the audience on some of these challenges and explore what the possible solutions are. Specific topics include the impact of FSMA requirements such as those related to Preventive Controls (PC) and Foreign Supplier Verification Programs (FSVP) regulations, to chemical contaminant surveillance programs, and how to balance programs that are focused on ensuring food safety with regulatory considerations (such as asynchronous global regulatory frameworks). Speakers will provide both a regulatory and industry perspective on these challenges, using example hazards such as pesticides, heavy metals, mycotoxins, and emerging contaminants.

S33 Food Safety Considerations in Alleviating Hunger and Food Insecurity

VIRGINIA TILL: *U.S. Environmental Protection Agency, Denver, CO, USA*
LARRY KOHL: *Retail Business Services an Ahold Delhaize USA Company, Salisbury, NC, USA*
MITZI BAUM: *Feeding America, Chicago, IL, USA*

The U.S. Department of Agriculture estimates the number of food insecure U.S. households to be 110.8 million as of 2016, meaning they were "uncertain of having, or unable to acquire, enough food to meet the needs of all their members because they had insufficient money or other resources for food." Such resources include, but are not limited to, transportation, cultural appropriateness, nutritive quality, and regularity of access. While the food industry works

to feed an estimated 9.8 billion people by 2050, the assumption cannot be made that each of them will have the purchasing power or other resources to access the available food.

As the industry works to increase available food, an estimated 30% – 40% of food in the U.S. is wasted. Recovering safe, surplus food is one strategy to ensuring that food is feeding people and not landfills. Partnerships between government, nonprofit, and industry organizations have been developed to do this work. Much of it is completed by volunteers, in which the depth and accuracy of the food safety culture/knowledge are greatly variable and could contribute to issues with food handling and security. Challenges that food safety educators experience include high turnover and varied previous understanding of food safety topics.

The objectives of this symposium are to inform the food safety community about partnerships to safely reclaim and redistribute food across our food system, and some of the food safety challenges hunger-relief organizations and their volunteers may experience. The discussion will focus on how the safe use of recovered and other foods can increase food security and prevent food loss and waste. This builds upon an IAFP 2015 symposium on food waste, which explored the utility of reclaimed foods to aid those who are food insecure.

S34 Food Fraud – Progress and Plans for Prevention and Management

SAMUEL GODEFROY: *University Laval, Department of Food Science, INAF, Quebec City, QC, Canada, Canada*
PETER BEN EMBAREK: *WHO, Geneva, Switzerland, Switzerland*
KAREN EVERSTINE: *USP, Rockville, MD, USA*
JENNIFER THOMAS: *U.S. Food and Drug Administration, Washington, DC, USA*
YONGNING WU: *CFSA, Beijing, China, China*
TBD TBD: *a, b, CA, USA*

Food fraud and economically motivated adulteration are of great concern in every region of the globe and have the potential to impact consumers from a food safety and nutrition perspective. Food fraud was a key topic of discussion on the agenda of the 40th CODEX Alimentarius Commission meeting in July 2017. Several working meetings held last year in Quebec and China, and recently in Ireland, brought together experts from regulatory agencies, industry, and academia for discussions to work towards creation of strategic plans and the identification of tools and protocols to address food fraud. This symposium will focus on providing progress updates on these key topic areas and to facilitate additional discussion, with a Q&A following.

S35 Converting WGS and Bioinformatic Jargon into Plain Language and Understanding the Science

KARI IRVIN: *U.S. Food and Drug Administration, CORE, CFSAN, College Park, MD, USA*
SHERRI MCGARRY: *U.S. Food and Drug Administration, Washington, DC, USA*
MARTIN WIEDMANN: *Cornell University, Ithaca, NY, USA*

As the fields of omics and bioinformatics become more integrated into the food safety arena, the emergence of new vocabulary terms is to be expected. Although many IAFP Members may be well versed in the common terms of these fields, many other members in the food supply arena are not exposed to the jargon used to describe results or the underlying science. Our objective is to provide a symposium where experts in their respective fields will present the most common words used in these disciplines and add a short description of the science behind the terms. The intent of these presentations is to bridge the gap between those who are well steeped in omics and bioinformatics to those who need to know these terms in order to proceed with their own work.

S36 The Saga Continues… What's on Your COA? How Can We Effectively Utilize This Tool?

BENJAMIN WARREN: *Land O' Lakes, Arden Hills, MN, USA*
TIMOTHY FREIER: *Merieux NutriSciences, Crete, IL, USA*
JENNY SCOTT: *U.S. Food and Drug Administration – CFSAN, College Park, MD, USA*

Due to the nature of the food, desired quality attributes of the product, global sourcing options, and cost of goods, food safety professionals are often faced with tough decisions on the safety of the ingredients used in new product development, particularly novel ingredients. Furthermore, evolving FSMA regulations have increased awareness on the safety of existing ingredients and the adequacy of the preventive controls to assure the safety of foods in the marketplace. However, in some operations, e.g., blending and packing operations, where there is no preventive control for ingredients where the expected level of protection against a biological hazard(s) is less than a 5-log reduction due to the undesirable quality changes, or in agricultural commodities, where mycotoxins can be of concern, manufacturers may have to rely on other control measures such as receipt of a certificate of analysis (COA) in addition to GFSI audit reports and a well-established environmental program. The value of COAs as a verification tool is widely recognized, but how reliable are they? As we continue the journey to enhancing a company's food safety programs, there is a need to understand how we can better utilize a well-established COA program to help enhance the safety of our food supply. The aim of this symposium is to dive into the details of properly implementing a COA verification program that help support supply chain preventive controls. This symposium will specifically discuss key considerations when establishing specifications and test parameters to be included in the COA and best practices when implementing COA verification activities in a food processing facility.

S37 International Recognition of National Food Safety Systems

LUCIA ANELICH: *Anelich Consulting, Pretoria, South Africa, South Africa*
CAROLINE SMITH DEWAAL: *U.S. Food and Drug Administration, College Park, MD, USA*
ROGER COOK: *New Zealand Ministry of Primary Industries, Wellington, New Zealand, New Zealand*

International trade in food continues to grow – and the safety of food exported and imported is critically important. Food coming from different regions can be subject to different hazards and almost certainly subject to different rules than the importing country. The Codex Alimentarius Commission continues to develop guidelines to facilitate one country recognizing the food safety systems of another country through judging the equivalence of sanitary measures and demonstrating the performance of national good control systems. These guidelines make it easier for countries to export and import foods from one another. Tools have also been developed that allow a country to assess their food safety systems against international norms, and assist in the development of a safe food supply within their country and facilitate international trade. This symposium will explain and demonstrate these guidelines and tools in the international arena.

S38 Norovirus and Hepatitis A Virus Contamination: Emerging Monitoring Methods and Their Future Applications

JOHN MESCHKE: *University of Washington, Seattle, WA, USA*
GEUN WOO PARK: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
LEE-ANN JAYKUS: *Department of Food, Bioprocessing, and Nutritional Sciences, North Carolina State University, Raleigh, NC, USA*

Human norovirus and Hepatitis A virus are the leading viral agents of foodborne illness. Common sources of contamination include water and environmental surfaces, but the relative importance of these sources is largely unknown. This can be attributed to deficiencies in sampling, testing, data interpretation, and the lack of routine environmental surveillance. The purpose of this symposium is to highlight improvements in methods that will facilitate routine sampling and monitoring for viral contamination on surfaces and in water (including seawater, wastewater, irrigation and source waters). Specifically, speakers will address advanced methods for surveillance of enteric virus contamination in the environment, providing examples of how they have been used to answer questions related to outbreak investigation, disinfection validation, and contaminant source. The advantages and limitations of these novel methodologies will be discussed, as will their validity and applicability for use in both industrial and regulatory settings. A better understanding of the source and extent of enteric virus contamination can assist in outbreak management, assessing viral prevalence, and implementing more effective control of these agents in the food supply.

S39 Validation and Verification – The Good, the Bad and the Ugly

PURNENDU VASAVADA: *University of Wisconsin-River Falls, River Falls, WI, USA*
ALVIN LEE: *Institute for Food Safety and Health, Illinois Institute of Technology, Bedford Park, IL, USA*
ROY BETTS: *Campden BRI, Gloucestershire, United Kingdom, United Kingdom*
JOHN O'BRIEN: *Ulster University, Nutrition Innovation Centre for Food & Health, Coleraine, Northern Ireland, Northern Ireland*

In the modern age in food safety, validation and verification processes are critical components of a good food safety plan and are complementary to each other. However, food manufacturers often confuse the meaning and function between the two terms. Most food products undergo a kill step of some form at the point of production and yet, most of these control points lack scientific validation. Similarly, some are unclear of details required in validation reports that could result in regulatory agencies not accepting validation reports. Once validation is completed, a food manufacturer needs to establish verification procedures to ensure that the implemented processes are effectively and consistently carried out and that a confirmation that the food manufacturer is doing what is intended and that it is effective and activities are properly documented.

Likewise in testing laboratories a fundamental requirement is to ensure that test methods actually work and will detect/enumerate the organisms of concern. To achieve this, we rely on method manufacturers to "validate" methods. But does this give the laboratory enough confidence to use the method? Should they undertake their own verification that the method works in their hands? What should they do? Does it depend on the extent of the validation process or the range of food that they test? All of these are good questions that will be reviewed within this session. The symposium will highlight the concepts of validation and verification, their differences, and how various food processing technologies and microbiological analytical methods are validated and verified to ensure they serve their intent, the parameters used in validation studies, and record keeping. An industry perspective and experience on implementation strategies, translating from lab-based studies to full production scale and proper documentation, will be highlighted.

S40 Alignment between Reference Microbiological Methods – Reality or Dream?

BERTRAND LOMBARD: *Université Paris-Est, ANSES, Maisons-Alfort, France, France*
ERIN CROWLEY: *Q Laboratories, Inc., Cincinnati, OH, USA*
THOMAS HAMMACK: *U.S. Food and Drug Administration, College Park, MD, USA*

Reference analytical methods are a key tool to guarantee food safety and quality in the food chain. Standard methods are widely used by official, industrial, and third party laboratories as part of official and quality controls. Standard methods are also required to validate alternative methods in comparison to them, allowing them to obtain equivalent results between food industry operators and between official controls.

The objective of this session is to present the methods recently published and the new methods under development by different organizations (ISO-CEN, FDA-BAM, AOAC, etc.), the alignment and differences between methods, and the impact and application of reference methods in the food sector.

S41 Can We Ever Accomplish a Standardized Protocol for Validating WGS-based Assays for the Detection of Foodborne Pathogenic Microbes?

HEATHER CARLETON: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
MARTIN WIEDMANN: *Cornell University, Ithaca, NY, USA*
KENDRA NIGHTINGALE: *Texas Tech University, Lubbock, TX, USA*

Over the last decade, NGS technology has significantly changed how we analyze foodborne pathogens and has become an integral part of food safety programs within the food industry and government regulatory agencies. As with many traditional microbiological methods used to identify and isolate foodborne pathogens, those used in the food industry, regulatory and food analytical laboratories have been subjected to a rigorous validation study. These include those that are bacteriologically-based as well as those that use molecular biology technology. Although both types of methods can undergo different means of validation, the end result is a product that can be used in the laboratory with a reasonable level of certainty and confidence. However, WGS technology is a comprehensive workflow which covers DNA isolation, library construction, sequencing platform, and computer analysis of the raw DNA sequences generated as megadata files. As a relatively new technology, changes in all of the above occur so often that any validation study may be rendered outdated as newer platforms, isolation, library kits, and bioinformatic software become available at such a rapidly evolving pace. Therefore, is it necessary to invest in validations studies for WGS, and if so, what will it look like? This symposium is not designed to resolve this issue, but rather to start the conversation in helping to describe future directions on validating WGS methods for food safety and public health.

S42 Building a Network of Accredited Governmental Human and Animal Food Laboratories: Benefits to Public Health and Industry

STEVEN LYON: *Chick-fil-A, Atlanta, GA, USA*
DANIEL RICE: *U.S. Food and Drug Administration, Bothell, WA, USA*
BRYANNE SHAW: *Minnesota Department of Agriculture, Saint Paul, MN, USA*

In 2012, the U.S. Food and Drug Administration (FDA) awarded cooperative agreements to state human and animal food testing laboratories to help it achieve or enhance its scope of accreditation to the ISO/IEC 17025 standard. At the same time, the FDA awarded a cooperative agreement to the Association of Public Health Laboratories (APHL), the Association of Food and Drug Officials (AFDO), and the Association of American Feed Control Officials (AAFCO) to provide training, guidance documents, tools, and technical assistance to the laboratories in its accreditation efforts. These efforts bring us closer to the ideal of an integrated food safety system and ultimately, improved food safety and public health.

An industry representative will describe a retailer's expectations of suppliers for environmental and finished product testing by accredited laboratories, engaging with regulators, and the overall importance of laboratory accreditation to the food industry. An FDA representative will describe the impact of accredited state laboratory data on the recall process. The representative will also describe engagement with industry during a recall, helping them to ensure companies are selling a safe, wholesome product to their consumers. These recalls remove contaminated product from the food supply, preventing additional (if any) human illness associated with that product. Finally, a state human and animal food testing laboratorian will describe how the ISO cooperative agreement impacted the laboratory; how accredited laboratory data was utilized in a recall; and highlight improvements to the laboratory's engagement with industry within his/her state.

S43 How Omics is Changing the Food-safety Landscape in Foodborne Parasitology: Sequencing, Not Just Seeing is Believing!

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RACHEL CHALMERS: *Public Health Wales, Microbiology and Health Protection, Singleton Hospital, Swansea, United Kingdom, United Kingdom*
YVONNE QVARNSTROM: *CDC, Atlanta, GA, USA*

In its 2016 report, WHO estimated 600 million cases of infection in 2010 caused by foodborne hazards, including apicomplexan parasites and some roundworms, among many others. Worldwide, foodborne illnesses occur when undercooked meat, seafood containing parasitic infectious stages, or produce contaminated with human/animal feces are consumed. A powerful analytical platform like next generation sequencing (NGS) could open the doors to better insights for understanding molecular pathogenesis, virulence processes, and strain-level molecular fingerprinting of these parasites for food safety. NGS has yielded a few annotated genomic, proteomic, and functional genomic datasets of *Toxoplasma* (*28 WGS assemblies)* and *Cryptosporidium (36)* so far. *Other apicomplexa, Giardia intestinalis (7)*, *Cyclospora cayetanensis (6)*, and the roundworm *Trichinella* (20) also potentially harbor a wealth of strain-level diversity and evolution trends relevant for food safety. The genomes of these foodborne parasites need to be extensively studied to understand complex life cycles and develop methods to mitigate their propagation. The advances in genomics have revolutionized the understanding of the biology, pathogenesis, prevalence, and modes of survival of other foodborne pathogens like bacteria and viruses. In particular, due to the high resolution capabilities of next generation sequencing (NGS) platforms, a visible impact is noticed in foodborne pathogen detection and subtyping. With 10s and 1000s of strain-level whole genome sequences available, the molecular fingerprinting of these pathogens is becoming easier every year. Functional and proteomic studies depending on their genomic sequences have enabled new diagnostic and detection methods. In contrast, the status of foodborne parasite genomics is highly uneven and just emerging due to inherent difficulty in sequencing these organisms. This symposium will discuss the impact of emerging omics research and its challenges in foodborne parasitology and how these advances are addressing specific food safety issues in this field. Speakers from the U.S. and abroad will provide practical examples about this topic in their presentations.

S44 Developments and Novel Applications of Microbiome Research for Pre- and Post-harvest Food Safety and Quality

K.C. JEONG: *University of Florida, Gainesville, FL, USA*
STEVEN RICKE: *University of Arkansas, Fayetteville, AR, USA*
MICHAEL ROTHROCK: *U.S. Department of Agriculture – ARS, U.S. National Poultry Research Center, Athens, GA, USA*

In the last few decades, DNA sequencing technology (next generation sequencing, NGS) has dramatically evolved and subsequently applied to numerous food-related ecosystems including human, animals, foods, and food processing environments to elucidate complex microbial community relationships. Microbiome 16S rDNA sequencing based on NGS technology has led to an enhanced level of resolution and profiling of overall microbiota in complex ecosystems related to food production systems. As sequencing data sets accumulate, tremendous quantities of biological data generated from microbiome sequencing have provided deep insights of bacterial functions and interactions among bacterial consortia. Microbiome sequencing based on the 16S rRNA gene amplicons can serve as phylogenetic markers to define the more complex relationships among microbiota in foods. In addition, sequencing technology is beginning to provide a wide range of information to identify the appropriate indicator microorganisms during the food manufacturing processes. This could help to better understand the microbial distribution of bacteria according to the presence or absence of specific foodborne pathogens. Food/feed supplements such as prebiotics, which have been considered antibiotic alternatives, are now being utilized widely in food industry to increase beneficial bacteria and decrease harmful ones. Identification of overall microbial populations in pre- and post-harvest foods supplemented with prebiotics across the food production spectrum, including poultry, cattle, dairy foods, and seafood, will allow for the identification of core indicator bacteria associated with food safety and quality. In this proposed symposium, we will organize three to four speakers who are actively working on different aspects of microbiome research in the area of poultry, food animals, and foods in academic institutes and governmental agencies. Perspectives from speakers who have experience in various areas will help audiences understand microbiome approaches for novel applications in food safety and quality. The strength of this proposed symposium will cover a wide range of food products consumed in both the United States and internationally.

S45 Food Safety and Hurricanes – The Eye of the Storm

DANIEL OKENU: *HEB Grocery Company LP, San Antonio, TX, USA*
ELIZABETH ORMOND: *U.S. Food and Drug Administration, Maitland, FL, USA*
SUMMER WILLIAMS: *Florida Department of Agriculture and Consumer Services, Division of Food Safety, Tallahassee, FL, USA*
JAMIE DEMENT: *Florida Department of Health, Tallahassee, FL, USA*
MICHAEL ROBERSON: *Publix Super Markets, Inc., Lakeland, FL, USA*

The 2017 hurricane season was like none seen before. The impact of Hurricane Harvey resulted in a massive flood-related event in Houston, Texas, the fourth most populous city in the United States. Several weeks later, Hurricane Irma led to the largest storm-related evacuation in the history of the United

States, with the entire state of Florida impacted by storm-related events. Puerto Rico was dealt a massive blow by Hurricane Maria, with half of the island still without power as of the New Year. The effects of these storms continue to be felt, with areas slowly regaining a sense of normalcy. Food retailers play a vital role in the critical infrastructure for storm preparation and recovery efforts. Wind, rain, flood, and loss of electricity all have a major impact on a retailer's ability to maintain food safety. Food safety inspectors play a major role assessing food safety and public health. This symposium discusses how retailers and regulators address these challenges. We will also discuss the importance of Public – Private partnerships as we work collaboratively to address these significant humanitarian public health crisis events.

S46 State and Local Regulatory Agency Foodborne Illness Investigations

RANDY J. TREADWELL: *Washington State Dept. of Agriculture, Spokane, WA, USA*
TOM SAFRANEK: *Nebraska Department of Health, Lincoln, NE, USA*
DAVID NICHOLAS: *New York State Department of Health, Albany, NY, USA*

Each year, state and local food regulatory agencies investigate thousands of potential foodborne outbreaks. This session will highlight the efforts of state and local regulatory agencies in the investigation of foodborne illnesses, including the U.S. Food and Drug Administration-funded state-based human and animal food rapid response teams (RRT). The session will include the use of techniques such as environmental assessments, environmental sampling, and whole genome sequencing in foodborne illness investigation at the state and local level. In 2015, the U.S. Center for Disease Control and Prevention continued to report that nearly 60 percent of foodborne outbreaks identified were associated with a single location of preparation, such as a restaurant. These outbreak investigations typically involve only state and local officials, but impact dozens and up to hundreds of individuals. The session will also discuss contributing factors identified during outbreaks and lessons learned by the agencies conducting the outbreak investigations.

S47 The Global Food Safety Impact of *Cyclospora cayetanensis*: An Issue Crossing Continents

BARBARA HERWALDT: *Centers for Disease Control and Prevention, Center for Global Health, Division of Parasitic Diseases and Malaria, College Park, MD, USA*
RACHEL CHALMERS: *Public Health Wales, Microbiology and Health Protection, Singleton Hospital, Swansea, United Kingdom, United Kingdom*
YNES ORTEGA: *University of Georgia, Griffin, GA, USA*

Cyclospora cayetanensis is a protozoan parasite that causes a human diarrheal disease called cyclosporiasis. Transmission of cyclosporiasis is commonly associated to the consumption of different fresh produce items contaminated with the parasite's infective stage, i.e., sporulated oocysts. According to the surveillance data from the Centers for Disease Control and Prevention, more than 1,065 cases of cyclosporiasis with 52 hospitalizations were reported from 40 states in the U.S. in 2017. Other countries have also reported outbreaks of cyclosporiasis. This symposium will discuss the impact of *C. cayetanensis* from a global perspective. Speakers from the U.S. and abroad will present lectures focusing on the impact that cyclosporiasis is creating in different countries, as well as the public health challenges associated with it.

S48 Food Safety of Hydroponic Fruits and Vegetables –What We Do and Don't Know

TBD TBD: *a, b, CA, USA*
MICHAEL EVANS: *University of Arkansas, Horticulture, Fayetteville, AR, USA*
SANJA ILIC: *The Ohio State University, Columbus, OH, USA*

Half of the fresh vegetables sold in the United States (U.S.) are greenhouse-grown, and the number of greenhouse operations is increasing at a phenomenal rate. Food safety is critical to sustainability of fresh produce industry as food safety issues can have an adverse economic and social impact. Recent studies have confirmed that human pathogens are present in the greenhouse environment and can grow in water and fertilizer solutions. While the implementation of the FSMA Produce Safety Rule is rolling out, solutions for meeting the requirements in hydroponic greenhouses remain limited. In this symposium we will 1) provide an industry perspective on the challenges of implementing food safety standards in hydroponic systems; 2) discuss water and sanitation food safety hazards associated with growing fruit and vegetables; and 3) present mitigation strategies to reduce or eliminate these hazards. Participants of this symposium will be representatives from industry, academia, and government.

S49 Novel Processing Technologies to Improve Food Safety and Quality

ROHAN TIKEKAR: *University of Maryland, College Park, MD, USA*
JEYAMKONDAN SUBBIAH: *University of Nebraska-Lincoln, Lincoln, NE, USA*
HARI NIWAS MISHRA: *Indian Institute of Technology (IIT), Kharagpur, India, India*

Although conventional thermal and non-thermal processing technologies have greatly improved food safety and quality, several specific challenges persist. These include: (1) How to improve sanitization practices in minimally processed food industry, such as fresh produce and meats to reduce the recurring incidents of disease outbreaks and food recalls; (2) How to improve the efficiency and uniformity of processing low-moisture foods such as spices and powders; and (3) Can non-thermal processing improve the quality and shelf life of foods? This symposium invites researchers who have developed or validated novel technologies to address these specific issues. One presentation will focus on photodynamic treatment using UV-A light and food grade ingredients exploiting synergistic interactions between food grade and generally recognized as safe (GRAS) compounds and UV-A light to enhance the microbial inactivation on the surface of fresh produce and in wash water. This technology attempts to address the limitations of chlorine-based sanitizers that are widely used in the industry. While thermal processing is an effective strategy for low-moisture foods, several challenges persist, such as long processing times or use of high temperatures that can adversely affect the quality of the products, and high energy consumption. Thus, there is a need to develop approaches that can lower the severity of processing. The second presentation will focus on the principles of radio frequency (RF) processing, determination of RF process parameters for microbial inactivation, and process validation for spices. Another presentation will focus on applications of diverse non-thermal processing techniques to improve quality and shelf life of different products, including fruits and vegetables.

S50 Environmental Pathogen Monitoring and Control for the Food Safety Modernization Act (FSMA) Preventive Controls Implementation

DOUGLAS MARSHALL: *Eurofins Scientific Inc., Fort Collins, CO, USA*
JENNY SCOTT: *U.S. Food and Drug Administration – CFSAN, College Park, MD, USA*
JOHN BUTTS: *Land O'Frost, Lansing, IL, USA*

Environmental pathogen monitoring is becoming an important tool in controlling major food hazards such as environmental pathogens, including *Listeria monocytogenes*, *Salmonella*, and *Cronobacter sakazkii,* in food processing environment. It is especially useful as a verification activity when an environmental pathogen is identified as a significant hazard, i.e., "hazard requiring a preventive control." The FSMA Preventive Controls for Human Food (PCHF)

requirements indicate that ready-to-eat food could be contaminated with environmental pathogens such as *Listeria monocytogenes* or *Salmonella*, which must be considered in hazard analysis. The environmental pathogen monitoring is also stressed in the FDA's draft guidance. Thus, the environmental pathogen monitoring program is helpful in verifying that the factory hygiene and sanitation and other preventive controls designed to assure food safety are working effectively. This symposium will focus on environmental pathogen monitoring program and its role in implementation of FSMA Preventive Controls for Human Food (PCHF). Speakers from industry, academia, and regulatory agency will provide updates and review key aspects of design and implementation of environmental monitoring program and analytical methods, including recent advances in rapid detection of environmental pathogens, as well as data interpretation, source tracking, and strategic use of environmental monitoring for food safety assurance and regulatory compliance. It will also include discussion of the FDA's expectation and guidance regarding environmental pathogens such as *Listeria monocytogenes.*

S51 Surreptitious Connections: Exploring the Emerging Role of Heavy Metals in Antimicrobial Resistance

SCOTT NGUYEN: *University College Dublin, Dublin, Ireland, Ireland*
KATHIA LUNEBERG: *Instituto de Geología, Mexico City, Mexico, Mexico*
DAN ANDERSSON: *Uppsala University, Dept. of Medical Biochemistry and Microbiology, Uppsala, Sweden, Sweden*

The intricate connection of tolerance to cationic heavy metals by bacteria exposed to pesticides and herbicides with their sustained mobilizable antimicrobial resistance (AMR) genotypes are shedding light on a widespread mechanism little studied until recently. Antimicrobials (AM) are extensively used in humans as drugs, and in food animals, additionally as growth promoter. It has been recognized as the primary cause for the resurgence of AMR. Recent studies have established that the frequent exchange of antimicrobial resistance genes (ARGs) between food- and food animal-borne bacteria exposed to heavy use of AM and human pathogens is threatening global human health. ARGs are often located on mobile genetic elements and can be disseminated to other microorganisms and pathogens by horizontal gene transfer (HGT). Once acquired though HGT, AMR can then migrate into the food chain after land application of amended soils. Though the overuse of antimicrobials in agriculture and medicine is to be blamed partly for the proliferation of AMR, other components and mechanisms such as the reliance on heavy metals in animal feed, pesticide, and herbicides might also contribute to the selection and dissemination of AMR. From recent investigations of the effects of the use of antimicrobials in foodborne pathogens at the molecular/genetic level, it has become clearer that the events leading to AMR are very complex. A deeper understanding of the genetics behind ARG exchange among strains from clinical, agricultural, and food processing environments may lead to more insights into the current ARG selective pressures within these environments. This emerging challenge of the interconnectedness between cationic (heavy metal) tolerance and AMR in food- and food animal-borne bacteria will be the major theme of this symposium that should be of keen interest to all members involved with food safety: food manufacturers, government regulatory personnel, and members associated with diagnostic companies.

S52 NGS Case Studies Beyond WGS and Outbreak Investigations

MICHELE SAYLES: *Diamond Pet, Meta, MT, USA*
MICK BOSILEVAC: *U.S. Department of Agriculture–ARS, Clay Center, NE, USA*
JESSE MILLER: *NSF International, Ann Arbor, MI, USA*

While advances in next generation sequencing continue to push our understanding of isolates, pathogens, and foodborne illness, there are additional NGS applications that can improve aspects of how we manage food safety and food quality. This symposium will provide unique and real-world examples of using next generation sequencing applications to improve the food supply chain. NGS applications include techniques such as metagenomics, targeted amplicon sequencing, antimicrobial resistance, and food authenticity. One of our biggest challenges with NGS is finding practical applications of use. This symposium will include plant applications in which some aspect of NGS is used to solve issues. A central theme of these applications will be metagenomics. Metagenomics is used to identify key changes in a microbiome. Complex data sets can become easily understood through applications such as shelf life monitoring, food spoilage investigations, cleaning, and sanitation verification.

S53 Enhancing Food Safety: Translating Molecular Biology to Microbiology: A Dialogue between Molecular and Traditional Microbiologists

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VIRGINIA DEIBEL: *Covance, McKinney, TX, USA*
ERIC BROWN: *U.S. Food and Drug Administration, Center for Food Safety & Applied Nutrition, College Park, MD, USA*

Molecular methods in general and sequencing techniques in particular (e.g., whole genome sequencing, next generation sequencing) have revolutionized aspects of microbiology, such as the speed of initial detection of pathogens; the potential for rapid and accurate identification of isolates; and even the possibility of characterizing the microbiome of a food plant through metagenomics. However, "traditional" microbiological methods remain the "gold standard" or reference methods against which molecular methods are judged. Moreover, traditional culture methods are the only way to isolate strains for further study and may be the only way to demonstrate causation of product spoilage, for example. Increasingly, the world of food microbiology seems to be polarizing into the "sequencers" and the "colonists," with the distance between the two becoming wider and the bridges of common language becoming narrower. This symposium attempts to illustrate the benefits and drawbacks of both approaches, to increase the pool of skilled interpreters, and to "amplify" a "culture" of mutual understanding.

S54 Improving Safety of Sprouted Seeds

KEITH WARRINER: *University of Guelph, Guelph, ON, Canada, Canada*
PATRICIA HOMOLA: *U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Division of Produce Safety, College Park, MD, USA*
RAYMOND JONES: *International Specialty Supply, Cookeville, TN, USA*

Sprouted seeds are gaining increasing popularity for their increased enzyme activity, higher nutritional value, easier digestion, and increased nutrient absorption. With this growing interest comes an ever-expanding array of uses and production practices. Seeds can be sprouted in soil or hydroponically. The tiny plants can be sold as sprouts, which are typically consumed as an entire plant, or as microgreens, which are harvested without the roots. They can also be dried and processed into powders and butters to be used as ingredients in beverages, dietary supplements, or other food products. The budding seeds can also be baked and sold as snacks.

Seeds are grown in an agricultural environment and can become contaminated with pathogens through a number of routes. The growing conditions used for producing sprouted seeds are conducive to microbial growth. While the risks associated with sprouts have been widely recognized, the recent outbreaks of Salmonellosis linked to chia powder and sprouted nut butter highlight the potential public health risk associated with sprouted seed products. These outbreaks also raise questions about the effectiveness of dehydration processes often used in the manufacture of these products in

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eliminating pathogens. Further research is needed to understand the survival and proliferation of pathogenic microorganisms, if present, during the production and processing of sprouted seeds and to identify key intervention strategies. In light of the continuing outbreaks associated with sprouts or sprouted seed products, the FDA has been concerned about the safety of seeds used in these products. Inspections have been conducted at firms that grow, process, and/or supply seeds and beans for sprouting to assess whether the production and manufacturing processes currently in place are sufficient to reduce food safety risks and if the Preventive Controls Rule applies to seed production procedures at these firms.

This symposium will provide an overview of the commercial application of sprouted seed products and production practices and summarize available data regarding the risk profiles of these products. The approaches that the FDA is taking to address the food safety issues associated with seeds for sprouting will be presented. Innovative approaches developed by seed suppliers to minimize the risks associated with seeds, including applicable seed treatment interventions, will also be discussed.

S55 Marrying Nanotechnology and Food Packaging: Benefits and Issues for Food Safety

JOZEF KOKINI: *Purdue University, West Lafayette, IN, USA*
PAUL TAKHISTOV: *Rutgers University, New Brunswick, NJ, USA*
JOAN SYLVAIN BAUGHAN: *Keller and Heckman, LLP, Washington, DC, USA*

Nanomaterials are making a name for themselves in food packaging with ever-increasing regularity. These up-and-comers bring advanced functional properties to packaging materials. To that end, nanotechnology-enabled food packaging can be divided into three main categories, according to Silvestre et al (2011) and Duncan et al (2011). These include *improved packaging,* whereby nanomaterials are mixed into the polymer matrix to improve the gas barrier properties, as well as temperature and humidity resistance of the packaging; *"active" packaging* in which nanomaterials are used to interact directly with food or the environment to allow better protection of the product, and "*intelligent/smart" packaging,* which is designed for sensing biochemical or microbial changes in food. Simply stated, nanomaterials are being developed to enhance the physical and mechanical properties of packaging in terms of tensile strength, rigidity, gas permeability, water resistance and flame resistance. Thus, invigorated, new food packaging materials with improved mechanical, barrier, and antimicrobial properties enable preserving of taste, color, flavor, texture, consistency, and nutrients of food. Boasting noteworthy strength and flame resistance, as well ultraviolet shielding capabilities, nanomaterials have the potential to completely transform the food packaging industry, insiders are saying. Benefits notwithstanding, research is underway to study migration and potential toxicological impacts of nanoparticles in food packaging. Consumer and food industry concerns about any possible downsides of nanotechnology, especially food safety issues and possible regulations, still present questions that need to be addressed. Nonetheless, nanotechnology is burgeoning and currently holds potential for application in the active food packaging industry.

This landmark symposium will cover the current uses, impacts, benefits, sustainability, and overall potential of nanotechnology in food packaging; the antimicrobial and pathogen detection capabilities of nanomaterial enhanced food packaging; and risk assessment and communications needs relative to consumer safety concerns and potential toxicological risks of nanomaterials in food packaging.

S56 Maximizing Food Safety and Quality Through Application of Hygienic Design

JOHN HOLAH: *UK:IE EHEDG & Holchem Laboratories Ltd., Bury, United Kingdom, United Kingdom*
VANESSA CRANFORD: *US. Food and Drug Administration-CFSAN, Division of Produce Safety, Office of Food Safety, College Park, MD, USA*
DUANE GRASSMANN: *Nestlé USA, Solon, OH, USA*

Use of equipment and facilities that are easy to clean (i.e., hygienically designed) is fundamental to ensuring food safety and quality. It is also a requirement by law and by Global food safety and quality standards. It is therefore surprising that hygienic design is often unknown – or overlooked – by those who purchase, use and supply equipment to the food industry. This seminar brings together information from regulatory bodies, the food industry, guidance providers, and equipment suppliers, with the aim of sharing valuable information about hygienic design.

Why Hygienic Design?: This introductory presentation provides information on what hygienic design is and about its basic principles; it highlights some of the hygienic design challenges faced by the food industry; explains how the application hygienic design can help; and provides details of the organizations that can offer support and of the training, guidance, certification and expert advice available.

Factoring Hygienic Design into Sanitation as a Preventative Control: Preventive measures used to eliminate or reduce hazards through sanitation procedures must meet stricter requirements than the sanitation procedures utilized in the past as part of prerequisite programs. The PCHF rule requires sanitation controls to include procedures, practices, and processes to ensure that the facility is maintained in a sanitary condition, adequate to significantly minimize or prevent hazards, such as environmental pathogens, biological hazards, and food allergens (CFR 117.135(c)(3)). How can factoring in hygienic design when developing a sanitation program help?

Hygienic Design – A Food Manufacturer Perspective: Food manufacturers still struggle to source equipment and facilities of good hygienic design, largely due to a lack of purchaser and supplier awareness or initial cost. How can we combat this?

S57 Understanding Antibiotic Resistance from an Environmental Perspective

YIFAN ZHANG: *Wayne State University, Detroit, MI, USA*
MONICA PONDER: *Virginia Tech, Blacksburg, VA, USA*
BEILEI GE: *Food and Drug Administration, Laurel, MD, USA*

Increasing evidence has shown that the evolution and spread of antibiotic resistance in the environment contribute to the occurrence of antibiotic resistance in clinical settings. This suggests an urgent need to minimize the public health risks due to the environmental exposure of antibiotic resistance. The problem is even more complicated in agricultural environment where multiple practices may exert unique impact on the prevalence and persistence of antibiotic resistance, and thus targeted control measures are needed. Multiple routes exist in agricultural environment for antibiotic resistance to enter the food chain. For example, soil microorganisms can be intrinsically antibiotic resistant and are a natural reservoir of antibiotic resistance for food. Soil amendment with different compost types can shape antibiotic resistance profiles in soil and pass them onto the produce grown in the field. Animal feed can also serve as a vehicle to introduce antibiotic resistance into the human food chain at the farm level. Collectively, this multifaceted issue requires integrated approaches that combine microbiological techniques and metagenomic tools in the context of produce and animal production environment. The purpose of this symposium is to explore antibiotic resistance dynamics in agriculture from an environmental perspective. The symposium will demonstrate the impact of soil microbiota, soil amendment, and animal feed on the prevalence and persistence of antibiotic resistance in food products. It will also address national surveillance efforts and possible control mechanisms at the pre-harvest level. Each topic will be presented from a different perspective of environmental conditions in agriculture allowing a comprehensive understanding of the issue across different sectors of agriculture. The symposium will attract not only food safety and agricultural professionals, but also members who are interested in the environmental dimension of antibiotic resistance.

S58 WGS and Mass Spectrometry: The Paved Road to Routine Food Applications!

THOMAS HAMMACK: *U.S. Food and Drug Administration, College Park, MD, USA*
ERIN CROWLEY: *Q Laboratories, Inc., Cincinnati, OH, USA*
DOUGLAS MARSHALL: *Eurofins Scientific Inc., Fort Collins, CO, USA*

Identification and characterization of microbial isolates provide crucial information to decision makers, from the tracking of foodborne pathogens to the monitoring of spoilage indicators or technological strains. For the past three decades, molecular phylogeny has significantly changed systematics and microorganisms classification, while conventional identification methods are still widely used despite some limitations which have been clearly demonstrated. Nonetheless, it is clear evidence that genomic and proteomic are now perceived as performing alternatives to characterize, identify, or routinely confirm microbial isolates.

Genomics tools are commonly linked to sequencing, providing (i) the average nucleotide identity between two specific sequences or genomes, and (ii) the evolutionary relationship between isolates. The predominant proteomics methodologies are based on mass spectrometry generating fingerprints that are compared to thousands of reference spectra from microorganisms, while specific biomarkers can be additionally detected for functionalities purposes, such as the resistance to antibiotics.

One of the pending challenges for the implementation of these technologies in routine testing laboratory is (i) to ensure the global acceptance of these new tools, and (ii) to facilitate the method implementation in routine testing laboratories.

Regulatory agencies have been investigating the use of these technologies as they offer new information for defining the risk profile associated to foodborne pathogen, for investigating outbreak, for tracking these pathogens, and finally for strengthening food safety regulations. This symposium proposes a 360° view on the adoption of these technologies by combining various horizons from regulatory agencies, standardization and certification bodies, and finally routine testing laboratories.

The first presentation will set the regulatory scene and the step of integration of these technologies in official food testing labs, including standardization schemes. The second presentation will update the audience about the on-going method certification development programs. Some key studies will conclude this symposium, showing the implementation in routine testing labs and the bottom-up approach.

S59 Utilizing Big Data to Revolutionize Food Safety, Traceability and Transparency in Food Systems

MARC ALLARD: *U.S. Food and Drug Administration, College Park, MD, USA*
RAMIN KHAKSAR: *Clear Labs Inc., Menlo Park, CA, USA*
FRANK YIANNAS: *Walmart, Bentonville, AR, USA*
TIMOTHY JACKSON: *Driscoll's, Watsonville, CA, USA*

With an increasing emphasis on data collection and transparency, big data is more readily accessible than ever in all scientific disciplines and food safety, and quality is no exception. Advances in next generation sequencing (NGS) technologies enable high-throughput metadata generation that can be used to revolutionize food safety and eliminate supply chains blind spots. NGS techniques can produce thousands or even millions of sequences, simultaneously enabling accurate identification of microbial taxa and allowing for determination of food authenticity. The sequences generated from such techniques can also be used for rapid identification of pathogens and spoilage organisms. In addition to the sequencing metadata provided via NGS, retailers and regulators can also take advantage of blockchain technologies that enable the generation of secure digital records to manage traceability of products and ingredients. Blockchains permit a digital footprint that will pin-point the location of a particular product or ingredient in a matter of seconds. Advances in NGS technologies coupled with the application of blockchains have the potential to revolutionize the food industry and vastly increase and improve food safety, quality, traceability, and transparency across the industry. This symposium seeks to discuss the applications' big data to the food industry and how to practically integrate these advanced technologies into existing food safety management systems.

S60 Risk Assessment of Listeriosis: Latest Developments for Food Safety Risk Management

ROLAND LINDQVIST: *National Food Agency, Uppsala, Sweden, Sweden*
FANNY TENENHAUS-AZIZA: *CNIEL (French Dairy Board), Paris, France, France*
YUHUAN CHEN: *U.S. Food and Drug Administration–CFSAN, College Park, MD, USA*
JANELL KAUSE: *U.S. Department of Agriculture–FSIS, Washington, DC, USA*

Many efforts have been taken by the food business operators to reduce the contamination by *Listeria monocytogenes* (Lm) in ready-to-eat (RTE) foods over the last two decades. However, risk from Lm contamination in foods continues to be a significant public health concern. Symposia presented at recent IAFP meetings focused on risk management and how to address challenges in preventive controls. A risk-based decision-making approach is more and more encouraged. Much progress has been made in recent years in understanding risk factors, susceptible populations (e.g., demographics, comorbidity), whole genome sequencing (WGS) of food and clinical isolates, and comprehensive surveys of Lm in RTE foods, and exposure and risk assessments. This symposium will bring together four speakers from government and industry in North America and Europe to present the latest advancements in quantitative risk assessments, taking into considerations food processing, characteristics of different food commodities, population subgroups and their consumption patterns, and other factors. Examples of how risk assessments can be used to evaluate the impacts of different control measures and intervention strategies for reducing listeriosis risk will be presented, along with current thinking about how to tap into the potential power of WGS data to strengthen risk assessments to inform risk management decisions.

S61 The Future of Food Microbiology is Extra CRISPy: Novel Applications of CRISPR Technology

RYAN JACKSON: *Utah State University, Logan, UT, USA*
CHASE BEISEL: *Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC, USA*
MATHEW HALTER: *DuPont Tate and Lyle Bio Products, LLC, Loudon, TN, USA*
KEVIN BROWN: *Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO, USA*

Clustered regularly interspaced short palindromic repeats (CRISPRs) and CRISPR-associated sequences (Cas) form an adaptive "immune system" that protects many types of bacteria and some archaea against invasive genetic elements. Although CRISPR-Cas technology was originally encountered within a food science context through the study of phage resistance mechanisms in lactic acid starter bacteria, it has been rapidly coopted as a versatile toolkit for genome editing in both prokaryotes and eukaryotes, as well as for a host of other valuable applications. While it is now used widely outside of the food science realm, the value and promise of CRISPR-Cas technology has not been lost on food microbiologists. In this symposium, leading researchers will discuss a variety of applications for CRISPR-Cas in food science and related fields, ranging from a broad overview of CRISPR biology, applications of CRISPR technology in foods and food microbiology, CRISPR-based control of bacteriophage infection in industrial fermentations and functional analysis of

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Toxoplasma gondii genes via CRISPR-based genetic manipulation of this pathogen's genome. These versatile, unique and rapidly deployable applications of CRISPR-Cas technology are expected to spark active discussion amongst symposium attendees.

S62 Use of Whole Genomic Sequencing Data for Source Attribution of Foodborne Pathogens

YI CHEN: *U.S. Food and Drug Administration, College Park, MD, USA*
KALLIOPI RANTSIOU: *University of Torino-DISAFA, Grugliasco, Italy, Italy*
BEN PASCOE: *The Milner Centre for Evolution – University of Bath, Bath, United Kingdom, United Kingdom*
WEIDONG GU: *CDC, Atlanta, GA, USA*

Evaluation of the role of various foods in causing human illnesses, i.e., source attribution, is important to design regulatory policies for prevention and control of foodborne diseases. Many studies use data from outbreak investigations for attribution, but it is likely that outbreaks do not represent the causal pathways in the population and in particular those of sporadic illness. Rapid advancements in whole genomic sequencing (WGS) have generated an increased amount of high-resolution genomic data of foodborne pathogens from various food sources. Comparative genomics and machine learning techniques can be used to identify genetic markers associated with specific foods for prediction of sources of human infection which promises an important tool for source attribution of non-outbreak foodborne illness. In this symposium, we will present the benefits and challenges of source attribution with WGS. We will discuss the use of evolutionary, comparative genomic fingerprinting, and machine learning methods to develop predictive models of food sources for major foodborne pathogens including *Listeria monocytogenes,* pathogenic *Escherichia coli,* and *Campylobacter*.

S63 Science, Safety, and Sanity: Hot Topics in Food Toxicology

ALEX EAPEN: *Cargill, Wayzata, MN, USA*
ALEXANDRIA LAU: *E & J Gallo Winery, Modesto, CA, USA*
PAUL HANLON: *Abbott Nutrition, Columbus, OH, USA*
JOANNA DRAKE: *FEMA, Washington, DC, DC, USA*

Consumers have been influenced to avoid ingredients in foods largely based upon safety concerns swirling in mainstream and social media outlets. Unfortunately, media reports about these substances often communicate that these chemicals can be hazardous (e.g., "Scientists caution harmful chemicals are seeping into our food.") without addressing the more important question of whether consumers are at risk from expected exposure to these substances. This session intends to provide information on a selection of hot topics in toxicology so that members can better understand whether the chemicals they are hearing about are indeed food safety concerns and that consumers could be at risk. Food toxicologists will give an introduction to these issues, allowing ample opportunity for the audience to have their questions answered. Topics include chemical preservatives (such as BHT and tBHQ); artificial colors and flavors; pesticides (such as glyphosate); GMOs; and process-formed chemicals (acrylamide and furfuryl alcohol).

S64 Closing in on the Research Gaps with *Listeria monocytogenes*, *Salmonella*, and Viruses in Low-moisture Foods

JEFFREY FARBER: *University of Guelph, CRIFS, Department of Food Science, Guelph, ON, Canada, Canada*
SABAH BIDAWID: *Health Canada, Ottawa, ON, Canada, Canada*
SOPHIA KATHARIOU: *North Carolina State University, Raleigh, NC, USA*
MEIJUN ZHU: *Washington State University, Pullman, WA, USA*

Low-moisture foods continue to be implicated in foodborne illness outbreaks and pathogen related recalls. Increasingly, public health officials are finding low-moisture ingredients as the source of contamination in compound foods. Most research on prevalence and the behavior of pathogens in foods has been conducted with mid- to high-water activity foods, but the data is significantly limited for low-moisture foods. Managing microbial risks in these diverse commodities remains a challenge. Importantly, some pathogens exhibit a tolerance to desiccation and interventions, such as heat, typically lethal in products of higher water activity. In the last 5–10 years, some meaningful research has been conducted on select commodities, such as spices and nuts, allowing scientists to gain insights into microbial mitigation for these foods. Yet, a vast array of low-moisture foods and associated pathogens has not yet been studied. Some of the more significant research gaps relate to *Listeria monocytogenes*, *Salmonella*, and select foodborne viruses' survival during storage, potential changes in virulence with time and temperature variations, and the overall effectiveness of various mitigation strategies when applied to low-moisture foods. Some of these gaps are being addressed using a variety of bacteria and viruses, including potential surrogates. This symposium brings researchers together to share new insights from their continuing work that begins to fill in some of the gaps in our knowledge on pathogen behavior in previously unstudied low-moisture foods, including pistachios, chocolate (both chocolate liquor and cocoa powder), corn flake cereal, dried apples, raisins, dried strawberries, skim milk powder, and almond flour/meal. Specifically, the role of water activity and the food matrix will be explored to better understand inactivation kinetics and overall survival.

S65 Starting Up after a Contamination-related Shut Down

ELIZABETH FAWELL: *Hogan Lovells, Washington, DC, USA*
JEFFREY KORNACKI: *Kornacki Microbiology Solutions, Inc., Madison, WI, USA*
KEVIN LORCHEIM: *ClorDiSys Solutions, Inc, Lebanon, NJ, USA*
MICHELE SAYLES: *Diamond Pet, Meta, MT, USA*

It is often a surprise when a regulatory action occurs that results in a plant shutdown for microbiological contamination. Companies are often left with questions, such as "What do I do now?" and "What can I expect to occur?" This symposium addresses these questions and provides practical suggestions for dealing with this difficult situation and getting back in business again.

S66 Culturally-targeted Messages and Methods: The Next Generation of Food Safety Education Strategies

JULIE ALBRECHT: *University of Nebraska, Lincoln, NE, USA*
RACHEL SINLEY: *Metropolitan State University, Denver, CO, USA*
ABBY GOLD: *North Dakota State University, Fargo, ND, USA*
JENNIFER QUINLAN: *Drexel University, Philadelphia, PA, USA*

Racial and ethnic minority consumers experience unique cultural and language barriers, which limit the effectiveness of conventional food safety education. This symposium will highlight the novel education messages and methods that were developed and evaluated to target minority consumers of different cultural backgrounds. All of the education presentations were developed based on strong formative research and evaluated for effectiveness. While developed for targeted populations, the unique and appealing formats of the education materials to be discussed may serve as a resource for educating all consumers. This session will highlight the needs of culturally-tailored food safety education strategies and discuss the applications of the novel methods to a larger population.

Julie Albrecht, University of Nebraska, will present an overview of novel education interventions and focus on Conceptual Change Teaching method. Rachel Sinley, Metropolitan State University, will follow up with the application of the Conceptual Change Teaching Method to develop food safety education programs for Native American and Hispanic communities. Abby Gold, North Dakota State University, will describe the effectiveness of discussion paps and cooking classes to educated non-English speaking immigrants and refugees. Jennifer Quinlan, Drexel University, will discuss the importance of photonovellas in health-related education and present the development and evaluation of a food safety program using photonovellas to reach a wide range of consumers with different cultural backgrounds. The session is designed to facilitate a dialogue informed by an overview of the current consumer research on culturally-tailored food safety education strategies and will focus on advancing effective food safety education to the next level.

S67 Spores in the Global Dairy Industry Significance, Issues and Challenges

PURNENDU VASAVADA: *University of Wisconsin-River Falls, River Falls, WI, USA*
NICOLE MARTIN: *Cornell University, Ithaca, NY, USA*
ANNIE BIENVENUE: *U.S. Dairy Export Council, Arlington, VA, USA*
JESSIE HEIDENREICH: *Hilmar Cheese Company, Hilmar, CA, USA*

Sporeforming bacteria represent global, food system-wide contaminants that significantly impact the quality and safety of dairy products. These organisms are known to contaminate dairy foods on the farm, where they are found ubiquitously in the environment, and in processing facilities where they have been shown to form biofilms in processing equipment. Sporeformer germination and outgrowth in dairy products lead to a number of quality defects, such as sweet curdling in fluid milk resulting from *Bacillus weihenstephanensis*, or late blowing in certain styles of cheese caused by *Clostridium tyrobutyricum*. Importantly, many traditional barriers to bacterial growth can be overcome by various sporeforming bacteria (e.g., psychrotolerant sporeformers grow under refrigeration conditions). Further, pathogenic sporeforming bacteria (e.g., *Bacillus cereus*) represent a particular challenge to the dairy industry as conventional processing methods (e.g., HTST pasteurization) do not effectively eliminate them, and traditional identification methods have been shown to be insufficient at distinguishing pathogenic strains from non-pathogenic strains.

Addressing the challenges posed by the presence of sporeforming bacteria in dairy foods requires a farm-to-fork approach. At the farm level, intervention strategies should address the transmission of sporeforming bacteria from environmental reservoirs (e.g., manure, bedding, etc.), where they are present in high numbers, into raw milk. At the processing facility, the approach to reducing sporeformer contamination will include preventing and eliminating the formation of biofilms in equipment as well as optimizing processing parameters to minimize spore outgrowth in finished products. Further, leveraging powerful genomics tools will be required to provide the resolution needed to reliably identify pathogenic sporeforming bacteria and detect associated food safety risks in dairy foods.

Roundtable Abstracts

SRT1 Shiga toxin producing *Escherichia coli* and Leafy Greens: Is it Déjà vu All Over Again?

KARI IRVIN: *U.S. Food and Drug Administration, College Park, MD, USA*
JAMES GORNY: *U.S. Food and Drug Administration, Sacramento, CA, USA*
MICHELE JAY-RUSSELL: *University of California-Davis, Davis, CA, USA*
CHANNAH ROCK: *University of Arizona, Maricopa, AZ, USA*
VICKI-LYNNE SCOTT: *Amigo Farms, Inc., Yuma, AZ, USA*

In 2006 a large-scale outbreak of *Escherichia coli* O157:H7 gastroenteritis lead to significant changes to the way in which food safety was managed in leafy green production. The California and Arizona Leafy Greens Marketing agreements were established and the principles behind them have been used to establish similar auditing programs for other commodities. This session will summarize the recent Romaine lettuce outbreak associated with *Escherichia coli* O157:H7 and compare the 2018 outbreak with the 2006 spinach outbreak, attempting to tackle some of the tough questions that these outbreaks continue to raise. What do we know? What don't we know? Are continued outbreaks simply inevitable? If so, how do we limit their frequency and scope? What is the role of traceback in these types of outbreaks? How has communication with the public changed since 2006?

RT1 Updates on the Impact of Sampling Plans on Microbiology Results

FREDERIC MARTINEZ: *Neogen Corporation, Lansing, MI, USA*
RABEB HENNEKINNE: *Danone Food Safety Center, Paris, France, France*
DAVID TOMAS FORNES: *Nestle Research Center - Nestec Centre De Recherches, Lausanne, Switzerland, Switzerland*
ERIC EBEL: *U.S. Department of Agriculture-FSIS-OPHS, Fort Collins, CO, USA*
MARC ALLARD: *U.S. Food and Drug Administration, Center for Food Safety & Applied Nutrition, College Park, MD, USA*

Newer, rapid pathogen detection platforms bring challenges to traditional sample preparation, size, type, number and collection stage methodologies. Additional challenges including analyzing and interpreting "big data" may impact actionable results with serious public health and economic consequences. This proposed round table brings together experts from different backgrounds who are studying the impact of sample variables paired with large databases on microbiology methods, results and data interpretation.

The panel will discuss the latest ideas and challenges that exist in pathogen detection methodologies in food safety, especially in an expanding global food market and the Q&A session will provide the audience with an interactive participation opportunity. What are the major challenges encountered in food microbiology when sample size may impact the results? Are your sampling plans and your analytical results a representation of the physiological and microbiological status of the sample? How do you filter and parse through "big data" to visualize and interpret the final results? The panelists will provide examples from their research and practical experiences to highlight how sample preparation variables can introduce complexity in the interpretation of newer testing technologies and when collecting large data-sets.

RT2 "One Size Does Not Fit All": Food Defense Planning for FSMA Compliance

AMY KIRCHER: *Food Protection and Defense Institute, Saint Paul, MN, USA*
JOSEPH SCIMECA: *Cargill, Minneapolis, MN, USA*
ASHLEY MILLER: *National Restaurant Association, Chicago, IL, USA*
MARK KAZMIERCZAK: *Gryphon Scientific, LLC, Takoma Park, MD, USA*
RYAN NEWKIRK: *U.S. Food and Drug Administration, College Park, MD, USA*
VICKIE LEWANDOWSKI: *Saputo Cheese, USA, Lincolnshire, IL, USA*

FDA published the FSMA final rule "Mitigation Strategies to Protect Food Against Intentional Adulteration" in May 2016. Domestic and foreign food facilities required to register under the Federal Food, Drug, and Cosmetic Act are required to address hazards that may be introduced into their food products with the intention to cause wide scale public health harm.

The rule requires a written food defense plan that identifies vulnerabilities, actionable process steps, mitigation strategies, procedures for food defense monitoring, corrective actions, and verification for each type of food processed, packed or held at the food facility. Key to the food defense plan is a vulnerability assessment (VA) of the food processing operation to identify actionable process steps and the establishment of mitigation strategies at those steps.

The requirements do not prescribe a specific methodology to conduct the VA; however, VAs must include consideration of access, potential public health impact, likelihood of success, and the possibility of an inside attacker. Similarly, requirements do not require specific mitigation strategies, and a variety of approaches may work for each step identified as significantly vulnerable.

In this session, presenters representing government, academia, consulting, and various sizes and focus of food industry will briefly explain their varied approaches for conducting VAs and identifying mitigation strategies within their specific contexts. Following comments by panel members, facilitated discussion will focus on the various approaches being taken as well as the reality that "one size does not fit all" when it comes to food defense planning.

Key discussion points will include: expectations as the compliance deadline of 2019 nears, effective and ineffective learnings during preparation for compliance, gaps identified, organizational adaptation to uncertainty, how food defense awareness and planning affects relationships across the food supply chain from grower through manufacturing and distributing to consumer.

RT3 Precious Water – The Tricky Business of Balancing Water Sustainability and Food Safety

PHYLLIS POSY: *VP Strategic Services & Regulatory Affairs, Atlantium Technologies, Har Tuv Industrial Park, Israel, Israel*
PEGGY TOMASULA: *Dairy and Functional Foods Research Unit USDA/ARS/Eastern Regional Research Center, Wyndmoor, PA, USA*
JEREMY TRAVIS: *Hilmar Cheese Company, Hilmar, CA, USA*

"When the well's dry, we know the worth of water", said Benjamin Franklin. Our wells are indeed running dry and as an industry, we need to take action and prepare for a future where even in highly developed countries access to abundant, clean water can no longer be taken for granted. Concerns about both the quality and quantity of water can be addressed by using water efficiently and wisely and in the food industry particular attention must of course be given to food safety. In the dairy sector, the holy grail of efficient water use is a zero discharge (closed-loop) production system but is that even possible in an industry that traditionally has been using an abundance of water as the means of ensuring food safety? One might also take the glass-half-full attitude, and claim that

reduced water usage actually could provide an opportunity. Excessive water usage during production can provide a means for dispersal of pathogens and spoilage organisms; hence future "dry manufacturing plants" may not only show reduced water usage, but also reduced microbial contamination. Economic incentives such as rising prices on water, increased fees or even caps on how much waste water is allowed are driving factors for innovation, technology and novel corporate water strategies. How do we embark on the journey toward ultimate water sustainability - what are the low hanging fruits? What are the food safety challenges we inevitably have to face - will we have to accept some level of trade-off? Reducing water consumption and discharge while maintaining the highest level of food safety is a tricky business and it is of utmost importance for a successful outcome that knowledge is shared between corporations, food sectors, and countries across the world.

RT4 How Much of a Mystery Remains with Whole Genome Sequencing?

PAMELA WILGER-BUKART: *Cargill, Inc., Wayzata, MN, USA*
KATHIE GRANT: *Public Health England, Glasgow, United Kingdom, United Kingdom*
MARTIN WIEDMANN: *Cornell University, Ithaca, NY, USA*
PETER GERNER-SMIDT: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
ERROL STRAIN: *U.S. Food and Drug Administration – Center for Food Safety and Applied Nutrition, College Park, MD, USA*

Whole genome sequencing (WGS) is transforming the field of food safety microbiology. The technology has already cemented its place as a tool for the investigation of foodborne illness outbreaks and as a prospective surveillance tool for the public health authorities and regulators. In the private sector, there is also a growing appreciation of the benefits of using WGS in source tracking of microorganisms and the wider potential of WGS to improve food safety. Advances in the sequencing technologies and the bioinformatics analytical tools are happening at a breakneck pace, leading to significant changes even within a short span of time.

When a new, complex and rapidly changing technology such as WGS is being implemented, there are always concerns about the reliability of the technology. It is important to understand how reliable and reproducible results can be generated using a technology which is constantly evolving. It is also important to understand what is changing and how that affects the interpretation and potential use of the technology. Clarification on these aspects will facilitate widespread use of WGS in industry.

This roundtable panel, comprised of leading scientists from the government, academia, and industry will discuss how the industry can cope with the rapid technological developments to apply WGS as routine. The panel will tackle questions that need to be answered to transition WGS from research to routine application. Cornerstones to obtain reproducible analytical results such as benchmarking, validation, harmonization, standardization and verification of WGS – both current status and future needs will be addressed by the panel. The panel will discuss how these concepts work when applied to WGS compared to traditional microbial analytics. Finally, identification and implementation of fit-for-purpose tools that meet industry needs will be discussed.

RT5 Identifying Knowledge Gaps Surrounding the Safe Production, Sale and Consumption of Cannabis and Cannabis-related Products

RUSTY ROCK: *Oregon Department of Agriculture, Salem, OR, USA*
MIEKO HESTER: *NORML, San Francisco, CA, USA*
ALEXANDRA TUDOR: *TEQ Analytical Labs, Aurora, CO, USA*
FRANCIS BOERO: *Famiglia Properties LLC, Plainfield, NJ, USA*
SCOTT RIEFLER: *Tarukino, Seattle, WA, USA*

While Cannabis remains illegal in many parts of the world, over the past few years, several nations and sub-national entities have legalized or are considering legalizing the possession and consumption of Cannabis and Cannabis-products (CCPs). Since the 2016 symposia, *Food Safety Concerns and Testing Challenges in the Emerging Cannabis Products Market,* additional states have legalized CCPs. The safety concerns surrounding CCPs are increasingly relevant for the IAFP community as California Proposition 64, and the Medicinal and Adult Use Cannabis Regulation and Safety Act will go into effect on January 1, 2018. This statute is the first attempt to legislate CCP safety, and allows for the sale and taxation of recreational marijuana, thus opening up the market from "smaller" growers to also the larger agricultural industry market.

Significant knowledge gaps regarding CCP food safety risks exist, acting as barriers to policy and protocol development, and thereby threatening consumer safety. Indeed, there is an increasing need for risk assessments to guide policy and legislation. As medical marijuana is frequently used by at-risk individuals, incorrect and missing data regarding CCP safety is particularly problematic. There are also concerns of distinguishing non-CCP foods from CCPS both in terms of fraud and within consumer's homes. This roundtable will seek to identify and address CCP safety concerns by: (a) reviewing current and future regulations and concerns; (b) identifying effective ways to communicate cannabis safety to all stakeholders, including to a migratory workforce; (c) discussing the role of government (both national and sub-national) in ensuring safe CCP production from bloom-to-bud; and (d) addressing knowledge gaps and future goals.

RT6 Food Safety Recalls in the Age of Online Grocery Stores

LINDA J. HARRIS: *University of California-Davis, Department of Food Science and Technology, Davis, CA, USA*
RICHARD BECKSTRAND: *Utah Department of Agriculture and Food, Salt Lake City, UT, USA*
ALLISON JENNINGS: *Amazon, Seattle, WA, USA*
MICKEY PARISH: *U.S. Food and Drug Administration, Washington, DC, USA*
WILLIAM HALLMAN: *Rutgers University, New Brunswick, NJ, USA*
BENJAMIN CHAPMAN: *North Carolina State University, Raleigh, NC, USA*

Online shopping for non-food items is well-established but purchasing food items online, especially perishable foods, is a more recent phenomenon. With promises of "same-day" or "one-day delivery", including weekends and holidays, the convenience and time savings of online groceries appeal to many shoppers. There are a variety of logistical approaches to fulfilling online grocery orders that vary widely in sophistication including infrastructure with regional warehouses and contracts with multiple delivery providers to storefronts for a variety of smaller merchants. Some online vendors never actually handle products, rather they coordinate delivery and aggregate orders. On a much smaller scale, individuals may also sell their own or commercially-processed foods through a variety of social networks and online mechanisms such as Ebay, Etsy, Facebook, and Craigslist. Online vendors may track purchase records that include lot codes or best before dates which are important in outbreak or recall situations and could allow individualized notification when a purchased item is withdrawn from the marketplace. The vast numbers of online retailers complicate complete marketplace withdrawal for food processors, especially for products with longer shelf lives that may be sold by secondary or tertiary retailers. Effective recall messaging is especially important when recalls expand beyond an initial limited number of product lot codes. The panelists will speak from their own personal and professional perspectives, and a range of issues from management and communication to regulatory challenges will be introduced and strategies for managing them will be discussed.

RT7 Global Perspectives on Strengthening Food Safety Performance: How to Keep up in a Changing World

XIUMEI LIU: *China National Center for Food Safety Risk Assessment, Beijing, China, China*
RICHARD ARSENAULT: *Canadian Food Inspection Agency, Ottawa, ON, Canada, Canada*
STEPHEN OSTROFF: *U.S. Food and Drug Administration, Silver Spring, MD, USA*
IAN MCWATT: *Food Standards Scotland, Aberdeen, United Kingdom, United Kingdom*
AMANDA HILL: *Dairy Food Safety Victoria, Camberwell, Australia, Australia*

Join regulators from Australia/New Zealand, Canada, China, Switzerland, U.K., and US as they share perspectives on the role of the regulator on strengthening food safety performance through a food company's organizational culture. Learn about each country's regulations and how inspectors/investigators impact organizational culture.

Get a glimpse into different countries' regulatory structure and current thinking about current/emerging food safety challenges. Expand your knowledge of international regulations and get a global regulatory perspective on protecting public health.

Conversational topics will include:

- Describing the structure of food safety regulation in each country. Is there more than one agency responsible for food safety oversight? Who creates the legislation? Who's responsible for implementing and inspection?
- Revealing each country's current and future focus.
- Explaining allergens of focus versus food allergen prevalence in their country. What's their country's position on thresholds versus zero tolerance?
- Discussion of the impact of Brexit on FSA UK. How will they ensure that their regulations are equivalent to UK continue European free trade? How will that affect the EU countries?

RT8 Best Practices for Safe Transportation of Food

ANSEN POND: *Pilgrim's Pride, Mt. Pleasant, TX, USA*
BETSY BOOREN: *OFW Law, Washington, DC, USA*
DONNA GARREN: *American Frozen Food Institute, McLean, VA, USA*
MICHELE SAYLES: *Diamond Pet, Meta, MT, USA*
KEITH JACKSON: *Performance Food Group, Richmond, VA, USA*
KEVIN SMITH: *U.S. Food and Drug Administration, College Park, MD, USA*

The FDA Food Safety and Modernization Act (FSMA) Sanitary Transportation of Human and Animal Food has been finalized with the goal to prevent practices during transportation that create food safety risks, such as failure to properly refrigerate food, inadequate cleaning of vehicles between loads, and failure to properly protect food. The objective of the round table will be to have various industries sectors including low moisture foods, pet food, meat industry discuss how each specific industry ensures safe transportation of food. The discussion will include their current best practices including cleaning and sanitation of vehicle and transportation equipment between loads, and temperature controls for safety. Aspects about compliance with the new FSMA transportation regulation, challenges and solutions will be addressed.

RT9 Do Lawsuits Play a Productive Role in Advancing Food Safety?

BRAD SULLIVAN: *L + G LLP, Salinas, CA, USA*
BILL MARLER: *Marler Clark, The Food Safety Law Firm, Seattle, WA, USA*
PATRICIA WESTER: *PA Wester Consulting, Alachua, FL, USA*
ROBERT WHITAKER: *PMA, Newark, DE, USA*
DAVID ACHESON: *The Acheson Group, Bigfork, MT, USA*
CRAIG WILSON: *Costco Wholesale, Issaquah, WA, USA*

When foodborne illness outbreaks are traced back to a particular product, they typically give rise to lawsuits that can cost companies and their liability insurers millions of dollars. Do these lawsuits do anything to advance food safety? Many believe that such lawsuits are too infrequent—about as likely as a lighting strike—to influence the decisions of food safety managers and company executives. Others suggest that lawsuits amplify the adverse publicity surrounding outbreaks and increase the pressure on companies to improve their food safety practices. In addition, fear of litigation has led many companies to purchase liability and recall insurance coverage, which increasingly includes consulting services to help reduce the risk of contamination. Recently, plaintiffs' lawyers have trained their sights on third-party auditors in the belief that exposing them to liability for professional malpractice can improve the quality of audits. This roundtable of experts from industry, auditing, insurance, law, and academia will address the following questions. How much do food safety managers and company executives worry about civil liability? Does civil liability influence the decisions that they make about food safety? Do insurance incentives to reduce the risk of contamination influence those decisions? Are lawsuits against auditors likely to improve the quality of private audits? Are there any negative effects of lawsuits, for example, driving up the cost of food? If lawsuits and insurance do advance food safety directly or indirectly, what practical steps could be taken to magnify their influence? For example, should indemnity clauses be deemed unenforceable or should liability and recall insurance be mandatory in the food industry?

RT10 Complex Risk Assessment and Classic Hazard Analysis on a Spectrum – Do We Really Need Both/Can We Really Do Both?

JANE VAN DOREN: *U.S. Food and Drug Administration–CFSAN, College Park, MD, USA*
DONALD W. SCHAFFNER: *Rutgers University, New Brunswick, NJ, USA*
MARIEM ELLOUZE: *Nestlé, Lausanne, Switzerland, Switzerland*
ROBERT BRACKETT: *Institute for Food Safety and Health, Bedford Park, IL, USA*
ROBERT BUCHANAN: *University of Maryland, College Park, MD, USA*
BALASUBRAHMANYAM KOTTAPALLI: *Conagra Brands, Omaha, NE, USA*

Complex quantitative risk assessment and classic hazard analysis have both been used to inform decisions in government and industry on how best to manage risks from food safety hazards in the food chain, although the two approaches are seemingly on the ends of a spectrum. Hazard analysis, widely used to determine hazards requiring preventive controls, involves hazard identification and hazard evaluation that is typically performed in a qualitative manner. Risk assessment, typically quantitative and sometime with complicated modeling, is often perceived as too difficult to apply, even though it can integrate a multitude of data and information on the pathogen, the food, and the consumer to predict effectiveness of control measures and intervention strategies. This roundtable brings together a panel of six experts who have a wealth of knowledge and experiences in the worlds of risk-based preventive

Roundtable

controls, predictive modeling and risk assessment. The roundtable will start with brief remarks from the panel on hazard analysis and risk assessment to set the stage. Industry perspectives will be presented by the two panel members from industry, giving the perspective of large firms and sharing perspectives obtained from co-manufacturers (small firms). Aspects for small firms will also be presented by other panel members such as Dr. Schaffner who has extensive experience working with small firms. The audience will be invited to pose questions in addition to questions prepared by the organizers and convenors. Facilitated roundtable discussion will address questions such as:

1. Do we really need both approaches?
   - Case in point: do we need challenge tests and predictive microbiology?
   - How do we bridge hazard analysis and predictive modeling/risk assessment?
2. Can we really do both?
   - Who are the key stakeholders that should participate in this process?
   - What are some of the real-world hurdles to applying risk assessment?
   - How does one determine what is a 'significant hazard' or' hazard requiring preventive control'?
   - What are some of the opportunities to evaluate hazards in a semi-quantitative and quantitative manner?
3. Is it possible to design food safety programs based on predictive modeling and risk assessment?
   - What type of information should be shared with regulators?
   - What is the perception among stakeholders of regulatory agencies' views towards predictive modeling and risk assessment?

Following a yes/no answer to the question based on opposite observations in the real world (not necessarily their own volitions), the panel members and audience are asked to share insights, lessons learned and case studies on what is needed and how to address the underlying issues, and point to tools and resources available to help bridge the gaps between hazard analysis and predictive modeling to integrate more quantitative elements to enhance science-based design and risk-based preventive controls.

RT11 Antimicrobial Resistance: Current Knowledge and Steps Toward Understanding the Relative Role of Food and Other Resistance Sources

RICHARD REID-SMITH: *Public Health Agency of Canada, Guelph, ON, Canada, Canada*
DENISE EBLEN: *USDA NIFA IFSN, Washington, DC, USA*
KENDRA WALDBUSSER: *Pilgrim's Pride Corp, Loveland, CO, USA*
VIRGINIA STOCKWELL: *U.S. Department of Agriculture – ARS, Corvallis, OR, USA*
PAUL MORLEY: *Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA*
FRANCISCO ZAGMUTT: *EpiX Analytics, Fort Collins, CO, USA*

Antimicrobial resistance (AMR) and the preservation of antibiotics to treat human illnesses are a growing concern worldwide. There are many sources of AMR: antimicrobial use (AMU) in humans, food production animals, pets, treatment of fruit or vegetable crops with antibacterial pesticides, etc.

The objective of the proposed roundtable is to discuss AMU and AMR in food production, and what's needed to better understand the role of foods and other contributors to AMR in humans and animals. The discussion will cover some of the following topics:

- AMR: do we know the relative contribution of food production, pets, humans, etc. into the overall AMR of drugs of medical importance?
- AMR and food production: what is the risk from foods (animal products, fruits and vegetables) versus the environment (soil, water, etc.)?
- What can be done to better identify and target the most important AMR sources - foods and others? What analyses and modeling could be performed? How can we take advantage of existing surveillance in foodborne illness and food production systems?
- Industry, academia and government: how to collaborate and communicate around this issue? What data sharing is needed to better support optimal interventions to reduce AMR?
- Could genome sequencing and metagenomics of surveillance sampling and clinical samples inform our understanding of illness from food production outside of foodborne illness?
- What further data collection is needed, or how do current surveillance systems need to be improved, to understand the optimal strategy to combat AMR?

Invited panelists will represent the food industry, government, and scientists working on food production and/or AMR. The format will be kept informal and will solicit participation from the audience.

RT12 Is There Such a Thing as Too Much Transparency? Different Perspectives on Deciding When to Communicate during a Food Safety Outbreak

ELIZABETH GREENE: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
SARA COLEMAN: *Health Canada – Communications and Public Affairs Branch, Ottawa, ON, Canada, Canada*
THOMAS GREMILLION: *Director of Food Policy Institute at the Consumer Federation of America, Washington, DC, USA*
AARON LAVALLEE: *USDA Food Safety and Inspection Service, Washington, DC, USA*

A consumer warning about a foodborne illness outbreak, if issued at the right time can save lives. In contrast, communicating about an outbreak without information on which consumers can react can lead to confusion. Different government agencies and stakeholders sometimes disagree on when it is appropriate to communicate information on outbreaks, often for very good reasons. Still others have regulatory requirements that dictate when they should share information. Despite those differences, close collaboration is essential when creating timely and clear messages for the public during outbreaks. In this panel discussion, communicators and public health experts from different organizations will discuss making decisions surrounding communications during foodborne illness outbreaks. The panel will look at balancing transparency, regulatory responsibilities, actionable information and public health when making decisions about communication information about outbreaks with the public. It will also explore the best ways to present limited information or uncertainties, how to develop useful, actionable information for the public despite those uncertainties, and how to meet the expectations of a public with increasing expectations for transparency.

RT13 *Salmonella* in Poultry: Where Do We Go from Here?

FRANK YIANNAS: *Walmart, Bentonville, AR, USA*
BARBARA KOWALCYK: *The Ohio State University, Columbus, OH, USA*
BETH RIESS: *The Pew Charitable Trusts, Washington, DC, USA*
MICHAEL ROBACH: *Cargill, Minneapolis, MN, USA*
ROBERT TAUXE: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
PAUL KIECKER: *U.S. Department of Agriculture – FSIS, Washington DC, DC, USA*

Salmonella in poultry still represents a significant public health concern. Industry, regulators, and researchers are working to address the issue. In recent years, companies have proposed new approaches to control risks and monitor food safety processes, non-profit institutions have published reports, and researchers have developed risk assessments to estimate the impacts of different microbiological criteria and control measures. However, the potential impacts and practical implications of these new efforts have not yet been discussed. This Roundtable will bring together a broad range of stakeholders to discuss how recent research findings could be applied to improve the microbial safety of raw poultry products.

Conveners will present the topic and provide the context for discussion (10 min). Each panelist will then share their opinion on the most important challenges and innovations in the sector, and propose their view for the future of *Salmonella* control in poultry meat in the United States (20 min). A short round of targeted follow-up questions will go more in depth into specific topics, e.g. microbiological criteria, interventions, and process control (15 min). The discussion will be then opened to the audience (40 min), followed by a summary (5 min).
Questions and topics to be discussed include:

What are the implications of recent risk assessments and research findings for your stakeholder group? How has new information changed (if at all) guidelines and practices within your sector?

- What is the main challenge faced by your stakeholder group in the next 5 years for reducing microbial risk in poultry?
- What information or tools are we missing (or do we need to better apply) to make progress?
- What policies and practices have worked in other contexts? Could they work in the U.S.?
- What stewardship partnerships or collaborative initiatives should be created/strengthened?

RT14 Responsible Use of Antibiotics – Are We Making Progress?

BIRTHE STEENBERG: *European Poultry Association, Brussels, Belgium, Belgium*
LINNEA NEWMAN: *Merck Animal Health, Madison, NJ, USA*
DON RITTER: *Mountaire Farms, Little Rock, AR, USA*
BRIAN LUBBERS: *Kansas State University, Manhattan, KS, USA*

Current and new marketplace food trends and consumer demands such as antibiotic free, cage free, gestational crate free, etc. are changing the way animals are raised for food production. Food industry stakeholders (producers, retailers, food service, etc.) are taking steps to address new consumer demands. Specific to the use of antibiotics in animals for food production; and in response to the concern of some regarding the contribution of food production to antimicrobial resistance (AMR), some companies have taken steps and learned valuable lessons that are worth sharing the with the wider audience at IAFP. As a continuation to last year's Pre-harvest symposium "Antibiotics in pre harvest and associated risks to food," during this round table we invite experts to share and discuss 1) How has AMR changed in systems that have reduced antibiotic use; 2) What antibiotics have been removed and what alternatives are available; 3)Europe's food safety experience in antibiotic reduction and responsible use; 4) How has food safety been improved perspective.

RT15 Help! I'm New Management. How Do I Convince My Colleagues Food Safety is Important?

TIMOTHY JACKSON: *Driscoll's, Watsonville, CA, USA*
KEVIN MURPHY: *University of Central Florida, Orlando, FL, USA*
LONE JESPERSEN: *Cultivate, Hauterive, Switzerland, Switzerland*
JORGE HERNANDEZ: *Wholesome International, Hinsdale, IL, USA*

New mid-level food safety managers or recent food safety graduates are often expected to communicate on many different levels in the food industry, from front level employees to leadership in order to be effective in their profession. Additionally, communicating effectively across the multiple ethnicities, languages, educational levels, socioeconomic levels, and generational levels of these employees requires multiple communication strategies. In order to build an effective food safety culture, the manager or recent food safety graduate must be persuasive, not only to those in the front line of the business, but to business stakeholders outside of the food safety department. Consequently, training is urgently needed to address the training gaps in communicating across ethnicities, educational and socioeconomic levels, languages, cultures, and positions within and outside food safety companies.
This session will directly address:

1. Effective and ineffective management styles of communicating food safety with business stakeholders within an organization across many (multiple ethnicities, languages, educational levels, socioeconomic levels, and generational) levels of these employees.
2. The drivers that affect restaurant and food service employees' to comply with food safety regulations.
3. The advantages of having the right management communication techniques in fostering an effective food safety culture.

RT16 Process Validations – Stories from the Trenches

TIM BIRMINGHAM: *Almond Board of California, Modesto, CA, USA*
BRIAN FARINA: *Deibel Laboratories, Inc., Gainesville, FL, USA*
ABDULLATIF TAY: *PepsiCo, Barrington, IL, USA*
LISA LUCORE: *Shearer's Snacks, Massillon, OH, USA*
NATHAN ANDERSON: *U.S. Food and Drug Administration, Bedford Park, IL, USA*

Validation of Process Preventive Controls are required by the Preventive Controls Rules for both human and animal food when a biological hazard is identified as requiring a Preventive Control. There has been much discussion about the design of validation studies that can take many forms, whether conducted in a lab or on-site at a food production facility. Validations of actual manufacturing equipment on-site using surrogate organisms are most challenging, and even the best designed study can result in failure if poorly executed on-site.

A panel of subject matter experts will discuss their successes as well as their disasters and resolutions encountered while conducting in-plant validations of a variety of Process Preventive Controls including thermal and irradiation processes.

Leading questions will be posed by a moderator and panelists will provide their perspective though their answers and discussion with the audience. These will include:

Roundtable

1. Introduction - What constitutes a "scientifically valid" on-site process validation study? Regulatory perspective?
2. The importance of validation documentation - What type of documentation is expected and should be available to auditors and regulators?
3. What are examples of the key factors leading to a successful on-site validation (e.g. appropriate design of the study, prework by the facility and validation authority, on site management of the study and communication)?
4. What are examples of validation studies performed under challenging conditions (e.g. lack of support from facility management, lack of environmental controls, missing process information)?
5. What challenges have been encountered with validation studies that rely on nontraditional or non-thermal approaches for control (e.g. cumulative risk reduction mitigation strategies, irradiation)?

RT17 The Conundrum of *Campylobacter* Source Attribution

KRISTEN POGREBA-BROWN: *University of Arizona, Tucson, AZ, USA*
BEAU BRUCE: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
ARIE HAVELAAR: *University of Florida, Gainesville, FL, USA*
MICHAEL BATZ: *U.S. Food and Drug Administration, Silver Spring, MD, USA*

Campylobacter remains one of the most important causes of foodborne disease in the world, yet our ability to quantify the relative roles of food, water, animal, and environmental sources of exposure is limited. Effective prioritization and policy development depends on such source attribution, but studies of *Campylobacter* sources point in different directions. For example, in the United States, case-control studies of sporadic disease point to chicken, yet a majority of foodborne *Campylobacter* outbreaks reported to CDC are associated with the consumption of unpasteurized milk. Studies of sporadic disease have found geographic variation, identified different risk factors in urban and rural environments, and suggested sources may differ by ethnicity. Other studies have found higher campylobacteriosis rates in areas of dairy production, pointed to the important roles of pets, and identified flies as a vector.

The methods used to estimate relative contribution of sources continue to evolve in ways that may allow improvements in understanding. Case-control studies are increasingly incorporating typing of isolates using whole genome sequencing and other molecular methods to improve elucidation of risk factors. Likewise, structured expert judgment studies grounded in data may provide a means to synthesize disparate and conflicting literature.

This roundtable discussion, comprised of international experts in epidemiology and policy, will focus on how to make sense of the varied and evolving work on this topic. Faced with uncertainties about sources, how do we appropriately target interventions to reduce illness risk? Similarly, how can we evaluate the effectiveness of policies and other interventions? Are we missing important transmission routes? What kinds of tools and studies could improve our understanding of *Campylobacter* sources? What are the findings from the most recent studies? What can we learn from other countries' efforts in this area? The discussion will illuminate the food safety challenges and opportunities around preventing *Campylobacter* infections.

RT18 The Grey Area of Science: "Predatory" Publishers and Questionable Conferences

MICKEY PARISH: *U.S. Food and Drug Administration, Washington, DC, USA*
ALLYSON MOWER: *University of Utah, Salt Lake City, UT, USA*
MARCEL ZWIETERING: *Wageningen University, Wageningen, Netherlands, Netherlands*
ELLIOT RYSER: *Michigan State University, East Lansing, MI, USA*

In the increasingly interconnected, digitized world of science, the number of venues to publish and present the results of scientific research continues to increase at a rapid pace. With the advent of the internet, the ability to present such findings is making obtaining the results of science easier. One major driving force of this is open access publishing. In the past decade alone, the percentage of articles published open access by English language publishers has doubled. Along with this growth, a number of other journals and publishers, termed "predatory," have appeared, essentially publishing papers with questionable peer review practices so long as the authors pay their publishing fees. However, the degree of review an article receives, and general quality of the article's content deemed worthy for publication varies drastically, even among questionable publishers. Additionally, a similar phenomenon has occurred with bare bones scientific conferences that focus more on providing a means for travel than for legitimate exchange of scientific ideas; again with varying degrees of quality and standards. Thus, a large grey area of science exists with different standards and experiences that must now be considered by many scientists when receiving a deluge of invitations for paper submissions and conference talks. This symposium hopes to provide insight into, and discuss the spectrum of different predatory publishers and conferences to help fellow scientists navigate this growing grey area of science, and consider how much review is "enough" to warrant a paper publishable. This symposium will inform attendees about the different ends of the spectrum of legitimate and predatory publishing, and provide attendees with useful elements and practices to look for when identifying potentially predatory publishers or conferences.

RT19 Insights into Food Safety Careers Roundtable

JENNY SCOTT: *U.S. Food and Drug Administration – CFSAN, College Park, MD, USA*
SEAN LEIGHTON: *Cargill, Wayzata, MN, USA*
DON ZINK: *IEH Laboratories & Consulting Group, Taylors, SC, USA*
MANAN SHARMA: *U.S. Department of Agriculture – ARS, Environmental Microbial and Food Safety Laboratory, Beltsville, MD, USA*
AMANDA KINCHLA: *University of Massachusetts, Amherst, MA, USA*
ADAM BORGER: *University of Wisconsin-Madison, Madison, WI, USA*

This roundtable will allow the audience to interact with representatives from different fields (industry, academia, government, etc.) to hear about experiences and hurdles associated with each career choice.

The roundtable will begin by giving each panelist a few minutes to introduce themselves, and give a quick summary of their career background. We'll then go into some questions that have already established, such as:

- What is some advice for potential applicants in your field to get them started?
- Is there anything candidates can do to prepare for a career in your field?
- What have been the biggest hurdles that you have had to face in your career?
- For those of you whose careers have covered multiple sectors, can you give guidance on the best order to purse each?
- For those that have covered multiple sectors, why did you choose to change fields?
- For those starting their career or looking to change career paths, what are some key aspects that an employer might look for?

We will then open the floor to audience questions, and finish the roundtable with a brief statement from each panelist about one final take home message for the audience to remember when looking for a career.

Technical Abstracts

T1-01 Assessing the Performance of *Clostridium perfringens* Cooling Models for Cooked, Cured Meat and Poultry Products

TIMOTHY MOHR[1] and Vijay Juneja[2]

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Introduction: Heat-resistant *Clostridium perfringens* spores may germinate and multiply in cooked, cured meat and poultry when cooling does not occur in a timely manner. In the event of a cooling deviation, establishments can support product safety by estimating the growth of *C. perfringens* that may have occurred using a validated predictive model.

Purpose: To evaluate the performance of five existing cooling models in predicting the growth of *C. perfringens* in cooked, cured meat and poultry products during chilling.

Methods: Time-temperature cooling profiles (185) of cooked, cured meat and poultry products and their intrinsic factors, along with the corresponding *C. perfringens* growth responses, were collected to analyze the performance of the five cooling models for estimating growth. The performance of the models was evaluated using the acceptable prediction zone (APZ) method. An accurate prediction is defined when the residual (observed value minus predicted value) is -1.0 to +0.5 log, a fail-safe prediction when the residual is <-1.0 log, and a fail-dangerous prediction when the residual is >+0.5 log.

Results: The combined percentages of accurate and fail-safe predictions based on the APZ method were 97.3% for the ComBase Perfringens Predictor, 81.08% for the United States Department of Agriculture (USDA) Pathogen Modeling Program (PMP) 7.0/8.0 cooling model for cured beef, 77.30% for the PMP 7.0/8.0 cooling model for cured chicken, 74.05% for the USDA Predictive Microbiology Information Portal (PMIP) cooling model for cured pork, and 100% for the PMIP cooling model for cooked beef.

Significance: The ComBase model and the PMIP cooling model for cooked beef are the most reliable models that food processors and regulatory agencies can use to evaluate the safety of cooked, cured meat and poultry products exposed to cooling deviations. The cooling models for cured beef, cured chicken, and cured pork are also reliable models that can be used to support the safety of affected product.

T1-02 STEAK-Safe Temperature Estimator at A Klick: A Simple, Spreadsheet-based Tool to Create Safe Cooking Time-Labels for Mechanically Tenderized Beef Steaks

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❖ ***Developing Scientist Competitor***

Introduction: Outbreak reports from Centers for Disease Control and Prevention and recalls associated with mechanically tenderized beef products are on the rise. Mandatory labeling requirements by the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) for safe cooking times of mechanically tenderized beef steaks require expensive and time-consuming validation studies. Additionally, determination of safe cooking times for customized steak cuts of different sizes and weights is tedious. Predictive modeling using a statistical approach could provide a quick, inexpensive solution to creating labels that identify appropriate cooking times and temperatures.

Purpose: To develop a simple spreadsheet-based tool utilizing predictive modeling to generate safe cooking time labels for a variety of mechanically tenderized steak cuts.

Methods: Steaks of various types (top round, knuckle, top sirloin, sirloin cap, flap, tri-tip, flank, and ribeye) and dimensions (n=3 each; n=160 total) with weight ranging from 117 to 567 g and thickness of 1.27, 2.54, and 3.81 cm, were used. Samples were tenderized, fabricated, vacuum-packaged, and refrigerated until cooking. Steaks were cooked based on USDA-FSIS guidelines. Data generated through dimension analysis and cooking time-temperature profiles were used for model development. Model building was performed using correlation and stepwise regression analysis of cooking time determining factors at P<0.01. Data from three replicates were analyzed and modeled using SAS software. The regression model, underpinning the spreadsheet-based tool, converted the qualitative information provided by the users into quantitative inputs for the model, generating an output of safe cooking time.

Results: Pearson correlation analysis (>60%) found thickness, weight, and cooking rate as significant factors affecting cooking time. The developed regression model accurately predicted safe cooking times with 80% accuracy. The spreadsheet was able to generate safe cooking time using three simple inputs: steak type, thickness, and weight.

Significance: A simple and accessible spreadsheet for calculating safe cooking times could be used to help address some of the shortcomings of cooking validation studies and to generate instant labels.

T1-03 Lis-RA: A Software Tool to Predict Listeriosis Risk in Different Ready-to-Eat Food Categories

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Introduction: The development of user-friendly tools to integrate probabilistic microbial risk-assessment methods is key to making this methodology accessible to a wide range of end users along the food chain.

Purpose: To design a user-friendly tool to perform probabilistic risk assessments of foodborne pathogens, focused on *Listeria monocytogenes* in ready-to-eat foods.

Methods: An Excel add-in, called *Lis-RA*, was developed based on Visual Basic for Applications, including a customized ribbon interface. The simulation capabilities were built upon functions from @Risk software. The application was optimized for Excel 2016 and @Risk 7.0 and 7.5.

Results: The tool can be freely accessed through the European Food Safety Authority community for food safety tools, Knowledge Junction, in the Zenodo research-sharing platform (*https://doi.org/10.5281/zenodo.822350*). The software tool allows users to load risk model spreadsheets, select scenarios, and define model inputs and simulation settings in an easy and intuitive way. The annual number of listeriosis cases estimated with the application corresponded to 2,318 (95% confidence interval: 1,450 to 3,612). Cooked meat and sausage presented most cases (median of 863 and 541, respectively). Specific scenarios were also simulated to determine the effect of the different variables considered in the model on the final number of listeriosis cases. Results confirmed the importance of controlling temperature and time in reducing listeriosis cases, but at the same time showed that their effects

varied depending on the type of food. This fact supports the hypothesis that more food-specific approaches should be considered to mitigate risk of *L. monocytogenes*.

Significance: Food stakeholders can benefit from the use of *Lis-RA* to assess the risk of *L. monocytogenes* in different food products, providing a suitable scientific basis to better support risk management decisions and derive suitable control measures.

T1-04 Quantitative Antimicrobial Risk Assessment: Data Gaps to Put Animal Source Foods in Perspective

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Introduction: Quantifying the contribution of antimicrobial resistance (AMR) from foodborne pathogens (FP) to AMR in humans (AMR_H) is key to prioritizing cost-effective control strategies.

Purpose: Our aim was to use risk assessment methods to identify data availability and gaps to quantify AMR FP attribution from animal source foods (ASF) to humans and its relative importance compared to other sources of AMR_H in the United States.

Methods: Using beef as a model, we assembled a collaborative team with industry, government, and academic partners to develop a simple stochastic model to estimate transfer of AMR FP from cattle to humans and FP contribution to overall AMR_H. Model complexity was increased incrementally to identify key data sources and gaps to properly estimate food sources of AMR FP and AMR_H.

Results: Key publicly available data included: FoodNet, National Outbreak Reporting System, and National Antimicrobial Resistance Monitoring System (NARMS) programs to longitudinally estimate incidence of foodborne illnesses with AMR; United States Department of Agriculture Economic Research Service and National Health and Nutrition Examination Survey data on beef consumption; Centers for Disease Control and Prevention National Hospital Ambulatory Medical Care Survey and National Ambulatory Medical Care Survey databases as a proxy of antimicrobial (AM) treatment failures (TF) for gastrointestinal infections; and source attributions estimated from published literature.

Crucial data gaps included: historical prevalence of FP and AMR in retail meats and from regulatory Hazard Analysis and Critical Control Points (HACCP) samples; consistent availability of Whole Genome Sequencing data to improve AMR source attribution; historical livestock AM use by class; links between resistance (NARMS) and FP illnesses (FoodNet) data, human AMR infections, and TF by pathogens.

Significance: Our findings resulted in undergoing research to fill key data gaps, particularly prevalence of FP and AMR in retail meats and longitudinal studies of AMR transfer from foods to humans. AMR results from regulatory HACCP samples are currently being incorporated in the model. The results of the updated model will be relevant to further understanding the role of foods in AMR in humans and targeting mitigation strategies accordingly.

T1-05 Machine Learning Methods as a Tool for Risk Assessment Applying Next Generation Sequencing Data

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Introduction: The massive generation of next generation sequencing (NGS) data presents an untapped potential to improve microbial risk assessment (MRA) through increased specificity and a potential fundamental change in the definition of the hazard.

Purpose: We propose machine learning approaches inputting NGS data from pathogens and potential pathogens in food and returning an estimate of the resulting risk and health burden at the population level. *Listeria monocytogenes* and *Escherichia coli* are used in case studies.

Methods: Sequencing data from 207 strains from food and food environment and 39 clinical strains of *L. monocytogenes* were assembled and blasted for 136 reviewed virulence and environmental stress resistance genes. The percentage of reported cases associated with each strain allowed for supervised machine learning models. Gene families were used as model input to predict and differentiate health outcomes in *E. coli* infections including diarrhea, bloody diarrhea, hospitalisation, haemolytic uraemic syndrome, and their combinations.

Results: Random Forest was selected as the best model for *L. monocytogenes* case (accuracy of 0.91 [95% confidence interval: 0.79 to 0.98]). Twenty of the most important predictive genes were selected based on importance analysis for genes more often used as splitter variables for each frequency of illness class. These genes occurred at highest prevalence among strains from ready-to-eat foods and vegetables and at lowest prevalence in dairy and composite foods. LogitBoost was selected as the best model in the case of *E. coli* (accuracy of 0.75 [95% confidence interval: 0.60 to 0.86]). Important new and reported gene combinations were proposed in predicting disease outcome and severity in *E. coli*.

Significance: Findings and approaches described offer the potential for developing a NGS-based web tool for MRA, which can be used for predicting health risks associated with food chain contamination, thereby creating opportunities for rethinking current approaches in MRA.

T1-06 Comparative Risk Assessment to Prioritize Pork Products for Potential Foodborne Hepatitis E Virus Transmission

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Introduction: Hepatitis E virus (HEV) is an opportunistic zoonotic virus that can cause liver inflammation, especially in transplant recipients, immunocompromised people, and those with underlying disease. The majority of exposed individuals develop no clinical symptoms. Reported case numbers rose in several European countries recently without a clear cause. Domestic pigs, wild boar, and deer are local reservoirs for HEV and suspected transmission routes to humans include environmental, direct contact, and foodborne transmission.

Purpose: To prioritize groups of pork products for the contribution to foodborne HEV transmission.

Methods: According to the Dutch National Food Consumption Survey, all consumed pork products in the Netherlands were grouped into one of seven groups based on the ingredients and processing steps: fresh meat, porcine liver (whole), liver sausage, raw meat products, cooked meat products, fermented products, and blood sausage. A quantitative microbiological risk assessment model was developed to compare estimated exposure doses among these food groups. As data scarcity made characterization of all required parameter distributions and the generation of absolute risk estimates impossible, parameters were quantified as ordinal, order-of-magnitude levels.

Results: Results showed that of the two sources of HEV-introduction in meat, i.e., blood and liver, the contribution of blood was negligible. The two food groups "fermented sausage" and "porcine liver (whole)" together were estimated to cause about 95% of pork-related HEV cases. Fermented sausages were contaminated due to carry-over liver when using the diaphragm as an ingredient, and refraining from using diaphragm in fermented sausages

reduced the estimated contribution by about 60%. Intervention on whole pig liver was related to proper heating by consumers, reducing the estimated contribution by about 25%.

Significance: The Dutch Meat Products Association issued an industry-wide guidance to stop using diaphragm in uncooked pork products to further improve pork safety.

T1-07 Modeling the Risk of Salmonellosis in the North American Market from Consumption of Walnut Kernels Produced in the United States

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Introduction: Salmonella has been isolated from inshell walnuts and walnut kernels, but this organism has not been associated with any known walnut outbreak in North America. A quantitative microbial assessment of the risk of salmonellosis from consumption of walnuts would provide a framework for evaluation of potential management strategies.

Purpose: The risk of salmonellosis from consumption of walnut kernels produced and consumed in North America was assessed through a Monte Carlo-based simulation model.

Methods: Postharvest walnut processing was modeled from receipt at the walnut handler, as this was the point at which inshell *Salmonella* prevalence data was available. Subsequent steps modeled storage (processor, retail, consumer), processing (including applications such as baking), and consumption. The model was based on data derived from laboratory experiments, published literature, and industry expert opinions. The base model assumed that contamination occurred only before the walnuts reached the processing facility. Uncertainty was analyzed via what-if scenarios to address variations in the model inputs. A total of more than 100,000 sample iterations were simulated using @Risk software.

Results: The estimated North American incidence of salmonellosis from walnuts averaged 330 cases per billion servings, or 700 cases per year, without considering *Salmonella* decline during storage, commercial processing, or consumer handling. Estimated cases of salmonellosis were reduced to <1 per year when 100% of walnut kernels were exposed to ≥3-log reduction treatment or when estimated *Salmonella* decline on both inshell walnuts and walnut kernels during storage was included.

Significance: This model can be used as a tool to aid walnut risk managers in enhancing the microbial safety of walnuts.

T1-08 Modelling *Salmonella* Contamination and Survival on Tomatoes at the Farm and Packinghouse

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Introduction: Fresh whole tomatoes have been linked to *Salmonella* outbreaks in the United States. Many of these outbreaks have been traced back to contamination at the farm or packinghouse.

Purpose: Mathematical models were developed to predict *Salmonella* transfer to tomatoes in the field, control at the packinghouse, and subsequent survival during storage.

Methods: The field contamination models were developed from unpublished data on the transfer of *Salmonella* from soil, plastic mulch, and agricultural waters to tomatoes. The packinghouse processing models used published data on the efficacy of peroxyacetic acid via a spray bar and roller application and chlorine via a dump tank application. The survival model used data from published literature to predict the survival of *Salmonella* on tomatoes during storage. Models were developed in Microsoft Excel.

Results: The log percent transfer of *Salmonella* from plastic mulch and soil varied between 0.5 to 2.5 and -1.0 to 3.0 log percent, respectively. The transfer of *Salmonella* from irrigation water to tomatoes was dependent on water volume and *Salmonella* concentration. Models predicted that chlorine was not very effective at reducing *Salmonella* concentration (~1 log CFU reduction/tomato), while peroxyacetic acid effectiveness was more dependent on flow rate than concentration to reduce *Salmonella* concentration. The literature data showed widely variable patterns for *Salmonella* growth, survival, and decline on tomatoes, which suggested multiple factors such as temperature, relative humidity, and *Salmonella* serovar may play a role. Some research showed growth up to 1.8 log CFU/day, and other research showed reductions up to 0.7 log CFU/day.

Significance: These models can be used as part of a farm-to-fork quantitative microbial risk assessment for *Salmonella* on tomatoes and used to identify suitable risk management measures for *Salmonella* on tomatoes. The models also show a clear need for a better understanding of the factors that influence *Salmonella* survival on whole tomatoes.

T1-09 Performance Evaluation of the Canadian Food Inspection Agency Risk Assessment Model Considering Multiple Food Commodities and Sub-Products

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Introduction: The Canadian Food Inspection Agency (CFIA) has developed a quantitative risk assessment model to assess the risk level of federal single- or multiple-commodity food establishments to help inform the allocation of inspection resources. The model performance was evaluated within meat (49) and dairy (29) establishments, resulting in a positive correlation coefficient for both (r=0.60, P<0.001).

Purpose: Herein, the model performance was evaluated in the maple sector and is ongoing within the honey and eggs sectors. This step also helped validate the data collection tool and refine the model based on sensitivity analysis.

Methods: A simple or stratified random sampling was used to identify 32 establishments from across Canada (for maple, honey, and eggs establishments, respectively). For the maple sector, data was collected and analyzed over two months in 2017 using CFIA's existing systems and an Excel questionnaire. Twenty-three maple CFIA senior inspectors, each with a minimum of five years of experience in food safety, participated in the performance evaluation. Each expert categorized 10 establishments, including two controls, based on their risk to human health and using an ordinal scale that was later translated to interval variables.

Results: Correlation between the model outputs, expressed as Disability Adjusted Life Years, and the CFIA inspectors' scores resulted low positive when all maple establishments were analysed (r=0.33, P=0.07), but moderate for medium (r=0.51, P=0.05) and small (r=0.56, P=0.20) establishments. Identified discrepancies included a variable interpretation of risk associated to product volume. Further examination of this potential cause factor showed that when fixing the volume variable, correlation values improved highly (r=0.77, P<0.001 for all maple establishments). Data analyses for the honey and eggs establishments are being completed and will be used to refine the model.

Significance: Evaluating the model's performance for multiple food commodities has been shown to enhance its applicability to the Agency's integrated risk management approach. Further assessment in the produce sector will follow.

T1-10 Burden of Disease as a Metric for Risk-based Sampling of Imported Foods

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Introduction: Globalization has led to an increase in the transport of food, and therefore foodborne pathogens, across the globe. No metric is available to rank the risks of pathogens in foods imported from specific regions.

Purpose: The purpose of this study was to develop a quantitative metric to rank the disease burden imported through food into the Netherlands by using publicly available data sources.

Methods: The number of illness per kilogram food for every pathogen-product-country combination was calculated using regional incidence estimations for foodborne diseases, regional food consumption data and Dutch foodborne disease attribution data. The calculated numbers of illness per kilogram were then multiplied with volume of imported products, inactivation estimations for frozen transport and Dutch disease disability weights.

Results: We calculated total imported Disability Adjusted Life Years (DALY) and DALY/kg in imported food for every pathogen-product-region combination. For the Netherlands, the most DALYs were imported via food from South America and Southeast Asia. This is mainly due to *Salmonella* in frozen poultry from Brazil and Thailand.

Significance: This metric can be used in combination with other information sources to design a risk-based monitoring scheme for imported foods.

T1-11 Integrated Risk Assessment of Nonylphenol and Bisphenol A through Dietary Intake in Taiwan

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Introduction: Nonylphenol (NP) and Bisphenol A (BPA) have been identified as endocrine-disrupting chemicals. NP and BPA through dietary intake in Taiwan are rarely well-studied simultaneously, as both are environmental hormones.

Purpose: The present study aims to monitor background levels of NP and BPA in local foodstuffs around Taiwan, and determine dietary exposure and potential health risk assessments, and use them to consider mitigation options for NP and BPA emissions.

Methods: In 2016, 278 food samples belonging to 11 different food groups were collected and LC-MS/MS was used for NP and BPA analysis. The average daily dose and hazard quotients (HQ) for different age-sex groups were assessed. The Monte Carlo method was used to determine variability and uncertainty.

Results: NP was detectable in all of the samples, and the highest content was found in seafood (mean: 73.1 μg/kg wet weight); the second highest was in canned food, livestock, and whole grain. The highest BPA concentration was measured in canned food (mean: 14.0 μg/kg wet weight), in beverages, and in oil. The HQ of NP and BPA exposure by age and gender showed that except for the 99% upper limit of HQ in the 0- to 3- and 4- to 6-year age groups, all HQ were lower than 0.5. If we further performed the sensitivity analysis in non-carcinogenic risk for NP and BPA together, the concentrations of NP in grain become the most influential variable, with the second being the intake quantity of whole grains.

Significance: Much research has revealed that BPA and NP intake were lower than the tolerable daily intake of 4 μg/kg bw/day established by the European Food Safety Authority. Grain, canned food, and vegetables accounted for more than 80% of BPA dietary intake. Grains, seafood, and livestock contribute more than 70% NP dietary intake in the current study.

T1-12 Identifying the Food Type and Location Source of Large-scale Outbreaks of Foodborne Disease

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Introduction: During large-scale outbreaks of foodborne disease, rapidly identifying the source, including both the food vector carrying the contamination and the location source in the supply chain, is essential to minimizing impact on public health and industry. However, tracing an outbreak to its origin is a challenging problem due to the complexity of the food supply system and the absence of integrated records.

Purpose: This project develops novel methods that leverage nontraditional data sources to build a "computational epidemiology" approach to probabilistically identify the food type and location source of emerging outbreaks of foodborne disease, as applied to available data in Germany.

Methods: Previous work developed a network-theoretic modeling framework to identify the location source of an outbreak given a network model of the food supply chain structure and reported locations of illness. This work builds on the existing framework to develop novel statistical methods to identify the food item vector carrying the contamination. Computational simulation is used to construct likelihood ratios, which are used to identify the food item network that best fits the observed distribution of illness data. The effectiveness of these methods are evaluated on recent outbreaks of foodborne disease using a model of the aggregated network structure of the German food supply chain for 50 product categories. Patient illness data is provided by the Robert Koch Institute.

Results: In application to two evaluated outbreaks, the 2011 outbreak of *E. coli* spread through contaminated sprouts and a 2012-2015 outbreak of listeriosis spread through contaminated pork belly, our methods are able to identify the food type and location source region with high accuracy using data from only a small fraction of the total number of reported illnesses.

Significance: Our results suggest the application of the combined statistical methods and network models could have substantial benefits for investigators during outbreaks of foodborne disease. Furthermore, our methods provide a "big data" framework for outbreak investigation in any country or international setting.

T2-01 Assessment of the Relationship between Foodborne Illnesses Due to Beef Contaminated with Antimicrobial -resistant Bacteria and Prophylactic Use of Antimicrobials in Beef Cattle

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Introduction: Prophylactic use of antimicrobials (AM) in food production animals has been targeted as a driver of antimicrobial resistance (AMR).

Purpose: Our objective was to assess the relationship between foodborne illnesses with AMR bacteria and prophylactic AM use in beef cattle.

Methods: A Bayesian model was used to compare the incidence of foodborne illnesses per beef meal contaminated with AMR bacteria and from cattle prophylactically treated ($\lambda_{Bf,AM}$) in 1999 vs 2010, the two most recent years with nationwide estimates of tetracycline and tylosin use in feedlots. Incidence of human illnesses from beef contaminated by STEC O157, *Salmonella* spp. or *Campylobacter* spp. with resistance to ≥3 drug classes was derived from FoodNet, NORS, and NARMS data. Per-capita incidences of beef meals from cattle treated with AM were estimated from USDA-ERS, USDA-EPA, and NAHMS data. Incidences were statistically compared using the probability that $\lambda_{Bf,AM}$ was higher in one of the two years ($PrHigher_{year}$).

Results: The fraction of beef cattle treated with tetracycline decreased from 30.6% in 1999 to 21.2% in 2010, while it increased from 42.3% to 71.2% for tylosin. $\lambda_{Bf,tetracycline}$ was not statistically different between years (all PrHigher <95%). For example, $\lambda_{Bf,tetracycline}$ for *Salmonella* was 3.9×10^{-7} ($2.2\text{-}6.2\times10^{-07}$) in 1999 and 4.3×10^{-07} ($2.3\text{-}7.4\times10^{-07}$) in 2010. In contrast, $\lambda_{Bf,tylosin}$ decreased from 1999 to 2010 ($PrHigher_{1999}$ of 99.9%, 100% and 99.4% for *Campylobacter*, *Salmonella* and STEC), as illustrated with $\lambda_{Bf,tylosin}$ for *Campylobacter* at 7.1×10^{-9} (95%CrI: $3.3\text{-}12.0\times10^{-9}$) in 1999 but 3.4×10^{-9} ($1.7\text{-}5.3\times10^{-9}$) in 2010.

Significance: Analysis of US surveillance data showed no evidence of a statistical relationship between prophylactic use of tylosin and tetracycline in beef cattle and AMR in foodborne illnesses from beef consumption.

T2-02 Effect of "Functional Ice" on *Salmonella* Inoculated on Raw Poultry Parts during Storage

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❖ ***Developing Scientist Competitor***

Introduction: The poultry industry uses antimicrobials such as peracetic acid to reduce *Salmonella* on raw poultry which do not possess residual antimicrobial properties to suppress the pathogen during storage and transportation. Existing methods of icing and refrigeration during storage and transportation to control *Salmonella* can be further improved. Novel methods for sustained antimicrobial release must be studied to actively suppress *Salmonella* throughout storage and transportation providing additional food safety hurdle.

Purpose: Research was conducted to design "Functional Ice" (FICE) (Patent Pending) and to study its antimicrobial effect on *Salmonella* inoculated on raw chicken over a 48h refrigerated storage.

Methods: FICE treatments were prepared by freezing aqueous solutions of phosphate (2.5% and 5% w/v) and lactate-diacetate (1% and 2.5% v/v) with tap water as control ice treatment. Nalidixic acid resistant (35 μg/ml) *Salmonella* Typhimurium suspensions were inoculated (100 uL) on raw boneless, skinless thigh meat pieces (n=15 samples/treatment x 5 trials), allowed 1h attachment time (final inoculum $10^{6\text{-}7}$ CFU/sample), placed in FICE treatments and sampled at 0, 12, 24, 36 and 48h. Viable *Salmonella* colonies were reported as log CFU/mL of rinsate. Yield (% pickup) was calculated for each treatment. Significant differences among treatments were determined using ANOVA with Tukey's LSD at $p<0.05$.

Results: Salmonella levels were reduced ($P < 0.05$) by >1 log within 12h by FICE made with 2.5 and 5% phosphate compared to control treatment during the 48h refrigerated storage. Also, soaking of the thighs in the melted 5% phosphate FICE caused a 10% yield increase after 48 h.

Significance: FICE can be used as a novel antimicrobial delivery method to improve food safety during storage and transportation of raw poultry.

T2-03 Antimicrobial Activity of Commercial Protective Cultures against *Listeria monocytogenes* and *Escherichia coli* O157:H7

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❖ ***Developing Scientist Competitor***

Introduction: The potential presence of pathogenic bacteria, including *Listeria monocytogenes* and *Escherichia coli* O157:H7 (O157), is a significant hazard in the production of cheese from unpasteurized milk. Protective cultures (PC) are commercially available to help control pathogens and spoilage microorganisms in the production of foods, including cheese.

Purpose: The objectives of this study were to compare the antimicrobial activity of commercial PC used individually and in combinations against *L. monocytogenes* and O157 and to characterize their interactions.

Methods: Cell free supernatants (CFS) from eight commercially available PC were collected after 24 h incubation at 35°C in de Man, Rogosa, and Sharpe broth (MRS). Six-strain cocktails of each pathogen were inoculated at 6 log CFU/ml in tryptic soy broth with yeast extract in the presence of varying concentrations of CFS from each PC, or binary combinations thereof, diluted in MRS and incubated at 35°C for 24 h. Concentrations of CFS that inhibited pathogen growth to half that of the control (IC_{50})were identified and compared. Diagonal measurement of n-way drug interactions methodology was used to identify and analyze interactions using fractional inhibitory concentration scoring calculated from observed and expected IC_{50} values.

Results: Supernatants from all PC were able to inhibit the growth of both pathogens. The supernatants from LPAL had the lowest single IC_{50} for *L. monocytogenes* at 3.4%, followed by HOLDBAC Listeria at 3.8%. HOLDBAC was the most effective PC against O157, with an IC_{50} of 7.7%, followed by LRB (8.4%) and LPAL (8.4%). Although no synergistic combinations were identified against O157, combinations of HOLDBAC and LRB (2.6%), as well as BLC-20 with either LRB (3.6%) or BS-10 (4%), were synergistic against *L. monocytogenes*.

Significance: This study characterized the efficacy of eight commercial PC for the control of *L. monocytogenes* and O157. A novel sampling and scoring method identified synergistic and antagonistic combinations.

T2-04 Inhibition of *Listeria monocytogenes* on Cured Ready-to-Eat Meats by Sodium-Free and Clean-Label Antimicrobial Ingredients

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Introduction: Listeria monocytogenes contamination and growth is a major concern in refrigerated deli meats. In response to consumer demands, the portfolio of reduced-sodium and naturally derived antimicrobials are being expanded to provide the option to inhibit this pathogen during long term refrigerated storage.

Purpose: To compare the inhibitory properties of Provian® K (potassium acetate and diacetate blend) and Provian® DV (neutralized dry vinegar) on the growth of *Listeria monocytogenes* in cured RTE meats.

Methods: Five treatments of cured deli-style ham (72-74% moisture, 1.75±0.1% salt, and pH 6.2-6.4, 156 mg/kg sodium nitrite and 547 mg/kg sodium erythorbate) included a control without antimicrobials and different concentrations of K (0.5% and 0.75%) and DV (0.5%, 0.65%). Cooked products were surface inoculated with 3-log10 CFU/g of a 5-strain mixture of *L. monocytogenes*. Inoculated slices (100g/package) were vacuum-packaged and stored at 4°C and 7°C for up to 12 and 8 weeks, respectively. Triplicate samples per treatment were assayed by enumerating on Modified Oxford Agar. The study was conducted twice.

Results: Control Ham supported >1 log increase of L. monocytogenes at 4 and 2 weeks storage at 4 and 7°C, respectively. In contrast, hams supplemented with 0.5 or 0.75% K or 0.65% DV inhibited the Listeria growth for 12 and 8 weeks at 4 and 7°C, respectively. Inhibition of Listeria on ham supplemented with 0.5% DV was further affected by pH. Ham supplemented with 0.5% DV in the trial 1 (pH 6.36) delayed Listeria for 12 weeks storage at 4°C, whereas individual samples of trial 2 (pH 6.43) supported growth (>1 log increase) at 8 weeks. Similar trends were observed at 7°C.

Significance: Results from this study validate the efficacy of sodium free antimicrobials and neutralized dry vinegar in inhibiting growth of *L. monocytogenes* on cured ready-to-eat meats.

T2-05 Effect of Storage Temperature on Injured *Salmonella* Bacteria on Apples Treated with Antimicrobial and Cold Plasma Combination

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Introduction: Most washing treatments that are designed to reduce bacterial populations on produce surfaces designated for fresh-cut processing result in survival of residual injured bacteria after processing.

Purpose: The objective of this study was to investigate the behavior of *Salmonella* bacteria inoculated on apple surfaces treated with antimicrobial solution in combination with cold plasma processing

Methods: Whole apples (Granny Smith) purchased from a supermarket were stored at room temperature (22°C) for 18 h to come to room temperature. Apple surfaces were dipped in *Salmonella* inoculums at 10^7 CFU/ml solution for 5 min. After inoculation and a 2-h waiting period, apples were treated as follows: 1) cold plasma treatments for 30 and 40 s; 2) submersion of inoculated apples in antimicrobial solution for 5 min; 3) application of the antimicrobial solution directly to the calyx area; 4) addition of antimicrobial solution immediately followed by cold plasma treatments; or 5) cold plasma treatments immediately followed by addition of antimicrobial solution.

Results: *Salmonella* population determined after inoculation of apples was 4.2 log CFU/g, while aerobic mesophilic bacteria and yeast and mold were 3.3 and 1.8 log CFU/apple, respectively. Cold plasma treatments alone at 30 and 40 s resulted in 1.42 and 1.43 log reduction of *Salmonella* population, while antimicrobial solution led to 2.8 log reduction. Population of injured bacteria on cold plasma treated apples averaged 67 and 43% at 30 and 40 s, respectively, and 50% on apples treated with antimicrobials. *Salmonella* was negative in fresh-cut apples treated with a combination of antimicrobial and cold plasma treatments and storage of prepared fresh-cut pieces at 5°C for 24 h.

Significance: The results of this study suggest that a combination of cold plasma and antimicrobial solution treatments will significantly improve the microbial safety of whole and fresh-cut apples by reducing bacterial pathogens.

T2-06 Antimicrobial Activity of Fermented Milk Protein after Maillard Reaction to Enteropathogenic Bacteria

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❖ ***Developing Scientist Competitor***

Introduction: A milk protein was recently discovered through fermentation of the Maillard reaction, called fermented Maillard reaction products (FMRPs). The FMRPs have anti-hypertensive and anti-thrombotic activities, and thus, adding these into dairy products has been suggested. However, their antimicrobial activity against enteropathogenic bacteria such as *Listeria monocytogenes*, *Clostridium perfringens*, and *Bacteroides fragilis* has not been examined yet.

Purpose: The objective of this study was to examine the antimicrobial activity of FMRPs against *L. monocytogenes*, *C. perfringens*, and *B. fragilis*.

Methods: To elucidate the mode of antimicrobial activity of FMRPs (whey proteins glycated [Gw] with galactose [Gal] fermented by *Lactobacillus rhamnosus* 4B15 [4B15], Gw-Gal-fermented by *L. gasseri* 4M13 [4M13], Gw-glucose [Glc]-4B15, Gw-Glc-4M13), a three-strain mixture of *L. monocytogenes*, a three-strain mixture of *B. fragilis*, and a six-strain mixture of *C. perfringens* were exposed to FMRPs. For the resulting bacterial cells, propidium iodide uptake assay was then conducted at 200 mg/ml, and the morphological changes of the pathogens were captured by field emission scanning electron microscope (FE-SEM). In addition, iron chelation activity was examined to elucidate the antimicrobial effects of FMRPs.

Results: The results of propidium iodide uptake assay and FE-SEM showed that FMRPs inhibited the bacterial cell growth by damaging bacterial cell membranes and cell walls. The mode of antimicrobial activity of Gw-Gal-4M13 was particularly obvious. However, iron chelation activity of FMRPs was not observed.

Significance: These results indicate that FMRPs have antimicrobial activity against *L. monocytogenes*, *C. perfringens*, and *B. fragilis* via damage to cell membranes and cell walls rather than chelation activity.

T2-07 Recombinant Probiotic *Lactobacillus casei* Expressing the Internalins AB or *Listeria* Adhesion Protein (LAP) Affect Specific Stages in the *Listeria monocytogenes* Infection Process In-vitro

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Introduction: Advanced understanding of its infection cycle and consideration of its virulence genes offers a strategy for its control. Probiotic bioengineering is a strategic approach to broaden efficacy for control of enteric pathogens.

Purpose: The current study investigates the ability of a *Lactobacillus casei* expressing internalins A and B (Lbc^{InlAB}) to control *L. monocytogenes*. This construct is compared with a previously developed *Lb. casei* expressing LAP (Lbc^{LAP}).

Methods: *L. monocytogenes inlAB* operon was cloned and expressed in *Lb. casei* using expression vector pLP401-T. Protein expression was confirmed by Western blot. Adhesion profiles of *L. monocytogenes, Lb. casei* (Lbc^{WT}) and Lbc^{InlAB} to Caco-2 cells were investigated. Competitive, inhibition and displacement of adhesion of *L. monocytogenes* to Caco-2 cells by these probiotic strains were investigated. Additionally, the inhibition of adhesion, invasion and translocation of the pathogen was determined Caco-2 cells, pre- exposed to probiotics for 1, 4, 16, or 24 h (MOI =10). All experiments were done three times in duplicates.

Results: Western blot confirmed expression of InlA and InlB on *Lb. casei*. There was a significant increase ($p<0.0001$) in the ability of Lbc^{InlAB} to adhere, invade and translocate through Caco-2 cells compared to Lbc^{WT}. In competitive and inhibition of adhesion of *L. monocytogenes* to Caco-2 cells there was a significant decrease by the recombinant probiotics. Lbc^{InlAB} reduced adhesion and invasion by 54% and 52%, respectively, while Lbc^{LAP} reduced these stages by 64% and 32%, respectively, after 24 h. Pre-exposure to Lbc^{WT} had no significant effect. Recombinant probiotics reduced adhesion and invasion of *L. monocytogenes* to Caco-2 cells; however, Lbc^{InlAB} reduced invasion better while Lbc^{LAP} reduced adhesion.

Significance: The results suggest that by employing specific virulence genes, bioengineering could be used to target specific stages in the infection cycle to inhibit pathogens colonization.

T2-08 Antimicrobial Hydrogel Patches to Control Gram-positive Bacteria on Food Surface

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Introduction: Beef tartare and sashimi are consumed raw; therefore, non-thermal decontamination technology needs to be applied. The technology should affect the flavor of meat and should not be toxic. Thus, hydrogels composed of edible compounds and antimicrobials should be appropriate as non-thermal decontamination.

Purpose: This study developed an antimicrobial hydrogel patch composed of edible compounds and antimicrobials to inhibit gram positive pathogens on food surface.

Methods: Alginate-based hydrogel was prepared by dissolving 5% sodium alginate powder in 25 ml distilled water, mixed with co-polymers (1% agar [weight/weight], 40% glycerol [weight/weight]), and mixed with crosslinker ($CaCl_2$). The mixture was heated at 80°C and stirred until the mixture became homogeneous. The alginate-based solution was spread in plastic plates and dried at 42°C for 24 h. The alginate gels were cut into 1 by 1 cm and physical properties (strength, elasticity, swelling, and antimicrobial loading capacity) of the hydrogels were determined. The hydrogels were placed in 2 ml of 0.1 to 0.5% natural antimicrobials (grapefruit seed extract and citrus peel extract) from 30 min to 2 h, and the hydrogels were placed on a lawn of *Listeria monocytogenes* on Palcam and a lawn of *Bacillus cereus* on MYP agar. Clear zones were then measured.

Results: Addition of $CaCl_2$ (0.2%) and agar (1%) into the alginate increased the hydrogel strength, and the elasticity of the hydrogel gel was increased by adding glycerol (40%) into the alginate. Among the tested natural antimicrobials, 0.5% grapefruit seed extract was the most appropriate to inhibit *L. monocytogenes* and *B. cereus* growth. Thus, an antimicrobial hydrogel composed of 5% alginate, 0.2% $CaCl_2$, 1% agar, and 0.5% grapefruit seed extract was prepared. The hydrogel completely inhibited *L. monocytogenes* and *B. cereus* growths.

Significance: These results indicated that the developed hydrogel can be used to inhibit the growth of foodborne pathogens on food surfaces, especially for food consumed raw.

T2-09 CRISPR/Cas9 Directed Inactivation of Polymyxin Expression in *Paenibacillus Polymyxa* for Sole Production of the Bacteriocin, Paenibacillin

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Introduction: Paenibacillin, a bacteriocin active against gram positive bacteria, has shown promise for food applications but is co-produced with an antibiotic, polymyxin E, in *Paenibacillus polymyxa* OSY-DF culture. Polymyxin is a non-ribosomally synthesized anti-gram negative antibiotic that could not be added to food products. Paenibacillin, however, could potentially be added to food through fermented protective ingredients if the polymyxin gene is knocked out. *P. Polymyxa* has been difficult to transform with traditional methods; therefore, clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9 (CRISPR/Cas9) techniques were adapted for *Paenibacillus* transformation for the first time.

Purpose: The objective of this study was to prevent polymyxin biosynthesis with CRISPR/Cas9 to allow for application of *P. polymyxa* fermentate to inhibit spoilage and pathogenic bacteria in food.

Methods: To knock out production of polymyxin, an adenylation domain in the polymyxin synthesis gene, *pmxE* was targeted. The guide RNA (gRNA) was designed to align Cas9 to the target site. The repair template was created by removal of 100 bp of the cut site flanked by 500 bp homologous regions. Plasmid components were ligated together into PB105 CRISPR/Cas9 plasmid. Standard electroporation methods used previously with *P. polymyxa* would allow for insertion of the CRISPR/Cas9 plasmid.

Results: A temperature-sensitive plasmid was successfully inserted and expressed in *P. polymyxa*, which showed plasmid could be introduced and expressed in the strain. The CRISPR/Cas9 plasmid has been verified with sequencing to contain the specific gRNA insert for the first adenylation domain in *pmxE*. All plasmid components were generated with specificity for *pmxE* in *P. polymyxa.*

Significance: A bacterium that produces paenibacillin only could be used to manufacture fermented food ingredients with antimicrobial properties (i.e., protective ingredients). This would bypass expensive purification steps to separate paenibacillin from polymyxin, and the antimicrobial would not need to be listed on product labels. The newly developed strain could protect humans from serious foodborne pathogens and spoilage bacteria.

T2-10 Synergistic Antimicrobial Effect of Eugenol and Biologically Synthesized Silver Nanoparticles against *Listeria monocytogenes*

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Introduction: *Listeria monocytogenes* is an important foodborne pathogen often associated with food processing environments. Effective sanitation methods are critical in minimizing such contamination. The essential oil eugenol and biologically synthesized silver nanoparticles (bio-AgNP) have shown potential effects against this pathogen.

Purpose: The aim of this study was to evaluate the antibacterial activity of essential oil eugenol and bio-AgNP against weak and strong biofilm producer *L. monocytogenes* isolates from food processing environments.

Methods: Bio-AgNP were obtained after reduction of silver nitrate by *Fusarium oxysporum.* Micro-dilution assay was used to determine the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the compounds, and microplate turbidometric growth inhibition assays were used to study the inhibitory action of eugenol and bio-AgNP, either alone or in combination, on *L. monocytogenes* isolates. All treatments were performed in triplicate and mean optical density (O.D.) values from microplate turbidometric assay were plotted *versus* time. Statistical analysis using Student's *t* Test was carried out to determine if significant differences ($p<0.05$) exists between different treatments.

Results: The results showed that when acting alone, the MIC and MBC of eugenol was 0.25% against the weak and strong biofilm-producing strain. MIC and MBC of bio-AgNP for the strong biofilm-producing strain was higher (630 µM) than that of the weak one (315 µM). Synergistic antimicrobial effect was observed when both compounds were used together and the MIC values were reduced for weak and strong biofilm producers.

Significance: Both eugenol and bio-AgNP exhibited anti-*Listeria* activity at relatively low concentrations. Eugenol in combination with bio-AgNP offers a potential solution for new sanitation treatment of food processing equipment and environments.

T2-11 Antimicrobial Resistance in the Food Industry – Is It Really Related to Sanitation?

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Introduction: Microorganisms becoming resistant to medically important antibiotics causes significant public health concerns. As such, there is renewed interest in ensuring that microbial management practices continue to be effective, not only in healthcare settings but also across the food system. Microbial resistance to biocides (i.e., disinfectants and sanitizers) in food manufacturing environments has been suggested by researchers and some international bodies (e.g., Codex Alimentarius) as a potential risk requiring further study and, in some cases, control measures. Since proper use of biocides plays an important role in food safety management, a balanced and risk-based approach is required to avoid deleterious impacts to public health protection.

Purpose: To review the proposed connection between resistance to antibiotics and biocides and to challenge the current research paradigm by proposing more appropriate evaluation methodologies based on real-world scenarios so valid implications to public health can be assessed.

Methods: Expert panel review of the currently available literature.

Results: A review of recent literature reveals the lack of connection between resistance to antibiotics and biocides, since real-world conditions are not consistently mimicked and there is a misunderstanding of terms. The most common method used for this type of research is the MIC method, which has been criticized by experts in the field as misrepresenting actual use conditions. Non-substantiated conclusions have been drawn by researchers against standard sanitation protocols that do not include effective cleaning followed by use of sanitizers under required conditions and concentrations.

Significance: More consistency of commonly used terms is needed. We propose definitions of key terms to promote understanding of information, as well as interpretation to implications in food facility sanitation. As new antibiotic resistance management strategies are being planned globally, it is important that relevant methods and accurate data are used to underpin recommendations related to practical biocide use, given their importance in promoting food safety.

T2-12 Comparison of Thermal Inactivation between *Staphylococcus carnosus* CS-299 and CS-300 As Potential Hepatitis A Virus Surrogates

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Introduction: Heat is traditionally used to inactivate pathogenic and spoilage organisms in food to ensure safety and extend shelf life. Recent foodborne viral outbreaks have necessitated research to determine their optimal thermal inactivation parameters (D- and z-values). Hepatitis A virus (HAV) is more resistant to heat ($D_{72°C}$ of 0.9 min in buffer) than known vegetative bacteria. To validate heat-inactivation processes, non-pathogenic surrogates are used. *Staphylococcus carnosus* is non-pathogenic and used in fermentation in the food industry.

Purpose: The purpose of this study was to compare the thermal inactivation (D- and z-values) of two strains of *S. carnosus* (Chr CS-299 and CS-300) as potential HAV surrogates.

Methods: Overnight cultures (~8 log CFU/ml) of *S. carnosus* CS-299 and CS-300 grown at 37°C in tryptic soy broth were washed, resuspended in phosphate buffered saline (PBS), and aseptically transferred to sterile 2-ml glass vials. Vials were heated at 50, 55, 60, 65 and 70°C in a circulating water bath for various time points, cooled on ice, plated on tryptic soy agar, and incubated for 24 to 48 h at 37°C. Each trial in duplicate was replicated at least thrice. D- and z-values were determined using the first-order linear model.

Results: *D*-values for *S. carnosus* CS-299 were 27.73±0.16 min (R^2=0.91), 10.68±0.10 min (R^2= 0.93), 0.81±0.04 min (R^2=0.90), 0.608±0.05 min (R^2= 0.89), and 0.51±0.04 min (R^2=0.90), at 50, 55, 60, 65, and 70°C, respectively, with a z-value of 6.49°C (R^2=0.93). *D*-values for *S. carnosus* CS-300 were 28.86±0.20 min (R^2=0.91), 11.7±0.11 min (R^2=0.90), 1.24±0.01 min (R^2=0.91), 0.77±0.02 min (R^2=0.92), and 0.53±0.03 min (R^2=0.89), at 50, 55, 60, 65, and 70°C, respectively, showing a z-value of 7.31°C (R^2=0.94).

Significance: Both *S. carnosus* strains at high titers show promise as potential HAV surrogates due to their ease of growth, enumeration, and non-pathogenicity. *S. carnosus* CS-300 is a marginally better surrogate than CS-299 in PBS for HAV heat-inactivation validation studies.

T3-01 Multi-regional Risk Analysis of Manure Use: Survival and Persistence of Foodborne Pathogens in Soil and Contamination Risk of Fresh Produce in Certified Organic Farms

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Introduction: Certified organic producers use animal-based soil amendments to improve soil fertility. However, raw manure may increase risk of contamination of fresh produce by foodborne pathogens. National good agricultural practices and National Organic Program (NOP) certified organic systems stipulate a time interval (90 to 120 days) between application of raw manure and harvest.

Purpose: The objective of this study is to investigate the persistence and survival of foodborne pathogens in soil and potential contamination risk of produce from certified organic farming systems in different regions of the United States.

Methods: Sixteen certified organic farms (eight in California [CA], four in Maine [ME], three in Minnesota [MN], and one in Maryland [MD]) were enrolled in a longitudinal multi-regional study. Manure, soil, irrigation water, and produce were collected for a period of eight months. Samples were cultured for non-O157 Shiga toxin-producing *Escherichia coli* (STEC), *E. coli* O157:H7, *Listeria monocytogenes,* and *Salmonella*.

Results: Manure samples were positive for non-O157 STEC (10.5%, 11 of 105), *E. coli* O157:H7 (0.95%, 1 of 105), and *L. monocytogenes* (6.7%, 7 of 105). The prevalence of non-O157 STEC in soil samples was 6% (6 of 100), 8.2% (15 of 196), 13.4% (48 of 358), and 13.9% (70 of 502) in MD, ME, MN, and CA

farms, respectively. *L. monocytogenes* was detected in 1% (1 of 100), 5.4% (27 of 502), 7.3% (26 of 358), and 12.8% (25 of 196) of soil samples in MD, CA, MN, and ME, respectively. *Salmonella* was found in 0.5% (1 of 196) of soil samples in Maine. Non-O157 STEC and *L. monocytogenes* were detected in soil up to 180 days and *Salmonella* up to 120 days post-manure application. Only one produce sample was positive for *L. monocytogenes*. All water samples were negative.

Significance: These findings contribute to filling critical data gaps concerning occurrence of fecal pathogens in NOP-certified farming systems used for production of fresh fruits and vegetables in different regions of the United States. Data will be useful in risk assessment and evaluation of appropriate manure-to-harvest wait time intervals for NOP-certified operations.

T3-02 Creek to Table – Investigating the Movement of Fecal Indicators, Bacterial Pathogens, and Total Bacterial Communities through Creek Water Irrigation of Kale and Radishes: A Conserve Study

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Introduction: Surface water, often used as agricultural water, may contain foodborne pathogens. However, the risk that these pathogens pose to consumers of fresh produce if present in irrigation waters is not well understood.

Purpose: This study tracked concentrations of fecal indicator bacteria, pathogenic bacteria, and total bacterial communities from a creek water irrigation source to irrigated produce.

Methods: Kale and radishes were drip-irrigated using Mid-Atlantic creek water in October 2017. Plant (five kale and five radish) and soil (10) samples were collected immediately before and after irrigation and for three consecutive days. All samples, including irrigation water, were tested for generic *Escherichia coli* and total coliforms (TC) using membrane filtration, and for *Salmonella* spp. and *Listeria monocytogenes* by selective enrichment. DNA extracted from all samples was amplified for the V3 to V4 region of the 16S rRNA gene for bacterial community profiling.

Results: In soil, TC levels were significantly higher immediately and three days post-irrigation compared to pre-irrigation ($P< 0.01$). *E. coli* levels in soil increased after irrigation, but not significantly ($P=0.31$), and no clear trends were evident throughout the study. No *E. coli* were detected on kale leaves and, although TC were detected, there were no significant differences by time point. TC increased over the study period on radish roots ($P< 0.01$) and in radish soil ($P< 0.01$), but *E. coli* did not change significantly. Although *Salmonella* spp. and *L. monocytogenes* were detected in irrigation water, only one field sample tested positive for a pathogen; *Salmonella* was detected from one post-irrigation kale sample. Results from the 16S rRNA data provide ecological context in the system.

Significance: This study addressed the possibility of transfer of specific bacteria from agricultural water to irrigated fresh produce, finding that TC were most strongly influenced by irrigation and pathogens were rarely transferred to the field.

T3-03 A Multi-regional Risk Analysis of Raw Manure Soil Amendment Use on Certified Organic Farms: Survival of Generic *Escherichia coli* in Soil and Produce

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Introduction: Certified organic producers use animal-based soil amendments to improve soil fertility. Raw manure use may increase the risk of contamination of fresh produce by foodborne pathogens. Microbial pathogens and contamination indicators may be transferred from amended soil to produce through different pathways.

Purpose: The purpose of this study was to evaluate generic *Escherichia coli* survival in soil and transfer to fresh vegetable crops under different environmental and management conditions in soils amended with raw manure in certified organic farming systems in different regions of the United States.

Methods: Sixteen certified organic farms were enrolled in a longitudinal multi-regional study (eight in California, four in Maine, three in Minnesota, and one in Maryland) and sampled from March to December 2017. Most probable number (MPN) methods were used to quantify *E. coli* soil samples (monthly) and fresh produce (at harvest). Quantitative and qualitative recovery of each sample was achieved via selective and differential culturing methods and confirmed via PCR.

Results: Overall, 55.6% of samples were positive for *E. coli* (755 of 1360, 55.5% soil and 3.7% produce). Average MPN values in California were 1,051 MPN/100g for soil (range 0 to 145,190) and 15.6 MPN/100g for produce (range 0 to 123). In Maryland, values were 2,026 MPN/100g for soil (range 0 to 33,776) and 11.5 MPN/100g for produce (range 0 to 22.5). Values in Maine were 1,220 MPN/100g for soil (range 0 to 87,736) and 149.7 MPN/100g for produce (range 0 to 2,514). In Minnesota, values were 2,963 MPN/100g for soil (range 0 to 33,776) and 885 MPN/100g for produce (range 0 to 15,750). The *E. coli* counts decreased considerably 120 days post-manure application on 10 of 16 farms. One irrigation water sample was positive for *E. coli*.

Significance: The survival rate of *E. coli* quantified from this study provide valuable data to conduct risk assessments critically needed to predict food safety risks associated with the application of untreated biological soil amendments of animal origin in certified organic systems.

T3-04 Molecular Characterization of Shiga Toxin-producing *Escherichia coli* and *Salmonella* Isolates from Untreated Cattle and Poultry Manure Sources at Livestock Farms and Composting Facilities in the Western United States

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Introduction: Manure generated from animal agriculture serves purposes such as soil amendment and feedstock for composting, but also represents a potential source of foodborne pathogens in the produce-growing environment.

Purpose: This study aimed to use whole genome sequencing (WGS) to characterize Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* isolates cultured from untreated cattle and poultry manure in California and Arizona.

Methods: Manure samples were cultured for STEC (cattle) and *Salmonella* (poultry) over a one-year period. Whole genome sequencing was performed on up to four isolates per positive sample; WGS data was submitted to the National Center for Biotechnology Information's (NCBI) pathogen database. Samples were compared genetically by phylogenetic analysis. Based on the WGS data, we predicted serotype, virulence, and antimicrobial resistance, and also determined membership of isolates within NCBI's pathogen database clusters (defined as isolates that differed by <100 single nucleotide polymorphisms [SNPs]).

Results: A total of 72 STEC and 66 *Salmonella* isolates were sequenced from 24 cattle and 29 poultry manure samples, respectively. The majority of STEC isolates belonged to 10 clusters and serotypes (O103:H11; O109:H10; O111:H8; O130:H38; O157:H7; O160:H12; O3:H12; O84:H2). *Salmonella* isolates grouped into 12 clusters and predicted serotypes (Alachua or IIIa 35:z4,z23:-; Altona; Anatum; Enteritidis; Hadaro or Istanbul; II 13,22:z:-; Liverpool; Mbandaka; Montevideo; Ohio; Schwarzengrund; Senftenberg or Dessau). Isolates from same facility ID (blinded) were found in different SNP clusters, while clusters were associated with individual facilities. Some isolates did not belong to existing NCBI SNP clusters and thus may represent endemic strains. Antimicrobial resistance genes were identified in 70 of 72 (97%) STEC and 18 of 66 (27%) *Salmonella* isolates.

Significance: The findings underscore the importance of preventing dissemination of zoonotic pathogens from raw manure in fresh produce production. This study reveals genetic diversity among the STEC and *Salmonella* isolates from manure sources and provides critical data for risk assessment relevant to the provisions of the U.S. Food and Drug Administration's Food Safety Modernization Act Produce Safety Rule.

T3-05 Infiltration of Bacteria through Leaf Stomatal Openings during a Vacuum Cooling Process: Mechanistic Understanding

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Introduction: Vacuum cooling is a common unit operation in the leafy greens industry and is considered a very efficient approach to extend the shelf life of fresh produce. However, during this process, bacteria can be passively internalized into the produce through available openings at the leaf surface such as wounds and stomata. Therefore, better understanding of the infiltration mechanisms and identification of the contributing factors to the amount and depth of infiltration is needed.

Purpose: Looking at one stomatal opening, a coupled multiphase transport model was developed to simulate infiltration of pathogenic bacteria into fresh leafy greens during the re-pressurization stage of the vacuum cooling process.

Methods: The model includes convective and/or diffusive transport of free water, bound water, vapor, bacteria, and heat into and out of a leaf section driven by the large pressure gradients during the vacuum cooling process. The simulation's results were validated for the prediction of the leaf temperature, pressure, and moisture content, as well as the amount of bacteria infiltration.

Results: The results showed that the prolongation of re-pressurization and larger stomatal opening size can promote infiltration of water into the leaf, which can further transport bacteria. In addition, leaves with higher initial moisture content undergo less infiltration. For example, a 10% increase in the initial moisture content led to 26 and 4% decrease in water and bacteria infiltration, respectively. The depth of the infiltration was significantly increased for the abaxial side of the leaf, higher bacteria diffusivity (motility), and longer re-pressurization duration. An increase of one order of magnitude in bacteria diffusivity resulted in 51% deeper infiltration into the leaf tissue.

Significance: The mechanistic understanding obtained from this work should help to better design and operate vacuum cooling processes to enhance microbial safety of minimally processed leafy vegetables.

T3-06 Evaluation and Validation of Non-living Bacterial Surrogates in Produce Wash Systems

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Introduction: The produce industry normally monitors the antimicrobial chemistry of the wash process, yet there is currently no practical way to rapidly monitor bacterial lethality and the risk of cross-contamination through the wash process. Finished product microbial pathogen tests have low probability of finding contaminants due to the small sample size and low incidence rate of pathogens. They are also slow and do not provide real-time information, requiring companies to place large amounts of finished product on hold until negative results are received.

Purpose: This study evaluated and validated a new class of non-living bacterial surrogates as rapid indicators of both *E. coli* (pathogenic and generic) decontamination and risk reduction of cross-contamination on produce in one-pass and recirculated flume wash systems.

Methods: Non-living bacterial surrogates were constructed by encapsulating DNA sequences into various food-grade materials. In a laboratory setting, their attachment to leafy greens surfaces and response to various sanitation schemes (e.g., water supplemented with various levels of chlorine and commercial products) were compared to *E. coli* O157:H7. Then the surrogate particles were applied to whole and cut leafy greens along with *E. coli* and the products were processed in recirculated flume and one-pass produce wash systems. The produce was tested before and after washing for *E. coli* via 3M Petrifilm and most probable number assay, and the surrogates via quantitative PCR.

Results: Under normal wash condition, very similar log reductions (2.15 log fg and 2.01 log CFU, respectively) and cross-contamination rates (0.07 and 0.01%, respectively) were observed for the surrogate particles and *E. coli*. The results validate the use of surrogates as a biomonitoring solution for produce wash systems to demonstrate pathogen reduction on produce surface and minimize the risk of cross-contamination.

Significance: These newly developed surrogates present several advantages over traditional methods: They are applied uniformly to the entire lot, thus reducing the probability of a sampling error; they are rapidly (<30 min) tested and quantified in-house, thus reducing the need for product hold pending microbial test results; and they are easily retrofitted on any wash line, enabling validation, verification, and monitoring of antimicrobial efficacy.

T3-07 Survival and Transfer of *Salmonella* on Fresh Cucumbers during Waxing

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Introduction: Cucumbers can become contaminated at numerous points during pre- and postharvesting and have been implicated in several multistate outbreaks of salmonellosis. Many cucumbers found in retail markets have been waxed to make them visually appealing and retard moisture loss. This waxing may affect bacterial survival.

Purpose: This study assessed the survival of *Salmonella* on waxed and un-waxed cucumbers and the potential for *Salmonella* cross-contamination during the waxing process.

Methods: Fresh, un-waxed cucumbers were spot inoculated with a four-strain cocktail of *Salmonella enterica* before or after wax treatment. Three different wax coatings (mineral oil, vegetable oil, or petroleum based wax) were applied manually, using polyethylene brushes. Samples were enumerated following storage for 0, 24, 72, and 168 h. *Salmonella* concentration was expressed in CFU/cucumber and *Salmonella* transfer from inoculated cucumber to brush or to un-inoculated cucumber was quantified.

Results: Higher *Salmonella* concentrations were observed on waxed cucumber during the first 72 h, but the final concentration on un-waxed cucumber (2.16 log CFU/cucumber) was higher than on waxed cucumber (1.48 log CFU/cucumber) after 168 h. *Salmonella* cells did transfer from contaminated un-waxed cucumbers to brushes used for waxing and then to un-inoculated cucumbers during waxing. Significantly higher log percent transfer to brushes was observed when cucumbers were waxed with vegetable oil (0.71 log %, P=0.00441) vs. mineral oil (0.06 log %) or petroleum (0.04 log %). Transfer to un-inoculated cucumbers via brushes was also quantified (0.18 to 0.35 log % transfer). *Salmonella* remaining on the contaminated cucumbers after waxing survived up to 168 h, and *Salmonella* survived better on the cucumbers with a petroleum-based wax.

Significance: This study shows significant bacterial transfer during waxing. Survival and transfer of *Salmonella* were affected by the type of wax coating. These finding should be useful in managing *Salmonella* contamination risk during postharvest handling.

T3-08 Establishing a Baseline for *Listeria monocytogenes* and *Listeria* spp. Prevalence 3 to 4 Hours into Production in Specialty Crop Facilities

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❖ ***Developing Scientist Competitor***

Introduction: Specialty crop processing facilities face numerous challenges in regards to food safety, including the categorization of produce as ready-to-eat and the absence of an in-process kill step. Environmental monitoring programs (EMP) are of increasing importance, as they are a key strategy for identifying environmental sources of *Listeria monocytogenes* that could lead to contamination of finished product.

Purpose: The goal of this project is to work with specialty crop packinghouses and processing facilities to develop *Listeria* EMPs in order to gather baseline data on *Listeria* prevalence and distribution over one year, with samples collected three to four hours into production, as is increasingly recommended.

Methods: Eight produce facilities from four states were selected to participate in this study. A site list was created during the initial visit to each facility. Once per month, 40 sites were selected for sampling in each facility. Samples were tested using the *Listeria* method from the U.S. Food and Drug Administration's Bacteriological Analytical Manual, with positive isolates being confirmed using *sigB* PCR and sequencing.

Results: Thus far, each facility has been sampled at least three times, with nearly 1,000 samples tested (73 to 165 per facility), excluding vector swabs. *Listeria monocytogenes* prevalence in a given facility ranged from <0.8% to 17.8%. *Listeria* spp. were found less frequently, with prevalence ranging from <0.8% to 3.2%. Thus far, results suggest that packinghouses have a higher prevalence of *L. monocytogenes* compared to processing facilities (8.7% versus 1.2%).

Significance: Overall, our data indicate that prevalence of *L. monocytogenes* in specialty crop facilities shows a range that is similar to what has previously been reported for other facilities, with packinghouses possibly showing a higher risk of environmental *L. monocytogenes* contamination. While our data support the challenge of controlling *L. monocytogenes* in specialty crop facilities, they also indicate that at least some facilities seem to be able to effectively control this pathogen.

T3-09 Harborage of *Listeria* spp. in Tomato Packinghouse Processing Equipment

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Introduction: Increasing focus has been placed on *Listeria monocytogenes* within the packinghouse given previous outbreaks associated with contamination during packing and new regulatory requirements. However, many produce packinghouses do not have environmental monitoring programs to monitor for *Listeria* species harborage within the packing environment.

Purpose: The purpose of this study was to identify potential harborage points of *Listeria* spp. on packingline equipment in tomato packinghouses.

Methods: As is common practice, *Listeria* spp. were used as an indicator species for *L. monocytogenes*. Three tomato packinghouses of varying sizes were sampled (n=565) after completing their sanitation program in four sampling periods during the packing season for the presence of *Listeria* spp. in zone 1 (food contact surfaces). A designated area (100 cm^2) was swabbed and the sample stored in Dey Engley neutralizing buffer. Bacteria were eluted in an additional 10 ml of buffered peptone water, and spiral plated on Modified Oxford Medium (MOX) and incubated for 48 h at 35 ˚C. In parallel, 1 ml was enriched in Buffered Listeria Enrichment Broth with supplements and streaked on MOX for basic detection. Presumptive positive cultures were confirmed with PCR via the *iap* gene and 16s rDNA.

Results: Overall, 62 of 565 (10.97%) samples were positive for *Listeria* spp. after PCR confirmation. Common sites for recovery included dump tanks (5/62; 8.06%), polyvinylchloride (PVC) rollers (21/62; 33.87%), and surfaces where two materials were joined together (15/62; 24.19%. Overall production throughput was not associated with the likelihood of *Listeria* spp. recovery ($p>0.05$).

Significance: Likelihood of *Listeria* spp. harborage was more closely associated with site-specific sanitation characteristics than throughput alone. Further work should focus on improving hygienic design of surfaces commonly found to harbor *Listeria* spp. and determining what cleaning and sanitation approaches are more successful at limiting *Listeria* harborage.

T3-10 Impact of Fusarium Fruit Rot Caused by *Fusarium fujikuroi* and *Fusarium oxysporum* on *Salmonella enterica* Newport Colonization and Growth on Melon

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Introduction: Presence of plant pathogens, such as soft rot or foliar-spot bacteria or fungi, have been reported to promote the growth of *Salmonella enterica* on plants.

Purpose: To investigate the impact of post-harvest *Fusarium* infection on the colonization of *S.* Newport on melon fruit.

Methods: Melon rind discs (surface area=158.5 cm^2) of the cultivars 'Arava'-Galia, 'Athena'-Cantaloupe, 'Dulce'-Honeydew, 'Jaune'-Canary and 'Sivan'-Charentais were inoculated separately with *F. fujikuroi* and two *F. oxysporum* isolates; A and B (100 µl of 6 log spore ml^{-1}), or water, and incubated at 25ºC for four days. The treatments were 1) no *Fusarium* infection (control), and *Fusarium* infection with 2) *F. oxysporum*-A, 3) *F. oxysporum*-B, or 4) *F.*

fujikuroi. After four days, *Fusarium* infection was followed by inoculation with 100 µl (~10^4 CFU ml^{-1}) *S.* Newport, adapted for rifampicin resistance, and incubated at 25°C for 24 h. The treatments were replicated six times (*n*=6 rinds/treatment). *Salmonella* Newport was recovered in 0.1% Buffered Peptone Water, plated onto Tryptic Soy Agar with rifampicin and cycloheximide (50 µg ml^{-1} each). Counts in Log CFU ml^{-1} were analyzed using a mixed model (JMP) and the means were separated using Tukey-Kramer HSD.

Results: *Salmonella* Newport was not recovered from water only and *Fusarium* only treatments. *F. fujikuroi*-infected melons supported lower levels of *S.* Newport (*P*<0.03; In Log CFU ml^{-1}, No Fusarium=4.74, *F. oxysporum*-A=4.64, *F. oxysporum*-B=4.55, *F. fujikuroi*=3.60). Melon cultivar was also a factor (*P*<0.001); smooth melon cultivars ('Dulce'=3.03 and 'Jaune'=3.34 Log CFU ml^{-1}) had significantly lower populations (*P*<0.05) when compared to netted melon cultivars ('Arava'=5.54, 'Athena'=5.27 and 'Sivan'=4.73 Log CFU ml^{-1}).

Significance: *F. oxysporum* infection had no effect on *S.* Newport populations, while *F. fujikuroi* infection restricted *S.* Newport populations on melon fruit. Rind surface (netted versus smooth) impacted of *S.* Newport on melons, with netted melons supporting higher bacterial populations.

T3-11 Use of Probiotics for Inhibition and Elimination of *Listeria monocytogenes* on Fresh and Caramel Apples

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Introduction: Apple and caramel apple processors have been challenged by reports linking listeriosis in consumers to the products they pack and produce. A control step for this problem is to use refrigeration in transport and retail. Refrigeration, which slows the growth but does not eliminate the presence of any pathogen, may not be entirely effective or practical. The produce industry needs an effective control measure for *Listeria monocytogenes* in apples.

Purpose: Inhibition and elimination of *L. monocytogene*s on fresh and caramel apples.

Methods: Experiment 1 evaluated the retention of the probiotic strains on fresh and caramel apples by using a dip and storing at 73°F for up to 6 days. The CFU/g apple of each strain was determined. Experiment 2 evaluated the effect of the probiotic dip on multiple strains of *L. monocytogenes* inoculated (ca. 10 CFU/g apple) onto fresh and caramel apples. Entire apples were selectively enriched for *L. monocytogenes* and tested for presence and absence over 6 days at 73°F. Three replications were performed for all experiments.

Results: Retention of probiotics on fresh and caramel apples remained high. Total numbers declined only by 2.7 log cycles between days 0 and 6 for fresh apples and less than 0.5 log cycles for caramel apples. The probiotic dip was effective in eliminating the presence of *L. monocytogenes* during storage of both fresh and caramel apples over a 6-day period at 73°F.

Significance: This approach, unlike refrigeration, can control *Listeria* during transportation, retail storage, and placement, in addition to uncontrolled storage by the consumer. This study validates the use of carefully selected, competitive probiotic organisms as a successful intervention for *L. monocytogenes* in fresh and caramel apples regardless of refrigerated storage.

T3-12 Protective Cultures and Caramel Apples: A Food Safety Mindset to Mitigate *Listeria monocytogenes*

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Introduction: One of the largest *Listeria* outbreaks in the United States was caused by contaminated apples in 2015. Seven deaths and 35 illnesses were reported. The need to reduce and eliminate future hazards from contaminated apples is a priority for many producers of caramel apples.

Purpose: Challenge tests were performed to evaluate the added value of a culture cocktail of four lactic acid bacteria (LAB) on the behavior of *Listeria monocytogenes* during the shelf life (19 days) of caramel apples when stored at room temperature.

Methods: Four *L. monocytogenes* strains associated with with the 2014 to 2015 caramel apple outbreak were applied specifically on the stem and the calyx. The LAB (or water for the control batch) was also sprayed on those two specific areas. A wooden stick was inserted in each apple which was then dipped into caramel (205°F) for 6 s. Three apples of each batch were used to enumerate *L. monocytogenes* and LAB at each of five time points: day 0 prior to and after caramel dip, and days 3, 7, and 19.

Results: The dipping in hot caramel allowed a reduction of 0.7 log for *L. monocytogenes* and 0.5 log for the LAB. During the caramel apple shelf life, the decrease in LAB concentration was moderate (0.9 log) and its use allowed an additional 2.1-log reduction of *L. monocytogenes*. The total *Listeria* reduction in the batch with LAB thus reached 2.8 log. In the control batch, a 4.4-log *Listeria* increase was measured between days 0 and 19.

Significance: These results demonstrate that properly selected protective cultures were efficient in inhibiting growth and reducing *L. monocytogenes* concentration on caramel apple. Protective cultures improve thus microbial safety of this sensitive product.

T4-01 Metagenomic Investigations of Antimicrobial-resistance in Beef, Pork, and Broiler Meat

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Introduction: Use of antimicrobials in livestock production is a public health issue due to concerns that administration of antimicrobials to food animals could increase antimicrobial resistance (AMR) in meat.

Purpose: The objective of this study was to use targeted shotgun metagenomics to characterize the resistome in beef, pork, and broiler meat rinsates.

Methods: Forty (*n*=40) meat samples were collected (16 pork, 16 broiler, and eight beef). Metagenomic DNA was isolated from meat rinsates, and a bait-pulldown system to enrich AMR genes was used to build DNA libraries, which were sequenced using an Illumina platform. Raw sequences were analyzed using AMR++ pipeline and MEGARes database. Ten classes of antibiotic resistance were identified across all samples; five were present at an average of more than 1% across production systems. Antimicrobial resistance genes that require confirmation of single nucleotide polymorphisms (SNPs) to confer resistance were excluded from downstream analysis. Proportions of these five classes of resistance were compared using the PROC GLIMMIX and PROC MULTTEST procedures of SAS software.

Results: The highest proportion of resistance reads in the broiler meat rinsate was attributed to multi-drug resistance at 49.9% (confidence interval [CI] 46.9 to 52.9%), which was a greater prevalence (*P*<0.05) for that class of genes than for beef and pork. The beef rinsate had the highest proportion

(*P*<0.05) of beta-lactam resistance, comprising 64.2% (CI 46.1 to 82.0%) of reads when compared to other species. The highest proportion of resistance genes in the pork rinsate was attributed to tetracycline resistance, where 56.8% of reads conferred tetracycline resistance (CI 44.4 to 69.2%), which was greater (*P*<0.05) than both other species.

Significance: These data provide an introductory view on the characterization and differences in resistome composition between meat products, which could inform public health research.

T4-02 Whole Genome Sequence Analysis and Antimicrobial-resistance Profiles of *Listeria monocytogenes* Isolated from Ready-to-Eat Meat Products in South Africa

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Introduction: Listeria monocytogenes is intracellular bacteria that is found in diverse environments and causes a disease known as listeriosis. Listeriosis is associated with high fatality rates of up to approximately 30% in humans. Contaminated foods that are considered "ready-to-eat" pose a high risk of listeriosis, and disease management may be complicated due to antimicrobial-resistant strains of *L. monocytogenes* that harbour virulence genes.

Purpose: The objective of this study was to sequence and analyse the whole genomes of six isolates of *L. monocytogenes* that were isolated from ready-to-eat meat products such as polony and biltong.

Methods: L. monocytogenes isolates were characterized using microbiological techniques and real-time PCR. Antimicrobial resistance profiles of the isolates were determined by testing 13 antimicrobial impregnated discs using the Kirby-Bauer disc diffusion method. The whole genome sequences of six isolates of *L. monocytogene*s were assembled with a view of identifying virulence and resistance genes, prophage sequences, SNPs, and markers for serotypes.

Results: The whole genome sequences of six isolates of *L. monocytogene*s were assembled with a view of identifying virulence and resistance genes, prophage sequences, SNPs, and markers for serotypes. Here we report the results of whole genome sequence analysis, biochemical tests and antimicrobial resistance profiles of the six isolates of *L. monocytogenes* from biltong and polony. The highest resistance was observed against penicillin and nalidixic acid for all six isolates included in the analysis. Further, 83.33% of the isolates (*n*=5) were found to be resistant to erythromycin, clindamycin, and enrofloxacin, followed by chloramphenicol and ciprofloxacin with 50% each (*n*=3) and 16.67% (*n*=1) against amoxicillin-clavulanic acid

Significance: This data contributes to a new knowledge that enhances the understanding and management of disease due to *L. monocytogene*s.

T4-03 Phage-like Plasmids are a Novel Class of Temperate Bacteriophages That Encode Antibiotic-resistance Genes of Clinical Importance

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Introduction: Phage-like plasmids (PLPs) are extrachromosomal temperate phage/plasmid hybrids that are inducible, encode antibiotic-resistance and heavy metal-resistance genes, and have been isolated from agricultural, food production, and clinical sources. Twenty-one PLPs have previously been reported in the literature and can be classified into two lineages, SSU5-like and P1-like.

Purpose: The objective of this study was to genomically characterize PLPs that are present within whole genome sequences (WGS) of bacteria in the National Institutes of Health GenBank genetic database to determine their lineage and scope within foodborne pathogens.

Methods: A custom script was used to blast signature phage-like plasmid genes against 9,118 assembled bacterial WGS in GenBank. PHASTER was used to confirm the presence of PLPs in the WGS. ResFinder and CARD were used to detect the presence of antibiotic-resistance genes within the PLPs, and PlasmidFinder was used for plasmid typing.

Results: Thirty-six PLPs were detected in the bacterial WGS of GenBank within six genera of the *Enterobacteriaceae* family, and thirty PLPs were found in *Escherichia coli, Salmonella enterica, Cronobacter sakazakii*, and *Klebsiella pneumonaie,* an emerging foodborne pathogen. All 30 encoded structural and lysogeny phage genes had nucleotide sequence similarity to either *Salmonella* phage SSU5 or *Enterobacteria* phage P1, which is in accordance with the 21 reported PLPs in the literature. All of the PLPs also encode plasmid genes *parA*, *parB*, and *repA* for plasmid maintenance and replication, and none were typeable by PlasmidFinder. Six of the PLPs encoded antibiotic resistance genes such as extended-spectrum *β*-lactamases (CTX-M-15 and CTX-M-28), *β*-lactamases (TEM-1), aminoglycoside resistance (aad5 and aac(3)-lla) and efflux pumps *(acrF*, *msbA*, *mdtB*, *acrD*, *mdtF*, *acrB*).

Significance: PLPs represent a novel class of temperate phages and this study suggests that they should be classified into two lineages, SSU5-like and P1-like. Furthermore, PLPs could potentially spread antibiotic resistance determinants among foodborne bacteria along the farm-to-fork continuum.

T4-04 Resistance Markers and Algorithm to Predict Antibiotic Resistance in *Salmonella* spp. by Whole Genome Sequencing

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Introduction: With the advent of whole genome sequencing (WGS), it is now possible to determine a full complement of resistance determinants and therefore predict the phenotypic resistance of a pathogen from its genotype. However, a major drawback of the genotypic method is that the underlying genetic mechanism, appropriate resistance markers, and optimum prediction algorithms should be known and validated for different classes of antibiotics in different pathogens before it can be used reliably.

Purpose: The study explored the suitability of various resistance markers and optimum prediction algorithms towards 31 antibiotics from 13 different classes in *Salmonella* spp.

Methods: ResFinder 3.0 was used to determine the resistance determinants from WGS data. Two rounds of prediction were carried out: an initial round without adjustments and a second round with adjustments in resistance markers and prediction algorithm to more accurately predict the phenotype from its genotype.

Results: Overall concordance improved from 89.4% initially to 95.9% after adjustments. Low concordance was observed for the prediction of β-lactam + β-lactamase inhibitors (66.0%) and fluoroquinolones (75.0%) initially. After adjustments, the concordance of the former improved to 91.7%, but this

study does not recommend WGS to predict resistance to fluoroquinolones due to a lack of reliable genetic resistance markers with acceptable correlation to phenotypic resistance.

Significance: This study highlights the need to validate the suitability of various resistance markers for various classes of antibiotics and to standardize and optimize the prediction algorithm before WGS can be reliably used to predict antibiotic resistance. According to these results, WGS should not yet replace the phenotypic testing methods but should only be used to complement the existing testing protocols until further validation studies are carried out.

T4-05 Metagenomic Profiling of Antibiotic Resistance Genes Associated with Lettuce Leaf Surfaces Grown in Soils Receiving Cattle Manure-based Amendments

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Introduction: While much attention has been directed towards preventing the transmission of pathogens via the food chain, little is known about the potential to spread antibiotic resistance. In particular, there is a need to understand the potential for manure-derived soil amendments, particularly those from antibiotic-treated livestock, to influence the "resistome" (i.e., carriage of antibiotic resistance genes (ARGs) on fresh produce.

Purpose: The objective of this research was to compare the resistomes of surfaces of lettuce leaves grown in different soil types and receiving dairy cattle manure-derived amendments: manure (with and without typical antibiotic administration) or corresponding composts, as compared to a chemical fertilizer only control.

Methods: Manure was collected from antibiotic-free or cephapirin- and pirlimycin-treated cattle, and static composting was conducted following FSMA guidelines for 42 days. Lettuce (n=3) were transplanted to either loamy sand or silty clay loam amended with manure, compost, or chemical fertilizer and cultivated to maturity in a greenhouse setting. DNA was extracted from lettuce leaf surfaces, subject to shotgun metagenomic sequencing, and compared against the Comprehensive Antibiotic Resistance Database to identify ARGs.

Results: Almost 700 putative ARGs from 23 antibiotic classes were identified from un-assembled reads. Overall, lettuce grown in compost-amended loamy sand contained greater relative abundances of total ARGs (3.3-fold) than lettuce from compost-amended silty clay loam, including macrolide-lincosamide-streptogramin (1.4-fold) ARGs ($P < 0.01$, Wilcoxon). The resistomes of lettuce grown in compost-amended silty clay loam were distinguishable from the lettuce grown in manure-amended silty clay loam (R>0.4, $P < 0.03$, ANOSIM).

Significance: Composting has established benefits for reducing pathogen loads and may provide additional benefit as a barrier to the carriage of ARGs on fresh produce, but interactive effects with certain soils can result in a different ARG profile on lettuce surfaces compared to raw manure soil amendments.

T4-06 Core and Accessory Genome-wide Association Studies to Investigate Genetic Determinants Involved in *Listeria monocytogenes* Cold Adaptation

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❖ ***Developing Scientist Competitor***

Introduction: Nowadays, whole genome sequencing (WGS) offers new opportunities to explain intraspecific variability of food pathogens. Various bioinformatic tools already exist for the treatment of bacteria WGS data. Some of these analyses have the aim to compare DNA sequences from strains with the phenotypes of interest (stress survival, growth, and/or virulence) in order to find markers (e.g., absence/presence of plasmids, genes, or variants) which are significantly associated with bacterial behaviors (genome wide association studies [GWAS]).

Purpose: The object of this study is to present and explore some existing bioinformatic methods to correlate phenotype(s) and genotype(s).

Methods: For this purpose, a data-set of 51 strains of *Listeria monocytogenes* was used. For each strain, a qualitative phenotype at low temperature (2°C) was established and whole genomes of all strains were sequenced. First, a GWAS based on the accessory-genome (gene level) was performed. In a second approach, an analysis on the single nucleotide polymorphisms (SNP) level was made.

Results: The outcome of the GWAS at gene level was a list of 114 genes with a statistical association with the tested phenotype. These genes included already involved genes in the cold adaption mechanism of *L. monocytogenes* and genes associated to mobile genetic elements. Concerning SNP-GWAS, a group of 184 highly associated SNPs were highlighted, including SNPs corresponding to positions in genes which were already likely involved in cold adaption, hypothetical proteins, and also SNPs in intergenic regions where, for example, promotors and regulators can be located.

Significance: Such methodologies will probably be systematically applied to identify genetic markers associated to a phenotype of interest in order to predict the behavior of foodborne pathogens along the food chain.

T4-07 Characterization of *Listeria monocytogenes* Isolates from Poultry Processing Plants

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Introduction: Genomic analysis of isolates from food processing facilities can yield insights into characteristics of *Listeria monocytogenes* such as phylogeny and evolution, virulence, and antibiotic-, stress-, and sanitizer-resistance. These insights may lead to better ways to target and inactivate *L. monocytogenes* in processing environments.

Purpose: The goal was to use whole genome sequencing (WGS) and subsequent genomic analysis to characterize *L. monocytogenes* isolates recovered from poultry processing plants.

Methods: Isolates were collected in two previous longitudinal studies of *L. monocytogenes* in poultry processing plants. In the present study, 156 genomes were extracted, sequenced on the Illumina MiSeq platform, *de novo* assembled, and annotated. Isolates were divided into lineages, MLST-typed, and screened for acquired antibiotic-, stress-, metal-, and sanitizer-resistance genes using Abricate.

Results: Fifty-six isolates belong to lineage I and 99 belong to lineage II. Eighteen unique MLST sequence types (ST) were found, the majority of which were identified as ST321 (*n*=41), ST5 (*n*=31), ST155 (*n*=27), and ST6 (*n*=20). All of the genomes contained *fosX*, *lmo0441*, *lmo0919*, *norB*, and *sul* antibiotic-resistance (ABR) genes; 14.1% (*n*=22) contained *aacA4* genes and 3.2% (*n*=5) contained *tetM* ABR genes. Of the genomes, 82.7% (*n*=129) had five total ABR genes identified, 16% (*n*=25) had six total, and 1.3% (*n*=2) had seven total. Of isolates screened, 82.7% (*n*=129) possessed the following genes related to stress-resistance: *lmo0444*, *lmo0445*, *lmo0446*, *lmo0447*, and *lmo0448*, all contained in a stress survival islet (SSI-1). The *bcrA*, *bcrB*, and *bcrC* genes related to sanitizer- or metal-resistance were in 73.1% (*n*=114) of the isolates.

Significance: The data provide insight into the characteristics of *L. monocytogenes* found in poultry processing plants. Additional analysis is needed to identify other genes and/or genetic factors that may be involved or interact with regulation of genes that influence ability of some strains to persist in food processing environments.

T4-08 Recto-Anal Junction Microbiota Composition in *Escherichia coli* O157:H7-Shedding Cattle

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Introduction: Cattle are the asymptomatic reservoirs of *Escherichia coli* O157:H7 (O157) that tend to preferentially colonize the bovine recto-anal junction (RAJ). Therefore, understanding the taxonomic profile, microbial diversity, and interactions between microbiota and O157 at the RAJ could give insights into O157 colonization in cattle and its persistence at this site.

Purpose: In this pilot study, we sought to determine if O157 colonization was associated with microbiota changes at the RAJ of cattle experimentally challenged with O157 in comparison to non-challenged cattle.

Methods: Four Jersey steers were orally challenged with 10^{10} CFU of a streptomycin-resistant O157 strain 86-24, and four steers were mock-challenged. RAJ mucosal swab (RAMS) samples were collected from all steers at 20 different time points and plated on sorbitol-MacConkey agar containing streptomycin and potassium tellurite, directly or after enrichment, to detect O157 colonization. DNA was also extracted from RAMS (*n*=160) and used for sequencing the V4 region of microbial 16S rRNA gene on Miseq platform (Illumina) to determine taxonomic profiles and assess microbial diversity using the CLC microbial genomics module (Qiagen).

Results: The Chao1 species richness (but not the Shannon Index) increased in samples collected after O157 challenge. The *Firmicutes* to *Bacteroidetes* (F:B) ratio and relative abundance of *Proteobacteria* were not associated with O157 colonization. Analysis of taxonomic assignments indicated significantly higher representation of the families *Fusobacteriaceae* and *Paenibacillaceae* in O157 shedding cattle. We also observed increased representation of *Solibacillus* and *Eubacterium* genera in non-shedding cattle. There was no difference in the beta-diversity between control and test samples before O157 challenge, but the microbial diversity was significantly different between the two groups after O157 challenge.

Significance: This study explores changes in microbiota composition at the bovine RAJ after O157 colonization, and understanding these changes may help develop strategies to reduce O157 colonization in cattle.

T4-09 Comparison of Automated and Manual Next Generation Sequencing Library Preparations for Analysis of *Salmonella* and *Escherichia coli*

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Introduction: The use of whole genome sequencing (WGS) to subtype foodborne pathogens is important to prevent outbreaks of foodborne disease and to identify sources of contamination in food manufacturing equipment and facilities. Although next generation sequencing (NGS) provides powerful information to identify and differentiate pathogens, considerable effort is still required to isolate DNA and prepare libraries necessary for NGS.

Purpose: The study objectives were to evaluate a fully automated system capable of isolating DNA and preparing NGS libraries and to compare the quality of this automated library preparation against manual preparation.

Methods: Six *Salmonella* and six *Escherichia coli* strains were used in this study. For the automated workflow, the Rheonix Encompass workstation was used to isolate DNA and prepare sequence-ready NGS libraries from the 12 strains. For the manual workflow, DNA was extracted using a commercial kit and NGS libraries were manually prepared using Nextera XT kits. Sequencing was performed on the Illumina MiSeq on two separate runs. Sequencing quality and WGS results were compared for all 12 organisms.

Results: Comparison of quality metrics provided by the MiSeq revealed that the automatically prepared libraries yielded higher pass filter scores (93 versus 82%), higher Q30 scores (90 to 97 versus 83 to 94%), better alignments, and lower error rates for the control than the manually prepared libraries. In addition, DNA isolation and library preparation using the automated process reduced the hands-on effort by three hours compared to the manual workflow.

Significance: The ability to reduce hands-on time and improve quality metrics using automated DNA isolation and NGS library preparation will help reduce costs, improve turn-around time, and provide consistently reliable data. Automation of the entire process on a single instrument will also reduce capital equipment costs and reduce required laboratory space compared to manual processes that use multiple pieces of equipment and require separation of work activities.

T4-10 Re-classification of *Bacillus cereus G*roup Dairy Isolates and Characterization of Their Pathogenic Potential

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Introduction: The *Bacillus cereus* group is a complex of nine closely related species that includes foodborne pathogens, food spoilage microorganisms, organic biocontrol agents, and anthrax-causing microorganisms. Phylogenetic analyses classify these species into seven phylogenetic groups.

Purpose: Recently, nine additional *B. cereus* group species have been validated. The purpose of this study was to reclassify 62 *B. cereus* group isolates, including type strain, and re-evaluate the distribution of virulence genes and cytotoxicity among phylogenetic groups.

Methods: We have defined nine new phylogenetic groups corresponding to new species based on *panC* gene and WGS sequences. This resulted in a total of 16 groups. BTyper was used for phylogenetic group classification and identification of virulence genes in 62 isolate genomes. The HeLa cell model was used to evaluate cytotoxic potential of 52 available dairy isolates and type strains.

Results: Classification of isolates from clades I, IV through VI, and VII remained consistent after introducing new clades. Two of the five isolates previously classified into clade II (*B. wiedmannii*) were placed into the *B. mobilis* clade (*n*=3) under the new classification system. Seven of 10 isolates that had been previously classified as clade III (*B. anthracis*/*B. cereus* group) were assigned to either the *B. tropicus* (*n*=3), *B. albus* (*n*=2), *B. pacificus* (*n*=4), or *B. paranthracis* (*n*=2) clades using the updated classification system. Genes encoding enterotoxin hemolysin BL (*hblACD*) were detected in 10 of 16 clades. Of the 12 clades for which phenotypic cytotoxicity has been evaluated, three exhibited no cytotoxic phenotype (*B. anthracis*/*B. cereus* group, *B. weihenstephanensis*/*B. mycoides*, and *B. tropicus* clades), while isolates in the *B. pseudomycoides* clade exhibited the highest degree of cytotoxicity.

Significance: Inclusion of new phylogenetic groups in the *B. cereus* group increased accuracy of phylogeny-based taxonomic classification and prediction of pathogenic potential.

Technical

T4-11 Metabolic Profiling and Transcriptomic Response: Synergistic Action of Electrolyzed Water and Mild Heat on Inactivating *Escherichia coli* O157:H7

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Introduction: Metabolite levels and gene expression in bacteria offer significant insights into the response of bacteria to disinfection intervention.
Purpose: The purpose of this study was to explore the possible mechanisms underlying the metabolic and transcriptomic changes of *Escherichia coli* O157:H7 in the response to electrolyzed water (EW) and mild heat treatments.
Methods: The efficacy of EW and heat treatment in inactivating *E. coli* O157:H7 was evaluated by direct plating on media. The oxidant-sensitive probe 2', 7-dichlorodihydrofluorescein diacetate (H_2DCFDA) was used to assess the intracellular reactive oxidative species. The metabolomic changes were investigated by UPLC-QToF-MS coupled with multivariate analysis.
Results: EW (4 mg/L free available chlorine) combined with heat treatment at 50°C resulted in 2.31-log CFU/mL reductions of *E. coli* O157:H7. The fluorescence intensity of EW at 50°C showed the greatest and was 10 times higher than the control group. The decreased ribose-5-phosphate level strongly indicated affected nucleotide biosynthesis, which was consistent with nucleotide level (e.g., uridine, guanosine) in the *E. coli* O157:H7 cells in response to EW and heat treatments. A 50-fold increase in heat shock regulatory gene *rpoH* expression was found in the 50°C group and combined treatment group compared to the control group, while for the EW group the gene expression remained unchanged. However, when treated with EW at 50°C, almost all the gene expressions (except *rpoH*) were dampened, with about 0.024- and 0.286-fold decrease for *udk* (encoding uridine kinase) and *gadA* (encoding glutamate decarboxylase alpha), respectively, as compared to the control group.
Significance: The results demonstrate a synergistic effect of EW and heat treatment on inactivating *E. coli* O157:H7. They also confirm and extend existing mechanisms of inactivation from gene expression and metabolite level.

T4-12 Detecting Genomic Contamination with Kalamari

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Introduction: Many public health laboratories use metagenomic profilers such as Kraken to detect contamination (i.e., conflicting taxonomic results) in individual bacterial genomes as part of foodborne disease surveillance activities. The standard Kraken database is based on RefSeq, which may have contaminated, misidentified, or atypical genomes. Additionally, the underlying taxonomy, taken from the National Center for Biotechnology Information taxonomy website, may be conflicting or incomplete.
Purpose: To address these issues, we created Kalamari, a database for quantifying potential contamination of foodborne bacterial genomes for use with the Kraken software. Kalamari contains a custom taxonomy and genome assemblies curated by subject-matter experts.
Methods: Several genomes in the following genera were tested against the Kalamari and standard databases for purity using the Kraken software: *Listeria* (*n*=28), *Salmonella* (*n*=72), *Escherichia* (*n*=27), and *Campylobacter* (*n*=23). Sensitivity was measured by quantifying reads matching the target taxon, and uncertainty was measured by quantifying unclassified reads.
Results: We quantified the number of reads assigned to the correct genus and compared the results from the standard and Kalamari databases using the Student's *t* test. The values for the standard and the Kalamari databases were as follows, respectively: *Listeria*, 88 versus 95% (*P*<7.49e-2); *Salmonella*, 90 versus 90% (*P*<4.98e-1); *Escherichia*, 56 versus 91% (*P*<7.85e-10); and *Campylobacter*, 73 versus 95% (*P*<3.57e-3). Additionally, we were able to decrease the number of unclassified reads as follows: *Listeria*, 11 versus 4% (*P*<8.90e-2); *Salmonella*, 5 versus 5% (*P*<7.93e-1); *Escherichia*, 5 versus 4% (*P*<5.6e-2); and *Campylobacter* 26 versus 5% (*P*<4.00e-2).
Significance: Most of our results showed more sensitivity and less uncertainty with the Kalamari database than the standard database. Therefore, Kalamari may allow for improved screening of foodborne bacterial genomic sequences for potential contamination.

T5-01 Effect of a Peracetic Acid-based Sanitizer on *Salmonella* Cocktail and Its Potential Surrogate, *Enterococcus faecium* NRRL B-2354, Inoculated on Chia Seeds, without Germination Loss or Mucilage Creation

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Introduction: Raw chia seed consumption is increasingly associated with *Salmonella* contamination. However, intervention technologies for chia seeds that maintain seeds in a raw state, without causing clumping due to mucilage production upon moisture exposure, are limited. Therefore, intervention methods for chia seed that meet these two criteria and inactivate *Salmonella* are needed.
Purpose: The suitability of a commercial sanitizing agent based in ethanol and peroxyacetic acid as an intervention method for chia seed was evaluated.
Methods: Samples (100 g each) of chia seed (*n*=5) were inoculated with either a cocktail of *Salmonella* Newport, Senftenberg, Oranienburg, Saintpaul, Typhimurium DT104, and Cubana or *E. faecium* NRRL B-2354. After overnight acclimatization, samples were treated with 4 mL of sanitizer, then held at ambient temperature for 1 h before triplicate plating of serial dilutions on tryptic soy agar. A separate 1-kg treated batch was evaluated for ability to germinate compared to untreated control (*n*=4) using methods approved by the Association of Official Seed Analysts. Following the post-treatment holding time, this batch was dried back to its original moisture content at 70°C to evaporate any residual sanitizer, thereby stopping treatment.
Results: An average of 0.76±0.13 log CFU/g *Salmonella* was recovered from treated seeds, compared to 4.94±0.32 log CFU/g from untreated controls (*P*<0.05; LOD 5 CFU/g). An average of 1.18±0.54 log CFU/g *E. faecium* was recovered from treated seeds, compared to 5.05 ± 0.11 log CFU/g from untreated controls (*P*<0.05; LOD 5 CFU/g). The log reductions for *Salmonella* and *E. faecium* were comparable (4.18 and 3.87 log CFU/g, respectively), although *E. faecium* showed higher resistance. The percent germination for both treated and untreated seed was 95%.
Significance: The sanitizer is an effective intervention method for chia seed, reducing *Salmonella* and *E. faecium* by >3 log without significantly impacting germination (*P*≥0.05). Furthermore, *E. faecium* NRRL B-2354 is an appropriate *Salmonella* surrogate for treatment of chia seeds with this sanitizer.

T5-02 Impact of Four Carriers and Storage Temperature on the Stability of Five-strain Cocktail of *Salmonella*: A Contribution for Challenge Tests of Low-water Activity Foods

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Introduction: The burdens of foodborne disease outbreaks involving low-water activity products (LWA) have demanded the development of protocols for assessment of the fate of bacterial pathogens in LWA. Despite this, limited information exists regarding the most suitable carrier agents and storage conditions of dry inoculum used in challenge tests.
Purpose: The objective of this study was to evaluate the performance of four carriers and two storage conditions on the maintenance of stability of *Salmonella* for up to 180 days of storage.
Methods: The dry inoculum was prepared in four different carriers (talc, calcium carbonate, and sands of two granulometry 0.05 to 0.2 mm [thin] and 0.2 to 0.6 mm [regular]), which were further stored in two temperatures (4 and 25°C) for up to 180 days. Five serotypes of *Salmonella enterica* were used for the preparation of the inoculum (Senftenberg 775W, Havana IOC 2310, Infantis IOC 2327, Mbandaka IOC 2317, and Typhimurium IOC 2328).
Results: Among the conditions studied, storage at 4°C resulted in a better protection of *Salmonella*. The sands (thin and regular) were the best carriers for *Salmonella*, once the time required to reduce 1 log of this bacterium was 118 and 123 days, respectively. When calcium carbonate and talc were used as carriers, the time required to reduce 1 log was shorter (18 and 6 days, respectively). Even though there was a aw variation of the carriers during storage, this variation did not influence the stability of the inoculum.
Significance: It was found that sand can be used in the dry inoculum preparation of *Salmonella* and that the survival of this bacterium was independent of the type of sand employed. Once prepared, the dried inoculum can be stored for up to 120 days at 4°C, which allows the use of the same inoculum for several challenge tests.

T5-03 Comparison of Five Methods for Inoculating Macadamia Nuts with *Enterococcus faecium* NRRL B-2354 for Industrial-scale Validation of Peracetic Acid-based Sanitizer Efficacy on *Salmonella*

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Introduction: Pathogen control interventions and validation methods are needed in the nut industry, because foodborne outbreaks and recalls in low-moisture foods, including macadamia nuts, have increased.
Purpose: To compare five inoculation methods for efficacy validation of a peracetic acid-based sanitizer on macadamia nuts using *Enterococcus faecium* NRRL B-2354 as a *Salmonella* surrogate.
Methods: Macadamia nuts (n=2X3) were inoculated (2mL/100g) using five inoculation methods (M1 to M5). Three inoculum types, grown at 35°C, were used: i) overnight tryptic soy broth (TSB) (**M1, M3**), ii) overnight TSB pelleted by centrifugation, re-suspended in 0.1% peptone water (PW) (**M2, M4**) and iii) overnight lawn collected from TSA in 0.1% PW (**M5**). The inoculated samples were either acclimatized overnight in a biohood (**M1, M2**) or dried in a dryer (40°C, 55 min) (**M3, M4, M5**). Post-inoculation, the samples were treated with 40 mL/kg of the sanitizer and dried (160°F, 55 min). Plate counts were analyzed by one-way ANOVA (α = 0.05), followed by post-hoc test (Sheffe). Previously, *E. faecium* NRRL B-2354 demonstrated to be a suitable surrogate, comparable but more resistant than *Salmonella,* when the sanitizer's efficacy was tested in inoculated macadamia nuts (i.e. 60mL/kg of sanitizer dried at 190°F, reduced *E. faecium* and *Salmonella* by 3.2 and 4.50 log CFU/g, respectively).
Results: All inoculation methods resulted in ≥ 6.4 CFU/g of *E. faecium* in the nuts, with statistically significant difference ($P < 0.05$) between the samples inoculated and acclimatized overnight (M1, M2) and those dried after inoculation (M3, M4, M5). The sanitizer reduced *E. faecium* by ≥2.97 log CFU/g, and no statistically significant difference was found for the log reduction produced post-treatment between all methods, except between M2 and M5.
Significance: Methods M1, M3, M4, and M5 were suitable for *Salmonella* surrogate (*E. faecium* NRRL B-2354) validation of the peracetic acid-based sanitizer for macadamia nuts. Furthermore, the sanitizer was effective against the *Salmonella* surrogate.

T5-04 Impact of Glass Transition on Bacterial Cell Survival: Relationship between Glass Transition Temperature and Desiccation Tolerance in *Salmonella enterica*

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❖ ***Developing Scientist Competitor***

Introduction: Pathogenic bacteria such as *Salmonella enterica* and *Escherichia coli* exhibit high desiccation tolerance, enabling long-term survival in low water activity (a_w) environments. Despite a few reports on the effects of low a_w on bacterial survival, the mechanism by which bacterial cells acquire desiccation remains unclear. As one hypothesis, we focused on the glass transition phenomenon of bacterial cells.
Purpose: We assumed that the glass transition phenomenon of bacterial cells was attributed to the acquisition of desiccation tolerance. To clarify the relationship between the aw level and the glass transition temperature (T_g), we determined the T_g of pathogenic bacterial cells.
Methods: Dried *Salmonella enterica* Typhimurium and Chester cells were prepared at several different aw levels (0.43, 0.63, 0.75, and 0.87) for T_g determination using thermal rheological analysis (TRA). TRA determines changes in the thermophysical properties of substances by applying constant pressure and elevated temperature. We focused on the softening of dried bacterial cells by TRA to determine their T_g values.
Results: Softening of the dried bacterial cells was clearly observed in all tested conditions and was considered a phase transition from a glassy state to a rubber state. There was a clear negative correlation between aw and T_g values, suggesting that bacterial cells with higher T_g values had stronger desiccation tolerance.
Significance: Glass transition in low-aw conditions plays a key role in bacterial acquisition of desiccation tolerance.

T5-05 Radiofrequency Inactivation of *Salmonella* spp. and *Enterococcus faecium* NRRL B-2354 in Cumin Seeds

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Introduction: A large number of reported outbreaks of *Salmonella* in spices have been reported in the United States and around the world. Radiofrequency (RF) heating is a novel thermal processing technology which volumetrically heats food products resulting in a shorter come-up time. This feature can be used to develop a high-temperature short-time pasteurization method to assure food safety with minimal deterioration in food quality.

Purpose: In this study, cumin seeds were inoculated with a cocktail of five strains of *Salmonella. Enterococcus faecium* NRRL B-2354 was evaluated as a potential surrogate for RF processing in cumin seeds.

Methods: A small 20-g bag of cumin seeds was inoculated with either bacteria and placed in the cold spot of 450 g of cumin seeds packed in a rectangular tray sealed by Press'n Seal film with a venting nut at the center. The whole tray was subjected to RF heating in a 6-kW, 27-MHz RF system for 70, 80, and 90 s. The cold spot was determined by temperature profiles from six fiber optic sensors at different locations in the sample during the same RF heating process.

Results: The results showed that there were reductions of 3.54±0.49, 5.77±0.06, and 6.07±0 log for 70, 80, and 90 s of RF heating, respectively. The corresponding reductions for *E. faecium* were 1.86±0.24, 3.38±0, and 6.23±0.17 log, respectively. After 90 s of RF heating, *Salmonella* cocktail population was under detection limit (nothing left on 10^{-1} petri dish), while the population of *E. faecium* was above detection limit.

Significance: This study concluded that *E. faecium* is a good surrogate for RF processing in cumin seeds. RF heating is a promising pasteurization technology for spices.

T5-06 Effects of Elevated Hydrostatic Pressure for Decontamination of Raw Milk from *Listeria monocytogenes* and Background Microflora

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Introduction: Recent epidemiological studies from the Centers for Disease Control and Prevention indicate that approximately 99% of illnesses caused by *Listeria monocytogenes* are foodborne in nature, leading to hospitalizations in 94% of cases and the deaths of 266 American adults every year.

Purpose: Current study evaluates effects of hydrostatic pressure at controlled temperatures for decontamination of raw milk.

Methods: Various time (0 to 12 min) of elevated hydrostatic pressure (310 and 380 MPa; e.g., 45 and 55K PSI) were investigated for inactivation of a four-strain mixture of *L. monocytogenes* (ATCC numbers 13932, 51779, 51772, BAA-2658) inoculated at target level of 6.0 log CFU/ml of raw milk. Temperature was monitored and maintained at 4, 25, and 50°C by a circulating water bath and a stainless steel water jacket. The experiments were conducted in two biologically independent repetitions as blocking factors of a randomized complete block design, containing three repetitions per time/treatment/pressure combination within each block. Study was statistically analyzed by analysis of variance using OpenEpi software. Inactivation indices were calculated using GInaFiT software.

Results: At 380 MPa and treatments of 0 to 12 min, *D*-values of 3.47, 3.15, and 2.94 were observed for inactivation of *L. monocytogenes* at 4, 25, and 50°C, respectiely. Reductions of up to 3.73 and >4.26 log CFU/mL (P<0.05) of *L. monocytogenes* at the planktonic stage were achieved using application of pressure at 380 MPa for 3 and 12 min, respectively. Similarly, background microflora counts were reduced (P<0.05) by 1.3 and >2.4 log CFU/mL after treatment at 380 MPa for 3 and 12 min, respectively.

Significance: Treatments below three minutes were less efficacious (P ≥0.05) against the pathogen and background microflora in vast majority of time and pressure combinations. Results of this study could be incorporated as part of a risk-based food safety management systems and risk assessment analyses for mitigation of the public health burden of listeriosis.

T5-07 High Pressure Superdormant Spore Characterization for Non-thermal Food Sterilization

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❖ ***Developing Scientist Competitor***

Introduction: High pressure (HP) processing is an emerging non-thermal technology to produce minimally processed, high-quality food products. However, most HP processed foods are not sterile due to a subpopulation of bacterial spores that are ungerminated and thus survive HP processing. This subpopulation is termed high pressure superdormant (HPSD) spores and have become the key target for further development of HP sterilization methods.

Purpose: To isolate HPSD spores and investigate the cause of their superdormancy, as well as possible germination solutions.

Methods: Bacillus subtilis wild type PS832 and a mutant FB115 that lacks genes for encoding three germinant receptors and one of cortex lytic enzymes were used in this research. Spores were treated at 150 or 600 MPa at 40°C and the subpopulation of spores that remained dormant after the treatment was isolated using fluorescence-activated cell sorting with flow cytometry. The germination capacity of HPSD spores under second HP treatment at various pressure/temperature combinations and in nutrients were investigated.

Results: HPSD spore yields varied between different HP processing parameters, indicating the influence of pressure and temperature levels on spore superdormancy. Isolated HPSD spores after treatment under 150 MPa showed lowered germination capacities after a second HP treatment. However, this was not the case for HPSD spores isolated at 600 MPa. This indicates that HP induces spore germination through different mechanisms at different pressure levels. The isolated HPSD spores showed similar germination in nutrients compared to their initial population, at least for the wild type. This suggests that by using combination of germination triggers, e.g, HP treatment with nutrients, higher germination can be achieved, which could serve as a gentle food preservation method.

Significance: This research provided information for constructing a potential HP combined spore germination-inactivation approach, which could produce high-quality foods with longer shelf life, even under ambient distribution.

T5-08 The Inactivation and Recovery of *Escherichia coli* O157:H7 Following High Pressure Processing at Different Stages of Drying during the Production of Dry Fermented Sausages

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Introduction: Dry fermented sausages (DFS) rely on a combination of ingredients, fermentation, and drying for pathogen control. However, this might not always provide the mandated pathogen reduction. High pressure processing (HPP) has been successfully used to inactivate pathogens in RTE foods; however, different food ingredients and physicochemical properties protect pathogens against high-pressure inactivation.

Purpose: Examine the effects of HPP at different time points during the drying of DFS on the inactivation *Escherichia coli* O157:H7 and reactivation during storage.

Methods: Sausages containing a five-strain cocktail of *E. coli* O157:H7 at 10^7 CFU/g were manufactured and subjected to HPP at 600 MPa for 180 s weekly throughout the drying process and stored at 4°C for 4 weeks. Changes in inoculated *E. coli* O157:H7, starter culture populations, and physiochemical properties of the sausages were tested before and after HPP and weekly during the four-week storage.

Results: Sausages treated with HPP on day 1 of drying exhibited an additional 4.9-log reduction of *E. coli* O157:H7, while days 7, 14, and 21 of drying displayed only 2.4, 1.2, and 0.7-log reductions, respectively, compared to untreated sausages from the same day. A significant decline in *E. coli* O157:H7 inactivation from HPP was seen after a week of drying, when the aw drops below 0.9. There was no recovery of *E. coli* O157:H7 during the four weeks of storage. Starter culture populations were not affected by the high-pressure treatment.

Significance: HPP rapidly loses its microbial inactivation capabilities during the drying of DFS, so it is critical to apply pressure treatment before the aw of the sausages falls below 0.9. The study demonstrates the beneficial effect of early HPP treatment in DFS production and could assist processors in selecting appropriate processing conditions to enhance the safety of dry-fermented products.

T5-09 Optimization of the Radio Frequency Power, Time, and Cooling Water Temperature for Pasteurization of *Salmonella* Typhimurium in Shell Eggs

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Introduction: Radio frequency (RF) heating has been found to have antibacterial effects comparable to industrial hot water (HW) processing (56.7°C, 60 min) in shell egg pasteurization, but with less time required. RF heating applies energy along with cooling water to rotating shell eggs. So far, no systematic investigation has been conducted to understand the effect of RF processing parameters on bacterial inactivation and egg quality.

Purpose: This study aimed to investigate the effect of RF power, treatment time, and cooling water temperature on the inactivation of *Salmonella* Typhimurium in shell eggs and on egg internal quality.

Methods: Shell eggs inoculated with *Salmonella* Typhimurium ATCC 53647 at a concentration of 6 log CFU/ml were processed using RF at powers of 30 to 45 W, times of 2.5 to 8 min, and cooling water temperatures of 30 to 38°C. Afterwards, eggs were treated using HW for 15 min, followed by cooling in ice water for 5 min. Cell counts were enumerated and quality (Haugh unit, yolk index, and albumen turbidity) of control was analyzed in eggs treated with either RF/HW or 60 min HW alone. Mean values were compared using analysis of variance.

Results: Higher power, time, and cooling temperature significantly (P<0.05) enhanced microbial inactivation; however, extreme conditions caused damage to egg albumen and yolk. To achieve >5-log reduction of *Salmonella* Typhimurium without observable quality change, the longest treatment was 8 min at 30 W and 30°C (5.11 log CFU/ml), while the shortest was 4.5 min at 35 W and 38°C (5.02 log CFU/ml). The industrial HW process increased both Haugh unit and turbidity and decreased yolk index, while RF/HW treatment only slightly increased the turbidity. Among RF/HW treatments, higher power and temperature affected the turbidity more than the lower ones.

Significance: The results indicate that milder conditions are better for maintaining egg quality, which will help the egg industry determine RF processing parameters.

T5-10 Inactivation of *Salmonella enterica* on Low-moisture Foods by Cold Atmospheric Plasma

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Introduction: Cold atmospheric plasma offers a dry, non-thermal, and rapid process for surface decontamination of food products.

Purpose: The purpose of this study was to evaluate the efficacy of cold plasma to inactivate *Salmonella enterica* on in-shell pecans and black peppers.

Methods: In-shell pecans and black peppers (kernels) were surface-inoculated with a mixture of five strains of *Salmonella enterica* (10^7 CFU/ml), followed by air drying. The inoculated pecans and black peppers were treated by cold plasma for 2, 5 and 10 minutes at a distance of 1, 2, and 5 cm from the electrodes. Log reduction of the viable *Salmonella* cells after treatment was compared with these without cold plasma treatment using ANOVA.

Results: *Salmonella* populations decreased with increasing exposure time. On in-shell pecans, an average of 0.75-log CFU reduction of *Salmonella* was achieved by 2-minute cold plasma exposure at all distances tested. A distance effect was observed at 5 minutes of exposure with a 3.99-log CFU reduction of *Salmonella* at 1 cm, which was significantly different from those at 2 cm (1.88-log CFU reduction) and 5 cm (2.05-log CFU reduction). An average of 4.04-log CFU reduction of the pathogen was observed at all distances tested when exposure time increased to 10 minutes. Similarly, an average of 1.1 log CFU reduction was achieved on black peppers when treated for 2 minutes at all distances. Higher reduction (2.39 log CFU) was reached when exposed to cold plasma for 5 minutes at 1 cm. Population reduction increased to 3.63 log CFU at 10 minutes of exposure at all distances.

Significance: Results from this study show that cold plasma has potential as a viable technology for inactivating foodborne pathogens in produce and spices.

T5-11 Mitigation of Furan in UV Light-treated Apple Cider

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Introduction: Furan is a five-membered heterocyclic compound with four carbon atoms and one oxygen atom that been classified as a chemical that is possibly carcinogenic to humans by the International Agency for Research on Cancer. Our previous study proved that UV light treatment induced furan formation in apple cider, possibly through a free radical mechanism; therefore, effective control measures are needed.

Purpose: The purpose of this study was to determine the effect of the main components of juices on furan formation during UV light treatment and to study the effect of antiradicals on the suppression of furan formation in UV light-treated apple cider.

Methods: The main components of most fruit juices, such as fructose, glucose, sucrose and malic acid, were selected to study their effects on furan formation when subjected to UV-C radiation. Antiradicals including ascorbic acid, gallic acid, and BHT at different concentrations were added to apple cider before UV-C treatment at different dosages; the concentration of formed furan was then examined.

Results: Our results showed that fructose is the predominant precursor of furan in apple cider during UV light treatment. By adding antiradicals such as butylated hydroxyl toluene, ascorbic acid, or gallic acid to apple cider during ultraviolet light treatment at 253.7 nm (UV-C), the amount of furan produced was significantly reduced. For example, the concentration of furan produced in apple cider during UV-C treatment at 9.0 J/cm^2 was 636 ppb, but was less than 20 ppb with 0.25 ppm BHT added, less than 3 ppb with 0.5% (weight/volume) ascorbic acid, and less than 1.0 ppb with 0.5% (weight/volume) gallic acid.

Significance: These findings demonstrated that antiradicals provided a safe and simple choice to control furan formation in fruit juices during UV light treatment.

T5-12 Plasma-activated Water and Intense Pulsed Light Processing for Decontamination of Deoxynivalenol in Raw and Germinating Barley

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Introduction: The contamination of barley kernel by *Fusarium* fungi constitutes a serious problem for malt-related industries. Deoxynivalenol (DON) is a secondary metabolite produced by *Fusarium* fungi.

Purpose: The present study aimed to understand the effects of plasma-activated water (PAW) and intense pulsed light (IPL) treatments on the degradation of DON levels in raw and germinating barley, and any changes in quality after these two treatments.

Methods: The effects of PAW and IPL treatments on DON detoxification in raw and germinating barley were investigated.

Results: Both non-thermal methods effectively degraded DON concentration in germinating barley. IPL treatment significantly reduced ($P < 0.05$) initial DON level of germinating barley samples by 32.5% after 180 pulses, and the PAW treatment effectively degraded DON level by 35% in germinating barley in the first 5 min. However, higher barley quality remained for PAW treatment (81 to 100%) than for IPL treatment (61 to 80%). On the other hand, for raw barley samples, although significant reduction (32.96%) was achieved after 135 pulses, obvious quality damage (41 to 60%) was recorded after IPL treatment. Significantly less DON degradation was achieved by PAW treatment on raw barley than germinating barley within 20 min.

Significance: These findings suggested PAW and IPL might potentially be used to reduce DON levels in barley-related industry applications.

T6-01 Detection of Norovirus Contamination in Outbreak-Associated Ice Cream Samples

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Introduction: Norovirus is the leading cause of epidemic and sporadic gastroenteritis worldwide. Although multi-ingredient foods are frequently implicated in outbreaks, there is a lack of analytical methods for sample preparation and virus detection in such items.

Purpose: Ice cream was implicated in a recent norovirus outbreak in Minnesota. An experimental investigation was undertaken to test the implicated food vehicle.

Methods: Fifty-gram portions of the implicated ice cream (raspberry flavored with chocolate chips) were tested. The protocol involved initially eluting the virus with a 0.1 M tris-HCl, 0.05 M glycine, 1% beef extract, pH 9.2 (tris-glycine-beef extract, TBGE) buffer containing 2% polyvinylpyrrolidone (PVP) and pectinase. The eluate was subsequently clarified with chloroform, and virus particles were concentrated with 10% polyethylene glycol (PEG) and 0.3 M NaCl overnight. The virus-containing pellet was re-extracted with TGBE and, to further concentrate the sample, the PEG precipitation was repeated. Viral RNA was isolated from the resulting pellet using a commercial kit (RNeasy PowerPlant Kit, Qiagen). Viral genome detection and quantification were performed using an in-house real-time RT-PCR assay and full-length RNA standards.

Results: Non-homogeneous, low virus contamination of less than 50 RNA copies/50-g sample was identified in several samples that were classified as presumptive positives. In a subset of these, the virus was subsequently confirmed by sequencing and the genotype further determined.

Significance: In conjunction with clinical and epidemiological findings, norovirus was confirmed as the etiological agent of this outbreak, demonstrating that a robust method to detect norovirus in different types of food samples associated with foodborne illness can be used as a critical tool during outbreak investigation and regulatory action.

T6-02 Norovirus in Imported Raspberries Linked to Illnesses

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Introduction: Several norovirus (NoV) and hepatitis A virus (HAV) outbreaks due to the consumption of frozen soft fruit have occurred during the past five years. In 2016, the United States had the third largest hepatitis A virus outbreak due to contaminated soft fruit. In addition, NoV was implicated in two outbreaks associated with the consumption of contaminated raspberries in 2016 and 2017. During both NoV outbreaks, the imported product was from the same country of origin.

Purpose: The objectives of this analysis were to detect, enumerate, and characterize NoV to confirm raspberries as a causative agent in NoV-associated outbreaks.

Methods: Frozen raspberries were analyzed for the presence of NoV using a high-pH eluent along with ultracentrifugation for concentration; murine norovirus was utilized as an extraction control. Commercial extraction kits and RT-qPCR assays were used for RNA extraction and detection, respectively. NoV levels were enumerated utilizing standard curves. Gel electrophoresis, conventional RT-PCR, and big-dye terminal sequencing of the capsid and polymerase regions of the NoV genome were used for characterization.

Results: NoV was detected at approximately 2 copies and 350 genomic copies per 50-g sample for the 2016 and 2017 outbreaks, respectively. Gel and sequence analysis of the amplicons demonstrated that the strain from the 2016 product was GII.17B, while the strain from the 2017 product was GI.3P/GI.3B. In addition, genetic analysis revealed 100% homology between the raspberries and the clinical strains from each outbreak.

Significance: NoV was detected and characterized from imported frozen raspberries and, therefore, confirmed as the source of illnesses in each outbreak. This and other outbreaks demonstrate the importance of having a sensitive and specific method for the detection of enteric viral pathogens. In addition, these findings emphasize the importance of routine surveillance of imported products.

T6-03 Presence of Hepatitis E Virus in Commercially Available Ground Pork

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Introduction: Hepatitis E virus (HEV) is an emerging foodborne virus. Increasing numbers of infections have recently been reported in industrialized countries. These foodborne HEV outbreaks are associated with the consumption of undercooked pork, wild boar, and deer meats. Limited information exists on the prevalence of HEV in commercially sold pork products in the United States.

Purpose: The purpose of this study is to evaluate ground pork sold at retail markets for the presence of HEV.

Methods: Twenty packages of ground pork were purchased from local markets and six 1-g samples from each package were collected (119 samples total). Prior to processing, 5×10^6 PFU/g of murine norovirus (MNV) was added to each sample as a process control virus. Samples were homogenized with PBS, then centrifuged and filtered, followed by total RNA extraction. RT-PCR was used to amplify the gene encoding the capsid protein of HEV and viral protein 1 of MNV. Presence or absence of viral RNA was determined by visualization of RT-PCR products using 1% gel electrophoresis.

Results: Prior to sampling for HEV, the detection limit for MNV in ground pork was determined to be 2.70 log PFU/g of ground pork. All samples screened for the presence of HEV (n=119) tested positive for MNV. Fifteen out of 119 samples (13%) were positive for HEV RNA, and 13 out of 20 packages (65%) contained at least one positive sample.

Significance: HEV was detected in ground pork, indicating this commodity is a potential source of HEV. Therefore, consumption of undercooked, HEV-contaminated ground pork may lead to foodborne illness outbreaks. Future studies should investigate the presence of HEV in pork muscle products.

T6-04 Disinfection Efficacies of Rotaviruses Attached to the Surfaces of *Brassica oleracea* 'Starbor' Kale and *Brassica juncea* Southern Giant Curled Mustard with Chlorine

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Introduction: Rotavirus (RV) is a cause of gastroenteritis worldwide especially for children under five years old and one of the most frequently detected viruses in water supplies. Therefore, it is important to ensure inactivation of RV-attached fresh produce, grown with RV-contaminated irrigation water.

Purpose: We aimed to identify if the efficacy of disinfection with chlorine, one of the most commonly used disinfectants in the food industry, is affected by either bacterial density or leaf properties when applied to RV attached to a leaf's surface.

Methods: In this study, we conducted disinfection experiments of RV (strain OSU, Wa and ST3) attached to either indoor- or outdoor- grown 'Starbor' kale (*Brassica oleracea*) and southern giant curly mustard (*Brassica juncea*). Rotavirus-contaminated leaf disks were exposed to chlorine, and plaque assay was conducted to quantify virus disinfection efficacies. In parallel with the disinfection experiments, bacterial density of vegetables was quantified by quantitative polymerase chain reaction (qPCR) with 16S rRNA gene-specific primers.

Results: All the RV strains had higher disinfection efficacies for southern giant curly mustard by chlorine (approximately 2-$\log_{10}$ reduction), than for 'Starbor 'kale (approximately 1-$\log_{10}$ reduction). However, similar disinfection efficacies ($P > 0.05$) for indoor- and outdoor-grown vegetables were observed with all the three strains, indicating that the difference of bacterial density from indoor- and outdoor- grown vegetables did not affect the chlorine disinfection efficacies. In conclusion, produce surface properties affected RV disinfection with chlorine, while bacterial density did not show a difference on inactivation efficacies. We are currently testing the hypothesis that the bacterial density does not affect RV disinfection efficacies of vegetables. Furthermore, we are currently conducting experiments to determine what RV capsid proteins are damaged by these disinfectants.

Significance: To conclude, produce surface properties are important to consider in food sanitation practice to control foodborne viral infection risks.

T6-05 Prevalence and Molecular Characterization of *Toxoplasma gondii* in Retail Meats in Canada

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Introduction: Toxoplasma gondii is a protozoan parasite which infects a very wide variety of mammals and birds worldwide, including humans. While human toxoplasmosis is thought to be most commonly transmitted through the ingestion of tissue cysts in the meat and organs of infected animals, there is a distinct paucity of information on the prevalence and molecular characteristics of *T. gondii* in retail meats in Canada.

Purpose: The purpose of this study was to establish a baseline prevalence of *T. gondii* in retail meats in Canada, as well as to identify the predominant genotypes.

Methods: A total of 421 samples of retail meats, including 93 packages of ground beef, 94 packages of ground pork, and 234 individual chicken breasts, were purchased at grocery stores in three Canadian provinces (British Columbia, Alberta, and Ontario). Following DNA extraction from 200-mg aliquots, screening of the samples for the presence of *T. gondii* DNA involved PCR amplification of the 529-bp fragment, as well as a fragment of the B1 gene. *T. gondii* genotyping was performed using multilocus PCR/RFLP on the *SAG2* 3′, *SAG2* 5′, *GRA6*, and *BTUB* genes, in addition to DNA sequencing.

Results: T. gondii DNA was present in 2.2% (2 of 93) of the ground beef samples, 3.2% (3 of 94) of the ground pork samples, and 3.9% (9 of 234) of the chicken breasts. Prevalence was similar in all three types of meat and from all three provinces. Of the positive samples, *T. gondii* Type II was present in 64.3% (9 of 14), while Type III was present in 35.7% (5 of 14).

Significance: This study demonstrated a relatively low prevalence of *T. gondii* in retail meats in Canada, representing the most common human and animal genotypes. These baseline data will allow for more accurate health risk assessments for the purpose of developing food safety guidelines and policies.

T6-06 Detection of *Cyclospora cayetanensis* in Agricultural Water by Combining the Dead-End Ultrafiltration Method with Sensitive Molecular Assays

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Introduction: Cyclospora cayetanensis is a protozoan parasite that causes foodborne and waterborne diarrheal illness outbreaks worldwide. Most of these outbreaks are associated with consumption of fresh produce where agricultural water may contribute to the contamination of crops.

Purpose: Sensitive and specific methods to detect *C. cayetanensis* in agricultural water are essential to ascertaining the presence of the parasite in such samples. In this study, we developed and evaluated a method to detect *C. cayetanensis* in water by combining dead-end ultrafiltration (DEUF) with sensitive and specific molecular detection.

Methods: Triplicates of 10-L agricultural water samples were seeded with decreasing numbers of *C. cayetanensis* oocysts (200, 100, 25). In addition, 12 replicates were seeded with six oocysts. All samples were processed by DEUF, followed by DNA extraction from concentrated samples using the U.S. Food and Drug Administration's Bacteriological Analytical Manual (Chapter 19B). The DNA extracts were analyzed by a qPCR targeting the 18S rRNA gene and a conventional PCR, followed by DNA sequencing that targeted the parasite's mitochondrial genome.

Results: All water samples seeded with 25, 100, and 200 oocysts were positive, while all unseeded samples were negative. Samples seeded with six oocysts gave a detection rate of 66.6% (8 of 12). DNA sequence analysis of amplicons was possible on the samples seeded with six or more oocysts.

Significance: We describe a method for detecting low concentrations of *C. cayetanensis* in agricultural water. The approach combined the DEUF method with sensitive molecular methods that could consistently detect *C. cayetanensis* DNA in 10-L agricultural water samples seeded with six or more oocysts. The method includes a qPCR and DNA sequencing analysis, which provided specificity for analysis of environmental samples.

T6-07 Mishandling of Poultry Products by Consumers: Identification of Gaps in Knowledge and Safe-handling Practices of Raw Turkey

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Introduction: Poultry is known to be a significant source of foodborne illness due to *Salmonella* and *Campylobacter.* Ongoing education campaigns have aimed to encourage consumers to handle raw poultry properly to prevent cross-contamination and to cook poultry to the proper temperature to ensure safety. These campaigns have generally focused on chicken and less is known about how consumers handle raw turkey. Additionally, the United States Department of Agriculture (USDA) currently discourages consumers from stuffing a whole turkey, but it is unknown what percent of consumers still engage in this traditional practice.

Purpose: The purpose of this research was to better understand consumer knowledge and practices around handling and preparing whole turkeys.

Methods: A brief survey comprised of 11 questions regarding preparing, cooking, and holding a whole turkey was distributed to more than 2,000 consumers nationwide in the United States through SurveyMonkey. Practices explored included washing a raw turkey, stuffing a turkey, cooking a turkey overnight, and holding of a turkey after cooking. The survey was administered immediately following the 2017 holiday season, when cooking and consuming whole turkeys is most common.

Results: Of 2,083 recipients across the United States, 71% (1,485) reported ever having cooked a whole turkey; of this population, 1,314 consented and completed the entire survey. Ninety percent (1,178) of respondents reported always or sometimes washing a raw turkey. Fifty-seven percent (745) reported always or sometimes stuffing a turkey prior to cooking, and 77% (989) reported holding cooked turkey at an uncontrolled temperature (warm oven or room temperature) after cooking.

Significance: Results suggest many consumers routinely wash and continue to stuff raw turkeys, despite the USDA's recommendation not to do so. This indicates a potential need for targeted consumer education to ensure consumers are aware of the most recent recommendations for how to prepare turkeys.

T6-08 Investigating Cross-Contamination to Fomite Surfaces in Consumer Kitchens Using MS2 as a Surrogate in Ground Turkey

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Introduction: Cross-contamination frequently occurs in consumer kitchens due to risky behaviors performed while handling raw meat and poultry products. Kitchen surfaces can become harborage sites for foodborne pathogens found in raw meat and poultry products and contribute to home-acquired foodborne illnesses. However, the frequency and level of contamination and the effect of food safety education on cross-contamination has not been well studied.

Purpose: This study was conducted to determine how frequently cross-contamination occurs in consumer kitchens, what items were most often contaminated, the level of contamination, and if a video on proper thermometer usage impacted food safety practices, including cross-contamination.

Methods: A meal consisting of turkey burgers inoculated with 10^8 PFU of bacteriophage MS2, a vegetable salad, and a lettuce garnish was prepared by participants (n=383) in a test kitchen. One half of the participants were shown a food safety video about proper thermometer usage before meal preparation (intervention group); the other half served as a control group. After preparation, sampling of the kitchen environment and equipment was completed and immediately tested for enumeration of MS2 using an RT-qPCR-based assay. Statistical analysis of prevalence and concentration of MS2 cross-contamination was completed using R.

Results: Spice containers and the refrigerator door handle were the most frequently contaminated surfaces. Levels of contamination ranged from a maximum of 4.23×10^7 PFU/surface and a minimum of 1.71×10^3 PFU/g. The average concentration of MS2 recovered from all contaminated surfaces was 2.56×10^5 PFU/surface. There was no statistically significant difference in the frequency or magnitude of cross-contamination when comparing the intervention and control groups.

Significance: This study provides insight into how pathogens may move around a kitchen, including location and prevalence. Refrigerator door handles and spice containers, shown as a potential source of indirect cross-contamination, are not often focused on in communication messages.

T6-09 Barriers and Strategies to Safe Food-handling among Financially Disadvantaged Families: An Observation and Self Report Study

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Introduction: Financially disadvantaged families experience unique cultural and language barriers that limit the effectiveness of conventional food safety education. This project utilizes both observation and self-report methods to evaluate barriers and strategies to safe food handling among financially disadvantaged families.

Purpose: Assess attitudes, awareness, and food handling behaviors among financially disadvantaged families with young children.

Methods: Primary food handlers from low-income minority families with young children (<5 years old) were recruited. Focus groups were held in community meetings and observations occurred in participants home. All sessions were translated and/or transcribed by two trained undergraduate researchers separately and reviewed by a third researcher. Paper-based surveys were conducted following the focus group.

Results: Thirty-two people participated in the observation phase and 81 in the focus group phase, with 41 Spanish-speaking and 72 English-speaking (17% Hispanic, 60% African-American, 10% Asian, and 13% Mixed). Most participants (90% in observation; 72% in focus groups) considered themselves knowledgeable or very knowledgeable about safe food handling. However, more than 85% of participants thought appearance, odor, and taste could determine when meat is adequately cooked, and none of the particpants used a food thermometer during observation. There was also a discrepancy between knowledge and behavior. Focus groups indicated that the majority of participants knew it was recommended to use separate cutting boards for raw meat and ready-to-eat produce. When observed, no one owned separate cutting boards. Some participants "solved" this problem by cutting food in their hands or on plates. The major barriers to safe food handling practice compliance were family tradition and belief in "grandma's magic," pseudoscientific information from multimedia, and lack of tools or space.

Significance: Food safety interventions tailored for financially disadvantaged families must consider space and equipment limitations, overcome some traditional family practices, and counter inappropriate behavior modeled in the media.

T6-10 Source Attribution of Illnesses Commonly Transmitted by Food and Water in the United States Using Structured Expert Judgment

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Introduction: Illnesses commonly transmitted by food and water create a major disease burden in the United States. These illnesses may also be transmitted by other pathways: person-to-person, animal contact, or through the environment. Limited data are available to estimate the proportion of disease attributable to each of these modes of transmission.

Purpose: To report findings from a structured expert judgment study to provide attribution estimates of illnesses transmitted commonly by food and water.

Methods: Forty-eight experts were assigned to one or more of 15 panels based on publication records and self-evaluation of professional interest and experience with each of 33 pathogens. Including subdivisions by serotypes, age, and clinical manifestations for some pathogens, a total of 47 target questions were asked to elicit the annual percentage of domestic illnesses that are transmitted through each pathway. Cooke's Classical Model, aggregated with equal and performance-based weighting, was used to synthesize the panels' collective judgments.

Results: The following are examples of the modes of transmission estimated for the 33 pathogens. Estimates for *Salmonella enterica* were 66% foodborne, 6% waterborne, 7% person-to-person, 11% animal contact, and 10% environmental. Estimates for norovirus were 19% foodborne, 6% waterborne, 70% person-to-person, 0% animal contact, and 5% environmental. Estimates for *Cryptosporidium* were 8% foodborne, 43% waterborne, 20% person-to-person, 21% animal contact, and 8% environmental.

Significance: This is the first study to estimate attributable proportions of disease in the United States across all modes of transmission. Rather than considering only one pathway, as in previous studies, our findings attribute transmission to a set of comprehensive and exhaustive pathways and may afford a more accurate understanding for each pathogen than before. Our findings can be applied to estimates of the number of illnesses caused by these pathogens in the United States.

T6-11 A Systematic Review and Meta-analysis of the Knowledge, Practices and Training Related to Food Allergies and Celiac Disease among Restaurant and Food Service Personnel

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Introduction: Up to 3 to 5% of adults may be affected by food allergies, and approximately 1% are affected by Celiac disease (CD). Food allergy reactions can be severe and potentially fatal, and CD can result in various symptoms. Restaurant and food service establishment staff have an important role in helping to prevent food allergy and CD risks among affected customers.

Purpose: A systematic review was undertaken to identify, characterize, and synthesize published research on the knowledge, practices, and training of restaurant and food service personnel toward food allergies and CD.

Methods: The review consisted of a comprehensive search strategy, relevance screening, article characterization, data extraction, and risk of bias assessment. Outcomes were stratified into comparable subgroups to examine trends across studies.

Results: A total of 38 relevant studies were identified. Most studies used a cross-sectional design (97%), were conducted in the United States (50%), and focused on food allergies (90%) compared to CD. Significant heterogeneity across studies was noted for most outcomes. Many knowledge gaps were identified, particularly on how to respond to food allergy reactions. Participants generally had a higher knowledge, self-efficacy, and usage of practices related to preparing and serving allergen-free meals compared with responding to food allergy emergencies. Participants' reported use of various risk prevention and response practices (e.g., posting of allergen information) related to food allergies and CD was generally low. Most participants had not received prior food allergy training and indicated that training was not provided to staff in their establishment (median prevalence of 65% across 12 studies and 64% across 9 studies, respectively).

Significance: Key knowledge and practice gaps were identified that could be targeted by future training programs. Research gaps were also identified, including a need for more studies to evaluate interventions to improve food allergy and CD knowledge and practices.

T6-12 Food Safety Considerations from Concept to Commercialization: An Extension Training Program Targeted toward Food Entrepreneurs

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Introduction: Local small food processing businesses can promote the local economy and help to add value to local commodities; however, small processors are challenged with navigating their way through regulation and food safety compliance while still delivering a safe, high-quality food product that can be profitable and make a local economic impact. Integrating the culture of food safety into the product development process from the beginning can better prepare these new businesses.

Purpose: This project developed a training titled "Product Development Considerations - Beyond the Concept" which was targeted directly towards small processing businesses and encouraged them to consider food safety from initial product concept to commercialization.

Methods: This training program aimed to provide a food safety curriculum that demonstrates the importance of food safety throughout the product development process. Key concepts covered in the training include principles of food safety, approaches to food safety risk management, quality and food safety controls, the product development cycle, food regulation, and commercial scale up. Paper program evaluations were fielded after the training to measure behavioral change and knowledge gained.

Results: This extension program has reached more than 100 food entrepreneurs, and program evaluations indicate that more than 63% of the participants will make changes in their food safety practices based on the knowledge gained from this training. Participants reported their overall knowledge of food safety concepts before and after the training using a three-point knowledge ranking score as 1.64 and 2.48, respectively, with the largest knowledge increase demonstrated with the product development life-cyle section.

Significance: This training helps build a strong understanding of food safety early in the development process, which can enable better business development for entrepreneurs.

T7-01 Molecular Comparison of New Strains of Shiga Toxin-producing *Escherichia coli* Isolated from Beef Product Samples with Human Strains

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Introduction: The United States Department of Agriculture Food Safety and Inspection Service (FSIS) analyzes raw beef products for Shiga toxin–producing *Escherichia coli* (STEC), the presence of which indicates adulteration and can result in recalls. Monitoring the results of this sampling and comparing pulsed-field gel electrophoresis (PFGE) patterns between beef and human STEC isolates may aid in identifying foodborne or zoonotic disease sources of human infections.

Purpose: To identify novel STEC strains from beef products that are indistinguishable from human isolates by PFGE.

Methods: During fiscal years 2015 to 2017, STEC isolates recovered from FSIS beef samples were analyzed by PFGE and uploaded to the Centers for Disease Control and Prevention's PulseNet, which also contains PFGE patterns from human isolates. If a pattern had not been seen before in PulseNet, the STEC isolate was considered to be a "new strain". All such new strains were selected for comparison with subsequent human isolates uploaded to PulseNet.

Results: During fiscal years 2015 to 2017, 397 STEC isolates from FSIS beef samples were uploaded to PulseNet, including 94 (23.7%) *E. coli* O157:H7 isolates and 303 (76.3%) non-O157 STEC isolates. From these 397 isolates, 362 distinct PFGE patterns were detected, of which 223 (61.6%) were new patterns. During this period, 14 human clinical STEC isolates were indistinguishable from eight (3.6%) FSIS new STEC strains, including one *E. coli* O157:H7, one STEC O111, and six STEC O103. The range of time intervals between the isolation dates of indistinguishable FSIS isolates and human clinical isolates was four to 33 months, with a median of 12.5 months.

Significance: Cattle are the principal reservoir for STEC. Identifying indistinguishable emerging strains from beef products and clinical specimens could be an early detection tool for human STEC infections acquired from food consumption or animal contact transmission routes. Study findings demonstrate the importance of food testing programs in identifying potential linkages between contaminated food and human illness.

T7-02 Thanksgiving Day Outbreak of Norovirus with Multiple Modes of Transmission – Tennessee, 2017

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Introduction: A restaurant manager notified the Tennessee Department of Health of gastrointestinal illnesses reports following Thanksgiving meals at his restaurant. We investigated to identify the outbreak's source and etiology.

Purpose: In this study, we demonstrate how reservation applications and environmental swabbing can support a multimodal outbreak in a restaurant setting.

Methods: We conducted a cohort study using an on-line reservation system to identify patrons and obtain phone numbers, seating times, and table assignments for dining parties. An environmental assessment was performed and clinical and environmental samples were collected. Probable cases were defined as diarrhea or vomiting in restaurant patrons with illness onset ≤1 week after the meal. Confirmed cases had norovirus detected in stool specimens using reverse transcription PCR. Logistic regression was used to assess associations between illness and exposures.

Results: A total of 130 people were interviewed, including 38 cases (one confirmed, 37 probable) and 92 controls (29% attack rate). Consuming pecan pie bars (odds ratio=3.0, 95% confidence interval [CI]: 1.3 to 6.8) and seating between 11 am and 1 pm (odds ratio=7.4, 95% CI: 2.9 to 18.7) were significantly associated with illness. Our parsimonious logistic regression model included significant risk factors and seating location (not significant). Only seating time between 11 am and 1 pm remained significant (odds ratio=7.3, 95% CI: 2.6 to 20.1). Two stool specimens and one environmental swab, collected on November 30 from the bottom of a table adjacent to the vomiting event, yielded norovirus Genogroup II.

Significance: In this norovirus outbreak investigation, laboratory, environmental, and epidemiologic data provided evidence supporting reports of a vomiting patron and multimodal transmission. This investigation demonstrates how the use of reservation applications and environmental swabbing can be used to support an outbreak investigation involving multimodal transmission.

T7-03 Foodborne Illness Source Attribution Estimates in 2013 for *Salmonella*, *Escherichia coli* O157, *Listeria monocytogenes*, and *Campylobacter* Using Multi-year Outbreak Surveillance Data, United States

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Introduction: The Interagency Food Safety Analytics Collaboration (IFSAC), established by the Centers for Disease Control and Prevention (CDC), the U.S. Food and Drug Administration, and the United States Department of Agriculture's Food Safety and Inspection Service, works to improve foodborne illness source attribution estimates in the United States.

Purpose: We present attribution estimates for *Salmonella*, *Escherichia coli* O157, *Listeria monocytogenes*, and *Campylobacter* for 2013.

Methods: Using data from CDC's Foodborne Disease Outbreak Surveillance System on outbreaks that occurred between 1998 and 2013, we analyzed 1,043 outbreaks where one of the four pathogens was the confirmed or suspected etiology and the implicated food was assignable to one of 17 food categories designated by IFSAC. Statistical models were developed to mitigate the influence of large outbreaks and to control for epidemiological factors.

Results: Seventy-five percent of *Salmonella* illnesses were attributed to seven food categories: seeded vegetables (e.g., tomatoes), eggs, chicken, other produce (e.g., nuts), pork, beef, and fruits. Eighty percent of *E. coli* O157 illnesses were attributed to vegetable row crops (e.g., leafy greens) and beef. Similarly, 86% of illnesses due to *L. monocytogenes* were attributed to fruits and dairy, though the rarity of these outbreaks makes these estimates less reliable than those for other pathogens. Almost 80% of non-dairy *Campylobacter* illnesses were attributed to chicken, other seafood (e.g., shellfish), seeded vegetables, vegetable row crops, and other meat/poultry (e.g., lamb, duck). Although most foodborne *Campylobacter* outbreaks were associated with unpasteurized milk, a high-risk product not widely consumed by the general population, other evidence suggests these outbreaks over-represent dairy as a source; we therefore excluded dairy from the *Campylobacter* estimates.

Significance: These results can support evidence-based food safety decision-making. Updates to these estimates will also enhance IFSAC's efforts to engage stakeholders and assess whether food safety prevention measures are working.

T7-04 Restaurant Grades are Difficult to Find and Understand

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Introduction: While there is increasing public availability of health department inspection scores and/or grades, there are significant impediments to understanding the data. Scoring and/or grading disparities create inconsistency.

Purpose: The lack of a nationwide standardized restaurant scoring/grading system makes comparison of health department scores and outcome measures across state and municipal lines nearly impossible. The purpose of this study was to evaluate variance and begin to create a national standardized restaurant food safety scoring and/or letter grading system.

Methods: Health department restaurant inspection data from multiple states, counties, and cities was obtained. Unique violation "report cards" were evaluated for format used. Conversion rules were created. Data was analyzed pre-and post-conversion rule adjustment.

Results: Using coded violation data and a universally accepted scoring curve of 100 to 90=A, 89 to 80=B, 79 to 70=C, 69 to 60=D, and 59 and below=F, the chances of an unadjusted "A" grade can range considerably. For example, the chances getting an "A" are 97.3 % in one Arizona county to 19.4% in another California city. In the same Arizona county, 29% of restaurants are non-participating with no score/grade publically published. In a large eastern metropolitan city, 41% of inspection results are not posted at the restaurant. In a southern Texas city, 4% of inspections are re-inspections from the same day. There are at least 19 different scoring systems using letters, numbers, color codes (green, yellow, or red), descriptors (excellent, superior, good, standard, fair, or poor), categories (basic, intermediate, high priority, Foodborne Illness Risk, Good Retail Practice, Core, Priority, Priority Foundation, Critical, Non-Critical, Demerits, or Violation Count). Multiple variant violation demerits were found to be applied to these scoring subcategories. Multiple variant public restaurant displays are used including letter grades, inspection numerical scores, color-coded placards, descriptors and emojis.

Significance: Health Department scoring/grading standardization can benefit food safety process improvement. There is inconsistency in health department scoring, including multiple systems and public displays, and redundant/confusing definitions. Violation demerits are variant. This inconsistency creates public confusion. Solutions are multilayered but can build consumer and restaurant management confidence.

T7-05 Cold-holding Compliance Rates in Food Establishments in North Carolina

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Introduction: The Centers for Disease Control and Prevention has identified improper holding temperatures as a risk factor that contributes to foodborne illness. In January 2019, North Carolina's cold-holding compliance temperature will change from 45 to 41°F. Proper cold holding below 41°F is vital in order to reduce growth of *Listeria monocytogenes* in RTE foods in food establishments.

Purpose: As part of the U.S. Food and Drug Administration's (FDA) National Voluntary Retail Program Standards, risk factor studies were conducted by environmental health specialists throughout North Carolina between 2010 and 2017 in seven counties. Data were compiled to determine compliance with the 41°F cold-holding temperature requirement.

Methods: The FDA risk factor study protocol contained in Standard 9 of the FDA National Voluntary Retail Program Standards was used. Environmental health specialists received training on the protocol before data collection began. Marking instructions developed by the FDA were used in data collection, and the establishments were divided into nine categories: full-service restaurants, delis, elementary schools, fast food restaurants, seafood markets, produce departments, institutions, meat markets, and hospitals.

Results: Over the seven-year data collection period, 1,830 cold-holding observations were made throughout the nine facility types. Overall compliance to cold holding at 41°F was 46%. The highest overall compliance rates (81%) were seen in meat markets. Compliance rates in full service restaurants were 31% and fast food restaurants were 36%, with lowest compliance seen in hospitals at 26%.

Significance: Results show that compliance with cold-holding temperature requirements has been challenging across all types of food service establishments; differences in compliance rates can be explained by the complexity of operation, design of establishment, and types and quantities of cold-holding equipment available. Results can be used as a compliance baseline as North Carolina moves toward cold-holding intervention strategies, and will assist in determining success of strategies used.

T7-06 Risk Factor Compliance of Food Establishments during Temporary Food Events

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Introduction: Temporary food events are growing in popularity and becoming more common throughout the United States. North Carolina has established rules for temporary food service establishments, and permits must be issued even when food is sold on a temporary basis. There are challenges associated with food safety during these events due to exposure to outdoor environments and lack of permanent fixtures for handwashing and temperature control.

Purpose: Risk factor studies were conducted by environmental health specialists during a large temporary event in North Carolina in August 2017. The large, full-service temporary establishments were evaluated for compliance with the five risk factors that contribute most to foodborne illness, as established by the Centers for Disease Control and Prevention.

Methods: The U.S. Food and Drug Administration (FDA) risk factor study protocol from Standard 9 of the National Voluntary Retail Program Standards was used to evaluate compliance. Environmental health specialists trained on and implemented this protocol for data collection over the course of the event. Twelve full-service temporary food establishments were surveyed twice during the event (24 total risk factor surveys conducted).

Results: The 2013 FDA Food Code was used to determined compliance. The 24 surveys conducted showed 22% compliance with proper hand washing and 22% compliance with avoiding cross-contamination. The lowest compliance rate was with proper cooling methods (16%), while the highest compliance was found in proper date marking (87%). Cold-holding compliance was found 30% of the time, compared with 54% compliance seen in permanent food establishments throughout North Carolina.

Significance: Results suggest that maintaining compliance with risk factors is difficult in outdoor, temporary food establishments, as they include challenges in maintaining cold-holding temperatures and non-permanent plumbing fixtures can lead to decreased hand washing compliance. Additional training and procedures are needed to ensure food served in temporary settings is equal in safety to food served at permanent food establishments.

T7-07 Deep Cleans, Optimized Sanitation Standard Operating Procedures and Management Engagement Can Reduce *Listeria monocytogenes* Prevalence in Retail Produce Departments

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❖ ***Developing Scientist Competitor***

Introduction: We recently conducted a six-month longitudinal study to determine the prevalence of *L. monocytogenes* in retail produce departments. This study was complemented by a practice and management survey that indicated changes in sanitation standard operating procedures (SSOPs) and departmental management practices could reduce *L. monocytogenes* prevalence in this environment.

Purpose: We evaluated the efficacy of SSOP changes and employee-led department deep cleans in reducing *L. monocytogenes* prevalence in retail produce environments with the goal of preventing cross-contamination to RTE foods.

Methods: Eight stores from the longitudinal study with >5.6% *L. monocytogenes* prevalence were selected for deep cleans and SSOP changes focused on eliminating standing water and enhanced harborage site sanitation. All stores were sampled monthly for at least three months; 15 surfaces were sampled and screened for *L. monocytogenes* using the ROKA Atlas platform. Positive enrichments were confirmed by secondary enrichment and plating.

Results: L. monocytogenes prevalence decreased by 53.1% in store 27 (x^2; P<0.0001), 49.9% in store 14 (x^2; P<0.0001), 41.5% in store 5 (x^2; P=0.0008), and 12.6% in store 22 (x^2; P=0.1566) after intervention. These four stores had markedly higher management engagement and capital improvement. The only food contact surface positive among them post-intervention was in store 27. There was no significant change in *L. monocytogenes* prevalence in the other four stores (8, 17, 21, and 28). Standing water and floor drains had the highest *L. monocytogenes* prevalence pre- and post-intervention. Storage pallets harbored *L. monocytogenes* in 22.2% (six of 27) of samples tested. In store 14, the squeegee was positive five to six months pre-intervention; after implementing a policy of soaking the squeegee in sanitizer each night, there were no further positives over three months.

Significance: L. monocytogenes prevalence in retail environments can be reduced by deep cleans and changes in SSOPs with management engagement.

T7-08 Three-level Longitudinal Analysis of the Antecedents of Distributive Food Safety Training in the Food Service Industry

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Introduction: An estimated 61% of foodborne illness outbreaks were attributed to employees' personal hygiene and improper food handling by employees in the food service industry in the United States. Although training programs have been carried out, they appear to be less effective when correlated with foodborne illness outbreaks over the same period. Researchers have suggested that food safety training should be developed based on firm behavioral and psychosocial theories.

Purpose: The objectives of the study were to examine the effects of factors in within-individual, between-individual, and between-organization levels on food safety training outcomes.

Methods: A food safety education program was delivered within a six-week span. The data was collected through a questionnaire from 25 foodservice operations. Overall, the sample included 39 managers and 394 food handlers. Each food handler was asked to complete the survey three times during the six-week period. Multilevel linear modeling was used for data analysis.

Results: The results showed that the intra-individual self-efficacy (π_{300}=0.63, P<0.01) and motivation to learn (π_{200}=0.39, P<0.01) had positive impact on the FSK growth at the within-individual level, while perceived enjoyment (β_{130}=0.39, P<0.01) and average motivation to learn (β150=0.39, P<0.01) significantly influenced FSK growth at the between-individual level. At the organization level, both safety priority (γ_{101}=0.39, P<0.01) and monitoring behaviors (γ_{102}=0.19, P<0.01) were significantly related to the FSK growth.

Significance: The results implied that managers play an important role in employee food safety training. Also, attitudinal factors vary within-person during the training. Therefore, it is critical to improve leaders' management skills and employees' attitudes toward food safety to enhance the effectiveness of food safety training.

T7-09 Food Service Small Medium Enterprises (SMEs) Contraventions Associated with Hygiene and Confidence in Management(CIM): Implications for Food Safety Culture

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Introduction: Good hygiene and an effective food-safety-management system (FSMS) is essential for the delivery of safe food and legal compliance; UK assessment/scoring occurs within the framework of the Food Hygiene Rating Scheme (FHRS) by Environmental Health Officers (EHOs) during enforcement inspections. SME management is responsible for FSMS and training; poor scores may be an indication of inadequate hygiene and a poor food safety culture.

Purpose: To determine the factors associated with hygiene and CIM in foodservice SMEs related to FSC to facilitate appropriate regulatory intervention and training approaches.

Methods: Hygiene and CIM scores from inspection reports for food-service SMEs (n=299) from two local authorities in England were determined. Scores accounted for 19 hygiene and 13 CIM subheading contraventions; data was coded according to contravention frequency and food hygiene score categories and analysed using content analysis.

Results: Cumulatively, 53.5-69.6% food-service SMEs reports included up to seven hygiene and five CIM contraventions. Common hygiene contraventions included cross-contamination in 72 (24.0%) of SMEs e.g. lack/ inadequate segregation of raw and cooked items and dirty food contact surfaces. Poor temperature control was recorded in 47(15.7%) of SME reports whilst personal hygiene issues recorded in 42 (14.0%). Frequent CIM contraventions included 63(21.1%) of food service SMEs reportedly failing to complete food safety checks or gaps in the FSMS, for example, temperature monitoring or safe methods not completed, lack of a documented FSMS 49(16.4%) and lack of training 66(22.1%). Final CIM scores included EHOs unquantifiable/ subjective elements such as prediction of future compliance based on past performance and observed practices.

Significance: Analysis of hygiene and CIM contraventions and recommendations could be used to identify factors contributing to the food safety culture of SMEs to facilitate targeted interventions and training to improve food safety culture.

T7-10 Genotypic and Phenotypic Diversity of *Staphylococcus aureus* Isolates from Retailed Frozen Flour and Rice Products in Shanghai

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❖ ***Developing Scientist Competitor***

Introduction: Frozen flour and rice products are important food for Chinese people, but they are easily contaminated by *Staphylococcus aureus*, which is a threat to public health.

Purpose: To investigate the prevalence and characteristics of *S. aureus* in retail frozen flour and rice products in Shanghai, China.

Methods: A total of 288 samples falling into three categories (meat stuffing, vegetable stuffing, and non-stuffing) were collected from September 2014 to May 2015 in Shanghai. *S. aureus* was isolated based on its biochemical properties. The positive isolates were identified by multilocus sequence typing (MLST). Meanwhile, they were assayed for the carrying of 18 staphylococcal enterotoxin genes (*sea~see*, *seg~ser*, and *seu*).

Results: The occurrence of *S. aureus* was found in 30.56% (88 of 288) of samples, which was an increase compared to historical reports. There was a remarkable difference in contamination rates among the three categories of samples (P<0.05), and the meat stuffing group had the highest rate of all, with 41.44%. A total of 124 *S. aureus* isolates were identified, and among the 124 isolates, 111 were positive for enterotoxin genes with a total carrying rate of 89.52%. The *sep* gene was the most prevalent (44.35%, 55 of 124), followed by *sei* (43.55%, 54 of 124), and *seg* (42.74%, 53 of 124). The classical enterotoxin genes (*sea~see*) were detected in 33 isolates, and 16 (48.48%) of them were from meat stuffing samples, higher than the other two categories. The 124 isolates were scattered in 36 sequence types (STs) by MLST and attributed to 19 clonal complexes by eBURST analysis, including 31 already known STs and five new suspicious STs. It is notable that ST7 possessed the most isolates and enterotoxin genes among all the STs.

Significance: The relatively high risk for *S. aureus* contamination in retailed frozen flour and rice products is a concern, especially for meat stuffing products.

T7-11 Evaluating Various Methods of Validating Sushi Rice Acidification in Retail Food Establishments

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Introduction: Acidification is employed as a frequent variance to the U.S. Food and Drug Administration's Model Food Code to hold sushi rice at ambient temperatures longer than four hours. The process involves adding a specific volume and concentration of vinegar to adjust pH and prevent growth of pathogens.

Purpose: Many jurisdictions require that a homogeneous sample of rice is at <4.2 pH before holding at room temperature. This study explores whether various methods of breaking rice grains to achieve homogeneity impact measured pH values. The study also investigates whether rice sample pH changes over time and if the outer bran on brown rice impacts acidification.

Methods: A batch each of white and brown sushi rice was prepared according to common retail preparation methods. During cooling, proper amounts of rice vinegar were added. Samples of rice were blended with deionized water after 5, 10, 20, 30, and 60 min. At the 30-min interval, samples were homogenized using four different methods for comparison against the blended control. The pH value of each sample was measured using a calibrated pH meter; the experiment was carried out in triplicate.

Results: No method of homogeneity yielded a significant pH compared to the control in brown or white rice (P>0.90). Additionally, the pH of neither type of rice underwent a significant change between 5 and 60 min (P>0.90). A paired t test revealed a significant difference in the pH values of white and brown sushi rice, where brown yielded a higher average pH (P<0.0001). All white rice samples returned an average pH value of <4.2, while the average pH value of 67% of brown rice samples exceeded 4.2 (n=9).

Significance: These results provide insight into different methods of validating sushi rice acidification, thus providing retail food establishments an array of convenient methods for performing validations and establishing evidence that brown rice acidification is less successful with common procedures.

T7-12 *Salmonella* Transfer and Survival on Fresh-cut Fruits

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Introduction: ***Salmonella enterica*** frequently causes foodborne disease outbreaks linked to fresh and fresh-cut fruits. However, the specific mechanisms that mediate its transfer and survival on fresh-cut fruits are not fully elucidated.

Purpose: To evaluate the transfer of *S. enterica* during fresh-cutting and its fate in fresh-cut produce during storage and to identify the genes and metabolic pathways relevant to the survival of *S. enterica* in fresh-cut fruits.

Methods: Fruits were surface-inoculated with *Salmonella* Newport strain 36796 and *Salmonella* Typhimurium strain LT2 19585 and cut once with a sterilized knife. The contaminated knife was then used to make subsequent cuts on four uninoculated fruit samples. Transfer rates of pathogens by each cut were statistically analyzed. The cut samples were stored in the form of slices or blended juice at 4°C for up to 7 days for bacterial survival counts. RNA-seq was used to identify differentially expressed genes in *Salmonella* Newport strain 36796 on fresh-cut tomatoes and cantaloupes after 1 h storage at 4°C, in comparison to that in 0.1% buffered peptone water.

Results: The transfer of pathogens was most significant in the first cut of an uninoculated fruit with a contaminated knife ($P < 0.05$). Cucumbers and apples showed higher bacterial transfers than tomatoes and cantaloupes. Pathogen population remained unchanged in tomatoes and cantaloupes and experienced an approximately 0.5-log reduction in apples and cucumbers during storage. A total of 273 and 330 genes in *Salmonella* Newport ($P < 0.05$, $\log_2|\text{fold-change}| \geq 2.5$) differentially expressed in fresh-cut tomatoes and cantaloupes, respectively. Genes involved in amino acids biosynthesis displayed most significant up-regulation ($P<0.05$).

Significance: This study clearly demonstrated that *Salmonella* can transfer through and survive well in fresh-cut fruits. Fruit textures play important roles in bacterial transfer. Genes involved in bacterial metabolism assist in adaptation and survival of *Salmonella* in fresh-cut fruits.

T8-01 Pesticide Monitoring of Foods Consumed in the United States

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Introduction: The United States Department of Agriculture (USDA) Pesticide Data Program (PDP) annually tests more than 10,000 samples of about 20 United States (U.S.) food commodities for pesticides. In 25 years, PDP has tested about 125 commodities for more than 500 pesticides. The commodities include fresh and processed fruit and vegetables, infant formula and baby foods, grains, nuts, dairy, meat, poultry, fish, and water. Emerging chemistries and newer pesticides including triazoles, pyrethroid, and neonicotinyl are tested, as well as older classes of pesticides such as carbamates and organophosphates.

Purpose: This monitoring program provides data on pesticides in foods for risk assessments that assist in developing policies on pesticide re-registrations and integrated pest management practices.

Methods: To represent more than 50% of the nation's population and all four census regions, the USDA National Agricultural Statistics Service has developed a statistically defensible framework for sample collection across the U.S.. State laboratories test the samples using program-wide validated QuEChERS-based methods for extraction and current liquid and gas chromatographic technologies using tandem mass spectrometers for analyses.

Results: A 10-year trend (2006 to 2015) reveals that PDP collected an average of 12,200 samples per year, with fruit and vegetables representing >80%. An average 0.36% of samples exceeded Environmental Protection Agency (EPA) tolerances, demonstrating that >99% of samples didn't contain residues above the safety limits. Selected analyses such as neonicotinoids in honey, fipronil in eggs, cyantraniliprole in milk, and selected trends will be discussed.

Significance: In addition to providing consumers with confidence on foods they consume, the EPA uses PDP data for re-registration of pesticides, thereby enabling growers with better integrated management of pesticides or active ingredients. Considered the "gold standard", PDP data is used in dietary risk assessments and by Codex Alimentarius in establishing maximum residue limits. On several occasions, USDA has used PDP data in trade promotion during negotiations and to advance compliance plans with trading partners.

T8-02 Data Mining for Developing Efficient Food Hazard Sampling Plans

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Introduction: Many government agencies monitor food supplies for chemical hazards with the goal of preventing consumption of foods containing unacceptable hazard concentrations. While random sampling can provide estimates of contaminant prevalence in the food supply, random sampling is not an efficient approach for preventing consumption of foods containing unacceptable hazard concentrations.

Purpose: Data mining was evaluated as a means to develop a targeted sampling approach to improve the ability of government agencies to efficiently identify unsafe foods and protect public health while minimizing the burden on the food industry.

Methods: United States Department of Agriculture Pesticide Data Program (USDA PDP) text files (containing approximately 2.3 million analytical results from approximately 10,000 commodity-location-date sampling events per year) were downloaded into Excel. Input variables included country and state of origin, commodity, commodity type (e.g., fresh, frozen), claim (e.g., organic), and collection month. The finalized Excel files were imported into SAS Enterprise Miner. Target variables were violations and total detects. Decision tree, regression, and neural network models were evaluated for developing a targeted sampling scheme using the USDA PDP database.

Results: Application of the optimal model to this database indicates that the resulting targeted sampling scheme will identify 90% of pesticide violations (397 of 441) by sampling only 35% of the number of samples analyzed (3,565 of 10,187) in the most recent year.

Significance: This presentation illustrates how data mining can be a valuable tool for developing efficient, targeted food hazard monitoring sampling plans. Additionally, interpretation of model output can also provide guidance to businesses that wish to identify foods with a lower probability of containing pesticide residues.

T8-03 Relationship of Metal Concentrations in Soil as Related to Fruit and Leaves of Apple Trees in Selected Orchards in Michigan

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❖ ***Developing Scientist Competitor***

Introduction: Historically, lead arsenate pesticides were commonly used in fruit orchards. Residues of metals from this historical use can persist in soils for decades, which can result in metal contamination in produce grown in these soils. These metals could be a potential risk for humans if their uptake by trees and incorporation into fruits results in concentrations high enough to cause toxicity.

Purpose: This research was conducted to assess lead and arsenic levels in apples, leaves, and orchard soils where the apples were grown to determine the relationship between metal levels in fruits and fruit products versus orchard soils.

Methods: Soil and tree tissues were collected from several Michigan farms and were analyzed using microwave extraction and inductively coupled plasma mass spectrometry. Soil samples were taken at depths of 20 and 40 cm at a distance of one meter from the trunk. Fruits were also processed into juice and pomace fractions to assess the partitioning of arsenic and lead during juice processing.

Results: Lead concentration (µg/kg) was significantly higher in topsoil (9.4±3.4) as compared to that in the subsoil (6.9±2.5); there was no difference in arsenic content between the two soil layers ($P>0.05$). Lead concentrations in apple leaves were significantly correlated to lead in top soil (0 to 20 cm, $P=0.03$), but lead concentrations in juice and pomace were not. Concentrations of total arsenic in all juice samples were less than 1 µg/L and show less potential than lead for uptake and translocation to fruits. There was no significant relationship among arsenic level in juice, residue, and leaf samples versus that in soil ($P>0.05$).

Significance: This was the first study characterizing the relationship between metal concentrations in apple tissues and orchard soils in Michigan. The results indicate that lead and arsenic concentrations in apples from Michigan orchards are unlikely to cause harm to human health.

T8-04 Occurrence of Perchlorate in Bottled Water, Beverages, and Tea from Taiwan Markets by High-performance Liquid Chromatography-Tandem Mass Spectrometry

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Introduction: Perchlorate (ClO_4^-) is a strong oxidizer and has been recognized as a thyroid-disrupting chemical. It has raised significant attention due to its reactivity, occurrence, and persistence in surface water, groundwater, soil, and food. Tea drinking is part of traditional culture in Taiwan, with average annual tea consumption at 2 kg/person. However, data on the occurrence of perchlorate in bottled water, beverages, and tea from Taiwanese markets are sporadic and rarely analyzed.

Purpose: The present study aimed to describe a rapid and simple method for the quantitation of perchlorate in bottled water, beverages, and teas stored in plastic bottles using solid phase extraction clean-up followed by liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS). In addition, the background levels of perchlorate in bottled water, beverages, and teas were also investigated.

Methods: A total of 35 samples of bottled water, tea beverages, and dried tea were purchased from the market or township with the highest food production according to statistics published by the Council of Agriculture and the national food consumption database in Taiwan. All food samples were extracted by deionized H_2O and acetonitrile, purified by Envi-Carb cartridge, and then analyzed by HPLC-MS/MS. Quality assurance/control were consistent with Taiwan Food and Drug Administration regulations.

Results: Concentrations of perchlorate in the samples of bottled water, tea beverages, and dried tea ranged of 0.33 to 0.49 ng/ml, 1.20 to 5.10 ng/ml, and ND-1603 ng/g wet weight, respectively. The analyzed results were consisted with other studies. In addition, the perchlorate levels of locally grown dried tea were significantly higher than those imported from other countries ($P<0.001$). After tea brewing, the migration rate of perchlorate from the dried tea were up to 118%.

Significance: The apparent higher levels of perchlorate were detected in the dried tea which were grown locally. The high migration rates were found after normal brewing.

T8-05 Microfluidic Paper-based Enzyme-linked Immunosorbent Assay for the Rapid and Sensitive Detection of Clenbuterol in Milk

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❖ ***Developing Scientist Competitor***

Introduction: Clenbuterol, a representative veterinary medicine for pulmonary diseases, has been illegally applied as a growth promoter in food-producing animals and has caused food poisonings throughout the food chain. Due to the negative effects in consumers, the maximum residue limit of clenbuterol is 0.05 µg/L in milk. Conventional detection methods such as liquid chromatography-mass spectrometry are accurate and sensitive, but require expensive equipment, complex sample preparation, and highly trained personnel.

Purpose: We aim to develop an affordable, simple, and easy-to-use technique for the detection of clenbuterol in foods.

Methods: Microfluidic paper-based colorimetric enzyme-linked immunosorbent assay (ELISA) was established to detect clenbuterol in milk. Wax printing was conducted to prototype paper-based devices. The device fabrication and antibody immobilization were investigated to optimize assay performance. Further, the limit of detection (LOD) of the developed assay was evaluated in both water and milk samples.

Results: A simple, cost-effective, and sensitive paper-based ELISA device was developed for the colorimetric detection of clenbuterol in water and milk (LOD=0.2 ppb). The total analysis time was about 1 h, which is substantially less than that required by conventional ELISA. Besides, this paper-based ELISA requires less volume of antigen-antibody solution (3 µl) than that of the conventional 96-well plate ELISA.

Significance: This device has the potential to be employed by the dairy industry as a cost-effective and rapid detection technique for preliminary screening of different chemical hazards.

T8-06 Risk Evaluation: Foodborne Titanium Dioxide Nanoparticles Pose Different Magnitudes of Adverse Effects in Obese and Non-obese Mice

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Introduction: Many food products contain engineered inorganic nanoparticles (NPs; e.g., titanium dioxide [TiO_2]) as a part of food additive, due to both unintentional and intentional additions. There has been increasing concern about the health risks associated with foodborne inorganic NPs. However, detailed understanding of potential adverse effects of foodborne TiO_2 NPs is not available despite a limited number of published toxicity studies on TiO_2 NPs.

Purpose: The purpose of this study was to evaluate the adverse effects of foodborne TiO_2 NPs in the obese and non-obese mice.

Methods: Two types of TiO_2 (anatase, 30 and 110 nm) were mixed with mouse diet at a dose of 0.1 wt% and fed to two populations of mice (i.e., high-fat diet-fed obese mice and low-fat diet-fed non-obese mice) for eight weeks. Blood biochemistry, redox state, composition of microbiota, and pro-inflammatory immune cells and cytokines were characterized.

Results: Our results demonstrated that: 1) TiO_2 NPs negatively affected the plasma and tissue parameters related to liver and kidney function with stronger adverse effects in the obese mice than the non-obese mice; 2) TiO_2 NPs, especially those with the size of 30 nm, significantly altered the composition of gut microbiota with stronger alterations in the obese mice than the non-obese mice, and the majority of these alterations were related to obesity and inflammatory bowel disease; and 3) the abundance of pro-inflammatory immune cells and cytokines was significantly increased by TiO_2 NPs in the mouse colonic mucosa, and the magnitude of the increase was much higher in the obese mice than the non-obese mice, indicating stronger pro-inflammatory responses to TiO_2 NPs in the obese mice than non-obese mice.

Significance: Overall, our fingdings provided new perspectives on the potential adverse effects of foodborne TiO_2 NPs among populations with different obesity status.

T8-07 *Listeria monocytogenes* Cell Envelope Physiology is Affected by Exposure to Dairy-Relevant Conditions

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Introduction: Most interventions used to control *Listeria monocytogenes* target the cell envelope. It is unclear, however, if changes in the environment will affect the physiology of the cell envelope and, in turn, the cell's sensitivity to cell envelope-acting antimicrobials.

Purpose: Determine if varying dairy-relevant conditions affect the physiological state of *L. monocytogenes* cell envelope.

Methods: Cells were grown on skim milk agar at different pH values (5.7 to 6.5) or temperatures (6 to 30°C). The cells were collected, washed, and standardized to a specific OD_{600}. Phage and cytochrome c binding assays were performed on collected cells. Phages LP-048 and LP-125 were used, as they bind to specific cell surface residues (*N*-Acetylglucosamine and rhamnose, respectively). Values reported from phage-binding experiments are the log reduction of PFU in the supernatant (i.e., approximate phage binding). Cytochrome c was used to measure cell wall charge as a negative charge indicator. Cytochrome c binding values reported are the reduction in OD_{600} due to cytochrome c binding LM.

Results: As pH increased from 5.7 to 6.5, phage binding increased from 0.76 to 0.90 (LP-048) and 0.38 to 0.62 (LP-125). Phage binding and pH were found to have a positive relationship for both phages ($P<0.05$). In the temperature study, LP-048 showed similar binding at 6 and 30°C (0.59- to 0.64-log reduction), but significantly greater binding at 14°C (0.95-log reduction). For cytochrome c binding, significant differences in binding were observed for both *L. monocytogenes* strains between 6 and 14°C. There was also a significant difference in cytochrome c binding observed between strain F2365 grown at pH 6 and 6.5.

Significance: The physiological state of *L. monocytogenes*' cell envelope is affected by dairy-relevant cell growth conditions. As the cell envelope is an important target of antimicrobials and other control interventions, these data suggest that control interventions can be targeted to specific conditions for greater impact.

T8-08 The Role of Farm and Bedding Practices in Reducing Mesophilic and Thermophilic Spore-Forming Bacteria Levels in Bulk Tank Milk on Dairy Farms in the United States

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Introduction: Mesophilic and thermophilic spore-forming bacteria are concerning to the dairy industry due to their ability to survive adverse conditions and eventually spoil value-added dairy products. Producers are facing increasing pressure to control spore-forming bacteria in raw milk at the farm level. Bedding of lactating cows has been targeted as a source for sporeformers in raw milk.

Purpose: Identifying the impact of farm and bedding practices and levels of sporeformers in unused and used bedding on the levels of mesophilic and thermophilic sporeformers in bulk tank raw milk (BTM), which will allow for tangible recommendations to producers to address reduction of BTM spore counts.

Methods: Farm and bedding management surveys were administered and unused bedding, used bedding, and BTM samples were collected from 189 dairy farms one to two times over 1 year. Bedding suspensions and raw milk were spore pasteurized (80°C for 12 min), followed by enumeration of mesophilic and thermophilic sporeformers. Structural equation modeling (SEM) analysis was used to determine direct and indirect pathways of causality among farm and bedding practices, levels of spores in unused and used bedding, and levels of spores in BTM.

Results: SEM confirmed that bedding material directly contributed to mesophilic and thermophilic sporeformer levels in unused and used bedding. Bedding practices contributed to unused and used bedding sporeformer levels for certain housing types. Notably, levels of mesophilic and thermophilic sporeformers in used bedding were positively related to those in BTM. Use of organic bedding material was indirectly associated with higher thermophilic sporeformer levels in BTM. Different farm practices were also confirmed to contribute to BTM mesophilic and thermophilic sporeformer levels.

Significance: This study revealed that used bedding directly and indirectly contributes to sporeformer levels in raw milk and identified practices that farmers may implement to produce low-spore raw milk, which will expand the raw milk supply for extended shelf life dairy products.

T8-09 Historical Overview of Fluid Milk-Related Incidents in California (1996 to 2017)

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Introduction: Dairy products represent the second most frequent source of foodborne illness reported in the US. The California Department of Public Health Food and Drug Branch performed a review of milk-related incidents, including outbreaks, in California from 1996–2017.

Purpose: To provide a historic overview of trends across California's milk-related incidents and conduct whole genome sequencing (WGS) analyses on isolates from environmental and product samples collected during investigations.

Methods: Environmental, laboratory, and epidemiologic information for all 28 California-linked incidents involving fluid milk was compiled and descriptive analyses were performed using SAS. WGS data was received for 187 isolates [*Campylobacter jejuni* (*n*=76), Shiga toxin-producing *Escherichia coli* (*n*=100), *Salmonella* (*n*=9), and *Listeria monocytogenes* (*n*=2)] from these incidents. CDC's PulseNet performed whole-genome multilocus sequence typing (wgMLST) analysis for *C. jejuni* isolates using BioNumerics and the Food and Drug Laboratory Branch was addressing the remaining pathogens.

Results: There was a total of 28 milk-related incidents in California from 1996-2017. Confirmed incidents (17; 61% of total) included 12 (71%) outbreaks, three (18%) complaints, and two (12%) surveillance. Most confirmed incidents (94%; 16/17) were associated with raw milk. Raw milk outbreaks constituted 59% (10/17) of confirmed incidents and, of those for which age range was known, 67% (6/9) involved cases that were one year old or younger. Fourteen confirmed incidents involved microbial hazard: seven (50%) associated with *C. jejuni*, four (29%) with *E. coli* O157:H7, two (14%) with *Salmonella*, and one (7%) with *L. monocytogenes*. *C. jejuni* strains were unrelated across outbreaks and WGS results matched pulse-field gel electrophoresis for 91% (69/76) of isolates.

Significance: Over 90% of milk-related incidents in California involved raw milk. Sale of raw milk is legal within California and trends across California's raw milk outbreaks mirror national ones. As raw milk consumption increases, it is essential to enhance prevention and response efforts.

T8-10 Determining the Efficacy of Protective Cultures for the Control of *Listeria monocytogenes* and Non-O157 Shiga Toxin-producing *Escherichia coli* in Raw Milk for Cheesemaking

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Introduction: Listeria monocytogenes and non-O157 Shiga toxin-producing *Escherichia coli* (STEC) are significant hazards in the production of cheese when using unpasteurized milk. Commercially produced protective cultures (PC) of lactic acid bacteria represent actionable natural interventions to these and other pathogens in cheese.

Purpose: The objective of this study was to determine the efficacy of two PCs to control the growth of *L. monocytogenes* and non-O157 STEC in raw milk when co-inoculated and exposed to a typical cheesemaking temperature profile.

Methods: Raw milk inoculated with six-strain cocktails of either *L. monocytogenes* or STEC at ~2 log CFU/mL were incubated overnight at 4°C. Freeze-dried PCs (*Lactobacillus plantarum* [LPAL] or *Lactobacillus rhamnosus* [LRB]) were either incubated overnight in reconstituted skim milk (RSM) prior to use or added directly to contaminated raw milk. In each case, PCs were added at ~8 log CFU/mL. Raw milk containing both pathogen and PC were then incubated under two conditions: 35°C for 24 h or a simulated cheesemaking temperature profile of 4 h at 35°C followed by 20 h at 20°C.

Results: Neither PC, added directly or in RSM, substantially inhibited STEC growth under either temperature condition. Direct inoculation of LPAL inhibited *L. monocytogenes* growth to ≤0.5 log CFU/ml after incubation under both temperature conditions. Pre-incubation in RSM enhanced antilisterial activity through the gradient incubation, reducing *L. monocytogenes* counts by >1 log CFU/ml from initial inoculation. Although LRB was ineffective when added directly, final *L. monocytogenes* counts were 1.6 log CFU/ml less than control at the end of the gradient incubation when added in RSM.

Significance: This work demonstrates the effectiveness of the PCs LRB and LPAL for the control of pathogens in an intended dairy matrix (raw milk) and under simulated use parameters (temperature and time). Differences in effectiveness based on pathogen and culture inoculation method inform future applications in the production of cheese and other dairy products.

T8-11 Inhibition of *Listeria monocytogenes* in a Model Cheese System Based on pH, Moisture, and Acid Type

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Introduction: High-moisture, low-acid cheeses have been shown to support *Listeria monocytogenes* growth during refrigerated storage. Previous studies have suggested that certain acids confer greater antilisterial activity than others, and that cheeses with lower pH vaues (e.g., 5.2) delay growth longer than cheeses with higher pH values (e.g., 5.8); however, no standard pH value for *Listeria* control exists.

Purpose: The purpose of this study was to determine the effect of pH, moisture, and acid type on the growth of *L. monocytogenes* in a model cheese system.

Methods: Cream, micellar casein, water, salt, lactose, and acid (citric, lactic, acetic, or propionic) were combined in 32 formulations to four pH values (5.25, 5.50, 5.75, and 6.00) and two moisture levels (50 and 56%). Each formulation was inoculated with 3-log CFU/g *L. monocytogenes* (five-strain mixture). Samples were vacuum-sealed and stored at 4°C for 8 weeks, with triplicate samples enumerated on modified Oxford agar weekly. All formulations were tested in duplicate trials and time to 1-log growth determined from average growth curves.

Results: Cheeses formulated to pH ≥5.25 with citric acid supported growth (≥1-log increase) of *L. monocytogenes* within 2 weeks, whereas no growth was observed at pH 5.25 for lactic acid and at pH ≤5.75 for acetic and propionic acids for 8 weeks. Cheeses formulated with lactic acid to 50% moisture and pH 5.50 delayed growth an additional 2 weeks compared to 56% moisture; moisture did not affect growth at pH ≥5.75 with lactic acid. Cheeses formulated to 50% moisture and pH 6.00 supported growth within 0.5, 1.0, 2.5, and 3.5 weeks when formulated with citric, lactic, acetic, and propionic acids, respectively.

Significance: These data suggest propionic > acetic > lactic > citric acids in antilisterial activity within the developed model cheese system and can be used in formulating safe high-moisture cheeses.

T8-12 Quantitative Risk Assessment of Listeriosis from Traditional Brazilian Minas Artisanal Semi-hard and Fresh Soft Cheeses

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Introduction: Traditional Minas cheeses are very popular in Brazil. These cheeses are of two types: artisanal ripened semi-hard cheeses (HC) produced with raw milk and refrigerated fresh soft cheeses (SC) generally produced with pasteurized milk.

Purpose: This study estimates the risk of listeriosis due to HC and SC consumption using quantitative microbial risk assessment (QMRA).

Methods: QMRAs were developed for both cheese types. The HC model contained a module for predicting *Listeria monocytogenes* decline during ripening. The SC model contained a refrigerated storage module for predicting *L. monocytogenes* growth during storage. HC modeling scenarios varied *L. monocytogenes* starting concentration over -2.4 to 6 log CFU/ml in raw milk and three ripening times (4, 22, and 60 days). SC modeling scenarios varied *L. monocytogenes* starting concentration (-2.4 to 4 log CFU/ml in milk). Inclusion of antilisterial lactic acid bacteria (LAB) was also examined. Simulations (100,000 iterations per scenario) were carried out using the @Risk add-in for Excel.

Results: Aging HC reduced risk, and risk was influenced by *L. monocytogenes* starting concentration. Aging 22 days with inhibitory LAB reduced risk more than 4 million-fold when *L. monocytogenes* was assumed to be 6 log CFU/ml in raw milk, and was lower risk than HC made without LAB and with a starting concentration of 1 log CFU/ml in raw milk. Inclusion of inhibitory LAB reduced risk of listeriosis from SC, but not as dramatically as for HC. Relative risk to vulnerable populations reduced 4.4 and 3.4-fold when *L. monocytogenes* starting concentrations were 1 log and 4 log CFU/ml in milk, respectively.

Significance: The results of the QMRA predict that consumption of contaminated Minas cheeses can present a high risk of listeriosis, especially for vulnerable populations. Scenario analyses indicate that aging of HC and inclusion of LAB in HC and SC are effective risk mitigation measures.

T9-01 The Identification of *Cronobacter sakazakii* and Its Traceability by Matrix-assisted Laser Desorption Ionization Time of Flight Mass Spectrometry

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Introduction: Infant formula powder is contaminated very easily by *Cronobacter sakazakii* if the production process is not controlled strictly. *Cronobacter sakazakii* is associated with neonatal diseases, including necrotizing enterocolitis (NEC), meningitis, and sepsis. Due to the limits of conventional methods, the rapid efficient microbial methods are developed for the *Cronobacter sakazakii* detection and its traceability.

Purpose: *Eighteen Cronobacter sakazakii* strains were isolated from the powder by a lab, two *Cronobacter sakazakii* reference strains, one *Enterobacter cloacae* reference strain and one *Citrobacter freundii* reference strain were identified using VITEK® MALDI-TOF MS, with the aim to research on the influence of sample preparation procedures on *Cronobacter sakazakii* discrimination and its traceability.

Methods: All the strains were prepared respectively by three different preparation procedures, including direct transfer method, formic acid extraction method, and formic acid-acetonitrile extraction method, and analyzed by VITEK MS, the results were compared with biochemical ones. Then the influence of sample preparation procedures on the identification results was evaluated, and the traceability of 20 *Cronobacter sakazakii* strains was also researched by the cluster analysis of VITEK MS.

Results: The 20 strains were identified by VITEK MS as *Cronobacter sakazakii*, which were in agreement with biochemical results and distinguished from *Enterobacter cloacae* and *Citrobacter freundii*. There was no significant difference in the results confidence among the three different preparation procedures, but the number of mass peaks and their relative intensities showed remarkable difference. The formic acid extraction method performed well on the number of mass peaks and their range. At similarity level of 80%, the 20 strains prepared by the formic acid extraction method were classified into 5 clusters by the cluster analysis of VITEK MS, and the contaminated pathway of the *Cronobacter sakazakii* isolated strains were also speculated.

Significance: The formic acid extraction method is more suitable for the identification of *Cronobacter sakazakii* by MALDI-TOF MS, which will be a potential technique for the *Cronobacter sakazakii* detection and its traceability.

T9-02 The United States Department of Agriculture Food Safety and Inspection Service Beef and Veal Carcass Baseline Survey

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Introduction: The Food Safety and Inspection Service (FSIS) within the United States Department of Agriculture (USDA) conducted the Nationwide Microbiological Beef and Veal Carcass Baseline Survey from August 2014 to December 2015.

Purpose: The purpose of the survey was to estimate the percent positive for pathogens *Salmonella, Escherichia coli* O157:H7, and non-O157 Shiga toxin-producing *E. coli* (non-O157 STEC) and to detect the presence of indicator organisms generic *E. coli*, aerobic plate count (APC), *Enterobacteriaceae*, and total coliforms.

Methods: The survey generated 2,736 carcass samples for beef and 548 carcass samples for veal from 149 establishments under FSIS. Samples were collected using sponge sampling methods at two points of the slaughter process (post-hide removal and pre-chill) from the same carcass halves; one half (4,000 cm^2 for beef and 2,000 cm^2 for veal) was sampled at post-hide-removal and the other half at pre-chill. Two sponges (posterior and anterior) for each sample location were composited and added to a final volume of 50 ml buffered peptone water.

Results: At post-hide, *Salmonella* was the highest for beef at 27.12% and non-O157 STEC was the highest for veal at 23.72%. At pre-chill, *Salmonella* was the highest for beef at 3.36% and non-O157 STEC was the highest for veal at 9.85%. For indicator organisms, at post-hide, APC was the highest for beef at 99.48% and for veal at 99.63%. At pre-chill, APC was the highest for beef at 80.50% and for veal at 75.00%. The national prevalence for *Salmonella* in beef was 0.72% and the national prevalence for non-O157 STEC in veal was 8.54%.

Significance: The data collected may be used in USDA risk assessments, to inform policy, or to develop guidance to industry on process controls.

T9-03 Prevalence of *Salmonella* in the Environment of New Zealand Egg Layer Farms

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Introduction: In contrast to other countries, New Zealand has a low incidence of egg-associated salmonellosis. This may reflect a low level of contamination of eggs and the environment in which eggs are produced. However, although routine testing for *Salmonella* is carried out by some layer farms, there is no nationally representative information available on *Salmonella* prevalence in the New Zealand egg production environment.

Purpose: This study sought to determine the prevalence and serotype(s) of *Salmonella* in the New Zealand egg production environment (caged, barn, and free-range), and potential sources of *Salmonella* contamination (e.g., feed).

Methods: A microbiological survey for *Salmonella* was undertaken in the egg production environment of commercial egg layer farms throughout New Zealand. Samples were collected from feed (n=33), laying sheds (faeces, dust, and boot/manure belt swabs; n=67) and packhouse egg contact surfaces (n=87).

Results: Salmonella was detected in 43 (13.3%) of 323 samples. Pooled dust samples had the highest prevalence (19 of 67, 28.4%), followed by boot/manure belt swabs (11 of 67, 16.4%), pooled faeces (7 of 67, 10.4%), and packhouse egg contact surfaces (5 of 87, 5.7%). Only one feed sample tested positive (3.0%), which may have been contaminated from the shed. Samples from caged farm environments were contaminated with *Salmonella* (33 of 75, 44.0%) more than cage-free (free-range or barn) systems (4 of 126, 3.2%) (P<0.001). Packhouse samples were positive only on farms with a high laying shed prevalence, and isolates were genetically related, suggesting cross-contamination between shed and packhouse surfaces. *Salmonella* serotypes included Infantis, Thompson, Typhimurium, Anatum, and Mbandaka. New Zealand has historically been considered free of *Salmonella* Enteritidis, which was not isolated in this survey.

Significance: Survey results establish a useful benchmark for *Salmonella* prevalence in the New Zealand egg production environment and serve as a reference point for assessing the impact on *Salmonella* prevalence resulting from changes to regulations or egg industry practices.

T9-04 Fate of *Salmonella* species within Refrigerated Ground Turkey Cooked in a Frying Pan

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❖ ***Developing Scientist Competitor***

Introduction: Poultry ranks first for estimated annual disease burden from its association with *Campylobacter* species and *Salmonella enterica*. From 1998 to 2012, 25% of outbreaks (279 of 1114) were associated with poultry. A multistate outbreak occurred in 2011 from the consumption of ground turkey across 34 states causing 136 persons to become infected.

Purpose: The purpose of this study was to evaluate the fate of *Salmonella* spp. in refrigerated ground turkey patties when cooked on consumer-scale frying pans.

Methods: Two batches of freshly formulated ground turkey (99% lean, 1% fat) were purchased directly from a local butcher. The meat was inoculated with a 9-strain cocktail of *Salmonella* spp. (ca. 6.3 log CFU/g) and then formed into weighing 112 g and 255 g. Patties were cooked in a non-stick frying pan using 15 or 30 ml of canola oil to final target internal temperatures of 135°, 150°, 165°, or 180°F. Internal temperature of the patties was measured throughout cooking with two thermocouples Type-J inserted close to the geometric center. After cooking, eight additional temperature points were taken using a handheld thermocouple throughout the patty.

Results: As expected, the higher the target internal temperature, the higher the volume of oil, and the thinner the patty, the greater the reduction of *Salmonella spp*. Pathogen reductions of ca. 2.8 to 6.0 log CFU/g were achieved in 144 turkey patties cooked to target internal temperatures of 135° to 180°F (36 at each target temperature). The average final internal temperature was ca. 142°, 158.2°, 171.5°, and 185.9°F for patties cooked to a target internal temperature of 135°, 150°, 165°, or 180°F, respectively.

Significance: These data validated that cooking turkey patties on a non-stick frying pan to the recommended target internal temperatures, that being ≥165°F, was sufficient to achieve a 5.0-log reduction of *Salmonella*. The data also suggests the importance of using a thermometer to ensure that the minimum endpoint required temperature is achieved.

T9-05 Evaluation of Cinnamaldehyde and Lactic Acid Spray Wash Formulations for Pre-slaughter Cattle for Meat and Hide Decontamination and Quality

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Introduction: Bacterial contamination on the hide of cattle serves as a significant challenge to the meat industry and food safety. Cattle serves as a host to several enteric pathogens. During hide removal from the meat of the cattle carcass, there is a high probability of cross-contamination from pathogens, thus increasing chances of food-related illnesses. To prevent cross contamination, cattle are frequently washed during processing. However, wash formulations may degrade quality of value added byproducts of the meat industry, such as hides, which are commonly processed into leather.

Purpose: This study was designed to evaluate the effectiveness of spray washing with cinnamaldehyde and cinnamaldehyde+lactic acid formulations to reduce pathogens of food safety concerns and concurrently investigate the formulations' effects on hides.

Methods: Samples of fresh hides (10 cm^2 each) from processing facilities were treated with 0.50 or 0.75% cinnamaldehyde, 0.50% cinnamaldehyde+0.50% lactic acid and 0.75% cinnamaldehyde+0.75% lactic acid (n=3 each). Hide samples were swabbed and plated within 5 min after antimicrobial applications on tryptic soy agar, sorbitol MacConkey agar (SMAC), cefixime tellurite SMAC, and XLT-4 for aerobic bacteria, enterobacteriaceae and related gram negative bacilli, *Escherichia coli*, and *Salmonella* counts, respectively. Leather was produced after treatment for mechanical and subjective property evaluations.

Results: There were significant differences between levels of mean values within solution treatments on all test bacteria counts (P≤0.001). The greatest mean log reductions were of 2.81 log CFU for total aerobic (P≤0.001), 2.06 log CFU for enterobacteriaceae (P≤0.001), 1.69 log CFU for *E. coli* (P<0.05), and 0.84 log CFU (P<0.05) for *Salmonella* using 0.75% cinnamaldehyde, 0.75% cinnamaldehyde+0.75% lactic acid, 0.50% cinnamaldehyde, and 0.50% cinnamaldehyde+0.50% lactic acid, respectively. Subjective and mechanical testing revealed no major differences during treatment.

Significance: Carcass pathogen intervention systems have been studied; however, limited intervention systems have concurrently investigated the food safety and byproduct quality aspects developed by cattle wash systems.

T9-06 Comparative Genomics Analysis of Multidrug-resistant *Salmonella* Dublin from Sick Cattle and Retail Meats in the United States

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Introduction: Salmonella Dublin is a host-adapted serotype associated with typhoidal disease in cattle. Although rare in humans, it usually causes severe illness, including bacteremia. In the United States, *Salmonella* Dublin has also become one of the most multidrug-resistant (MDR) serotypes of *Salmonella*.

Purpose: This study is to investigate the genetic makeup of its virulence and resistance features.

Methods: Sixty-one strains of *Salmonella* Dublin (49 from sick cattle and 12 from retail meats) were selected for sequence analysis using the Illumina MiSeq, and five strains were closed genomes using the PacBio sequencing platform. Eight human strains of *Salmonella* Dublin genomic data were downloaded from the National Center for Biotechnology Information for comparative analysis.

Results: Fifteen pathogenicity islands (PIs) and *spv* operons (*spvRABCD*), which encode important virulence factors, were identified in all 69 *Salmonella* Dublin strains. All 15 PIs were located on the chromosome of the five closed genomes, with each of these isolates also carrying one or two plasmids with sizes between 36 and 329 kb. Multiple resistance genes, including bla_{CMY-2}, bla_{TEM-1B}, *aadA*12, *aph(3')-Ia, aph(3')-Ic, strA, strB, floR, sul1, sul2, tet(A)*, and *spv* operons were also identified on these plasmids. Comprehensive resistance genotypes were determined, including 20 genes encoding resistance to five different classes of antimicrobials and two housekeeping gene (*gyrA* and *gyrB*) mutations associated with resistance or decreased susceptibility to fluoroquinolones. Together these data revealed that *Salmonella* Dublin commonly carried 15 PIs, MDR/virulence plasmids, and several classes of resistance genes.

Significance: Genomic structure may contribute to the severity of disease and failure of treatment in both humans and cattle.

T9-07 Off the Radar: Identifying Food Safety Practices and Educational Resource Needs of Small Farm Owners and Processors Exempt from the Food Safety Modernization Act

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Introduction: Many small farmers and processors are either excluded or exempt from the regulations of the U.S. Food and Drug Administration's Food Safety Modernization Act (FSMA), but these food producers provide food to many consumers through direct markets and small wholesale accounts. Even if local food producers are subject to FSMA regulations, small farm and food processing operations may lack the resources to comply with the rule.

Purpose: The purpose of this study was to identify the food safety practices and resource needs of small farm owners and food processors who supply local markets.

Methods: A needs assessment survey was built to gather food safety perceptions of local food producers, to assess if the food safety practices they have implemented align with FSMA requirements, and to identify resources that local food producers want to assist them with the implementation of food safety practices. Additionally, the survey provided an opportunity for local food producers to share information about food safety trainings, audits, limitations to implementing food safety practices, and preferred methods of outreach and learning. The survey was distributed nationally through Local Food Safety Collaborative partnership channels, both electronically and by paper.

Results: A total of 1,176 surveys were submitted with 599 respondents reporting that they sell over 50% of their food locally, defined as within 275 miles from their farm or facility. Sixty-two percent of local-serving farmers indicated they did not have farm food safety plans, while 53% had not had a 3rd party audit since January 2016. Three hundred forty-seven (347) respondents reported gross annual sales under $24,999, representing a significant pool of FSMA exempt food operations.

Significance: This is the first national survey designed to identify the food safety practices and needs of local food producers, including farmers and processors. Findings further clarify local food producer needs and the impact they have on the food supply.

T9-08 Persistence of Generic *Escherichia coli* and Enteric Pathogens in Blueberry Preharvest and Post Harvest Environments

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Introduction: Blueberry producers in the southeastern United States (U.S.) rely on overhead irrigation to protect blossoms and fruit during freeze events. Surface waters are typically used. Blueberry contamination with human pathogens from surface waters could pose a significant risk to consumers.

Purpose: To establish the baseline microbial quality of surface waters used in blueberry production and to determine the survival of generic *Escherichia coli* from blossom to the packinghouse.

Methods: Blueberry blossoms were inoculated during freeze events with generic *E. coli* (rifampin-resistant at 50 mg/ml, EcW778) at log 5 CFU/ml. Water used during freeze events came from two different reservoirs. Inoculation took place through the two independent irrigation systems. The microbial quality of each reservoir was established for eight months at 25-day intervals. The presence and persistence of enteric pathogens and EcW778 was determined at three- to four-day intervals for 15 days, and at 45 and 75 days post-inoculation (DPI) from blossoms, berries, and the harvester/packinghouse (swabbing). Sanitation practices were implemented to remove microbial contaminants.

Results: Both reservoirs met generic *E. coli* limits established by the Produce Safety Rule of the U.S. Food and Drug Administration's Food Safety Modernization Act. Both reservoirs were contaminated with Shiga toxin-producing *E. coli* (STEC) and *Salmonella*; however, greater prevalence ($P<0.05$) was observed in one reservoir (STEC 100% versus 50% and *Salmonella* 50% versus 0%). The concentration of EcW778 decreased over time and was only detected by enrichment after 15 DPI. A linear die-off rate of log 0.3 CFU per day was established for EcW778. At harvest (75 DPI), all nine 150-g blueberry samples were positive for EcW778 after enrichment. Harvester (45%) and packinghouse (25 to 50%) swab samples were positive for EcW778. STEC and *Salmonella* were present in 2 and 1.5% respectively and were not associated with a specific reservoir. A comprehensive sanitation process eliminated the presence of EcW778, STEC, *Salmonella*, and *Listeria* spp.

Significance: Results indicate that strain EcW778 could persist for more than 75 days and was transferred from fruit to the harvester and the packinghouse.

T9-09 Metagenomic Characterization of Alfalfa Sprout Spent Irrigation Water from *Salmonella*-contaminated Seeds

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Introduction: An increasing number of foodborne disease outbreaks have been associated with the consumption of raw sprouts since 2000. Microbial testing of sprout spent irrigation water (SSIW) is an important part of a multi-hurdle strategy to enhance sprout safety.

Purpose: In this study, a metagenomics approach was used to characterize the temporal changes of the microbial community in SSIW to provide insight into dynamic interactions between *Salmonella* and sprout microbiota.

Methods: Alfalfa seeds were contaminated with *Salmonella enterica* serovar Cubana at varying levels (0, 0.2, 2, and 10^4 CFU/g of seed) and sprouted in an Easy Sprout sprouter. SSIW was collected at 0 h after 8 h of soaking, and every 4 h between 24 and 48 h. Genomic DNA from filtered SSIW was extracted and shotgun sequenced. Sequencing data was analyzed with CosmosID to characterize the bacterial community. SSIW and alfalfa sprouts were also evaluated for *Salmonella* contamination by the most probable number method and direct plating.

Results: At 0.2 CFU/g inoculation level, *Salmonella* population remained similar during the entire sprouting process, while a 2- to 20-fold increase in *Salmonella* was observed at 2 CFU/g. Shotgun metagenomic analysis revealed a core SSIW microbiome comprising few bacterial genera dominated by *Klebsiella*, *Enterobacter*, *Pantoea*, and *Cronobacter*, with a strikingly high relative abundance (RA; 90.0±6.9%) across all sampling points and inoculation levels. The RA of *Pantoea* decreased drastically from more than 45 to below 10% in the first 24 h of sprouting at all levels except 10^4 CFU/g. Shifts in *Salmonella* relative abundance were observed between 24-h and 32-h time points. Slight decreases in *Salmonella* RA occurred after 32 h of sprouting.

Significance: The data suggest a dynamic interaction between *Salmonella* and the microbial community in SSIW. The microbial community pattern observed in SSIW may suggest their functional importance in this dynamic.

T9-10 Thermal Inactivation of *Salmonella* Surrogate and Indicator Microorganisms in Turkey Litter Compost during Physical Heat Treatment Process: A Plant Validation Study

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❖ ***Developing Scientist Competitor***

Introduction: Turkey litter compost, commonly used as a biological soil amendment, was subjected to a commercial physical heat treatment to reduce human pathogens such as *Salmonella*. In order to validate the effectiveness of this commercial thermal processing, the thermal inactivation of a *Salmonella* surrogate/indicator was investigated.

Purpose: The objective of this study was to quantify the effects of thermal processing on populations of the *Salmonella* surrogate *Enterococcus faecium* NRRL B2354 and presumptive indigenous enterococci in a commercial turkey litter compost.

Methods: E. faecium was inoculated at ca. 7 log CFU/g into composted turkey litter adjusted to 36 and 44% moisture. Tyvek pouches containing 50 g of inoculated compost were placed into customized metal baskets and run through an industrial dryer with an inlet temperature of >400ºC and outlet temperature of >65ºC. Compost samples before and after heat treatment collected from the processing line were served as process controls. All samples were shipped with cold packs overnight to the laboratory for physical, chemical, and microbiological analyses. Four plant trials in three different seasons were performed with two separate runs per trial, each with 5 to 6 samples.

Results: The median residence times of the compost samplers inside the driers for the four trials were 51, 45, 43, and 35 min. In all four trials, a >5.5-log reduction of *E. faecium* was achieved after physical heat treatment in the processing dryer. Additionally, there was a >3-log reduction of presumptive indigenous enterococci inside the Tyvek bags, a level similar to the log reduction observed in the process control samples.

Significance: Because the heat resistance of the *E. faecium* surrogate is greater than that of *Salmonella*, the industrial drying of turkey litter compost is sufficient to eliminate enteric pathogens even if they were present at levels well in excess of 5.5 log.

T9-11 Changes in Susceptibility to Ciprofloxacin and Ceftriaxone in Epidemic *Salmonella enterica* Strains after Exposure to Simulated Gastrointestinal Conditions in Chicken Breast

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Introduction: Salmonella enterica is estimated to cause 93.8 million infections and 155,000 deaths worldwide each year. Epidemic *S. enterica* strains frequently acquire multiple drug resistance phenotypes. Ciprofloxacin (CIP; fluoroquinolone) and ceftrixone (CTX; third-generation cephalosporin) are currently the antibiotics of choice to treat salmonellosis. Emergence of CIP- and CTX-resistant *Salmonella* strains is a cause for global concern.

Purpose: This study evaluates the effects of exposure of epidemic *S. enterica strains* in chicken meat to simulated gastrointestinal conditions on CIP and CTX susceptibility.

Methods: The MIC of CIP (64 to 0.008 µg/ml) and CTX (512 to 0.01 µg/ml) were determined against 15 distinct human outbreak-linked *S. enterica* serovars by microdilution broth (brain heart infusion) assay before and after exposure to simulated gastrointestinal conditions. The MIC data were interpreted according Clinical and Laboratory Standards Institute breakpoint values (µg/mL). In vitro digestion was performed at 37°C by exposing each strain in chicken breast (20 g; inoculated at 5 log CFU/g) to a nine-step continuous system simulating the mouth (1st step), esophagus to stomach (2nd to 7th step), duodenum (8th step), and ileum (9th step) conditions. Mechanical agitation was used to mimic the conditions in each digestive compartment.

Results: Three strains were CIP-susceptible (≤0.06) and 12 strains showed reduced susceptibility to CIP (≥0.125 to 0.5 µg) before in vitro digestion. Four strains were CTX-resistant (≥4 µg/ml) and 9 strains were CTX-susceptible (≤1 µg/mL), while two strains showed CTX-intermediary (2 µg/ml) phenotype before in vitro digestion. After exposure to simulated gastrointestinal conditions, the 12 strains with reduced susceptibility to CIP showed CIP-resistant phenotypes. One CTX-intermediary strain showed a CTX-resistant phenotype and two CTX-susceptible strains showed CTX-intermediary phenotypes after in vitro digestion.

Significance: The exposure to simulated gastrointestinal conditions may change the susceptibility of epidemic *S. enterica* strains to CIP and CTX and should be considered in the selection of antibiotic therapies for human salmonellosis.

T9-12 Die Off Kinetics and Preharvest Intervention Practices to Reduce Contamination of *Enterohemorrhagic Escherichia coli* (EHEC) and Shiga Toxin-producing *E. coli* (STEC) from Cilantro Surfaces

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Introduction: The Food and Drug Administration (FDA) since 2004 has reported the presence of *Salmonella* and other human pathogens in over 28 samples of fresh cilantro from both U.S. and non-U.S. origin. Concerns specific to growing and harvesting practices in cilantro and the need for strategies to remediate and/or prevent contamination close to harvest when using surface or well water for irrigation are necessary to improve consumer safety.

Purpose: To evaluate die-off kinetics of surrogate and pathogenic EHEC-STEC strains in cilantro leaves and potential preharvest remediation practices to reduce pathogen contamination of this herb.

Methods: Cilantro was grown following commercial practices and then transferred to a BSL2 greenhouse for inactivation studies. Generic E. coli-W778 (*Ec*778), pathogenic and avirulent *E. coli* O157:H7 (*Ec*O157), *E. coli* 045 (EcO45) and *E. coli* 0111 (*Ec*O111) were spray and spot inoculated at log 6.0 CFU/mL and log 3.0 CFU/mL. Bacterial recovery was performed at 0, 2, 5 and 7 days post-inoculation (DPI) by direct plating on ChromAgar O157, ECC and STEC amended with rifampicin or kanamycin at 50 mg/mL and/or after enrichment with mEHEC. At 4-DPI plants were treated with chlorine (100ppm), peroxyacetic acid (PAA, 40ppm) and sterile water.

Results: Similar die-off kinetics and linear rates were observed between *Ec*O157 and *Ec*778. Linear die-off rates were 0.67 and 0.37 log CFU/day at the high and low inoculum dose ($P < 0.05$). Linear die-off rates were different at both inoculum doses for *Ec*O111 and EcO45 (0.75 and 0.62 log CFU/day, respectively, ($P < 0.05$), high inoculum dose). All die-off kinetics were best described by double Weibull models (biphasic inactivation kinetics). No significant difference ($P > 0.05$) in strain inactivation was determined between Chlorine and PAA.

Significance: Linear die-off rates for all pathogens varied with inoculum dose and did not adjust to current in field die-off rates listed by the Produce Safety Rules.

T10-01 A Comparison Study between Conventional and Mathematical Modeling on the Antimicrobial Effect of Cinnamon Oil, Encapsulated Curcumin, Zinc Oxide Nanoparticles and Their Combinations against Foodborne Pathogens

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❖ ***Developing Scientist Competitor***

Introduction: No single validated method for studying antimicrobial interactions is available. Over the past decade, more than 60% of the dual antimicrobial studies used the fractional inhibitory concentration index (FICI) method and 36% applied the time-killing method. Each method generates different outcomes that might not be comparable with each other. Most importantly, synergy between antimicrobials can be over- or under-estimated, resulting in misleading decision after further research and development. Mathematical modeling can offer a better accuracy and be used not only to identify synergism, but also to evaluate interactions at different levels of antimicrobial potency (MIC or MBC), which provides more precise and meaningful outcomes for the application, stress response, or mechanism studies of the synergistic combinations.

Purpose: The aim of this study is to discover an antimicrobial synergistic effect against *Campylobacter jejuni*, a leading foodborne pathogen that causes human gastroenteritis, by cinnamon oil, encapsulated curcumin, and zinc oxide nanoparticles (ZnO NPs).

Methods: We compared three approaches to study the antimicrobial interactions including time-killing method, FICI method, and a mathematical concentration-effect model. Nonlinear isobologram analysis was performed to evaluate the synergy in different combinations, and a median-effect equation was applied to identify the combinations of synergistic effects at median, bacteriostatic, and bactericidal reduction levels.

Results: The time-killing method overestimated the synergistic interaction between antimicrobials, while the FICI method failed to detect an existing synergistic phenomenon. This lack of accuracy and sensitivity was mainly due to combining antimicrobials based on their MICs or sub-MICs without comprehensive understanding of their concentration-effect curves. Our results showed that each targeted antimicrobial had a unique concentration-effect relationship. Specifically, encapsulated curcumin showed a sharp sigmoidal curve, while cinnamon oil and ZnO NPs had a hyperbolic curve. A mathematical model was successfully constructed to study the interaction between antimicrobials with different shapes of concentration-effect curves. Although some binary combinations showed different interactions, the tertiary combination of antimicrobials had the greatest synergistic interaction.

Significance: This novel mathematical model could accurately study antimicrobial interactions against different foodborne pathogens and provide an alternative method to develop new effective combinations.

T10-02 The Effect of pH on the Antimicrobial Activity of *Cryptolepsis sanguinolenta* and *Psidium guajava* against *Salmonella* and *Escherichia coli*

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❖ ***Developing Scientist Competitor***

Introduction: There are several plants indigenous to West Africa that have potential as antimicrobials based on their uses in tradition medicinal practices. Preliminary studies of *Cryptolepis sanguinolenta* and *Psidium guajava* crude extracts against various foodborne microorganisms have been conducted and the extracts showed greater antimicrobial activity against gram-negative than gram-positive bacteria.

Purpose: The purpose of this study was to determine the antimicrobial activity of ethanolic extracts of *C. sanguinolenta* and *P. guajava* against *Salmonella* and *Escherichia coli* and evaluate the influence of pH on the activity.

Methods: The MICs were determined against *E. coli* ATCC 2196 and *Salmonella* Typhimurium in tryptic soy broth (TSB). Concentrations of 0.75% volume/volume P. *guajava* and 0.25% volume/volume *C. sanguinolenta* were then added to inoculated TSB with adjusted pH values of 6, 5, or 4.5. Controls included extracts in TSB with unadjusted pH values and TSB at the adjusted pH values without added extracts.

Results: MICs against both organisms were 0.5 and 1.0% for *C. sanguinolenta* and *P. guajava*, respectively, for both organisms. Adjusted pH values had no effect on the antimicrobial activity of *P. guajava* against *Salmonella*; however, at a pH value of 6 and 5, *C. sanguinolenta* lost effectiveness, rising to more than 2 log CFU/ml greater than the sample with unaltered pH after 24 h. The inhibitory effect of both extracts against *E. coli* was decreased by the lowered pH; at an unaltered pH, it was inhibited to below detectable limits of 1.00 log CFU/ml after 24 h. However, at a pH of 6, cell numbers rose to over 8 and 6 log CFU/ml, respectively, when *P. guajava* and *C. sanguinolenta* were applied.

Significance: This study suggests that *C. sanguinolenta* and *P. guajava* may be useful as alternative antimicrobials against select pathogens causing foodborne illness, however antimicrobial efficacy may be lowered when applied in acidic conditions.

T10-03 Effect of *Thymus vulgaris* Essential Oil on the Fatty Acid Profile of the Antibiotic-resistant *Bacillus cereus* Cell Membrane

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Introduction: Bacterial resistance to antibiotics has been increasingly reported worldwide and is one of the major causes of failure in the treatment of infectious diseases. Natural-based products, including plant secondary metabolites (phytochemicals), may be used to solve or reduce this problem.

Purpose: To investigate the action of *Thymus vulgaris* essential oil on the fatty acid profile of the *Bacillus cereus* cell membrane.

Methods: The effect of thyme oil on *B. cereus* was evaluated by bio-assay preparation, MICs, and scanning electron microscopy (SEM). Fatty acid profile of *B. cereus* cell membrane was assessed using gas chromatography with flame ionization detector (GC-FID).

Results: From the results observed, thyme oil depleted both saturated and unsaturated fatty acids of *B. cereus*. In addition, the oil demonstrated a decrease in bacterial saturated C16:0 and C18:0 and unsaturated C16:1, C18:1n9, C18:2n6 (cis), C18:2n6 (trans), and 18: 3n6 fatty acids in comparison to the control sample. Moreover, the decrease in the amount of saturated fatty acids when compared to untreated cells resulted in a gain of membrane fluidity and as a consequent a decrease in membrane rigidity as noticeable by SEM examination. The results indicated that thyme oil may prove to be a useful alternative antimicrobial agent for *B. cereus*. This study also demonstrates that thyme oil has significant antimicrobial activity against *B. cereus*, acting on the cell surface and causing disruption of the bacterial membrane, which ultimately leads to cell death.

Significance: It was evident in this study that thyme oil has the capability to target the bacterial sites of *B. cereus* that most antibiotics failed to target. Moreover, the results propose that the selected essential oil can be a natural-based alternative to conventional synthetic antimicrobials to control bacterial infections caused by *B. cereus*, particularly topical infections.

T10-04 Antimicrobial Effect of Conjugated Linoleic Acid Over--producing *Lactobacillus* with Berry Phenolics on Enteric Pathogens

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❖ ***Developing Scientist Competitor***

Introduction: The growing threat of multidrug-resistant pathogens, specifically enteric bacterial pathogens, is urgently increasing the need for alternative antimicrobials. Probiotics, specifically modified *Lactobacillus*, with increased production ability of bioactive compounds like conjugated-linoleic-acid (CLA) can act as antimicrobials, along with other beneficial effects. Further, prebiotic-like components such as bioactive phenolic compounds from berry pomaces and byproducts could enhance the antimicrobial/beneficial effects of probiotics.

Purpose: The purpose of this study was to evaluate combined antimicrobial properties of *Lactobacillus* with over-expressed linoleate isomerase gene (LC-CLA) and berry phenolic extracts (BPEs) against growth, adhesion invasiveness, and virulent gene expression of two major enteric pathogens.

Methods: Growth pattern of enterohemorrhagic *Escherichia coli* EDL933 (EHEC-EDL933) and *Salmonella enterica* serovar Typhimurium LT2 were determined. Adhesion and invasiveness assay was carried out in a cell-culture model. Expression of virulent genes was determined with quantitative reverse transcription PCR. Analysis of variance was used for statistical analysis.

Results: In mixed-culture conditions (LC-CLA with 1.0 mg/ml BPEs), EHEC-EDL933 was competitively excluded (100%) and growth of *Salmonella* Typhimurium LT2 was reduced >4.0 log CFU/ml within 48 h ($P<0.05$). Cell-free cultural supernatant of LC-CLA with BPEs (1.0 mg/ml) showed >4.0-log reduction for EHEC-EDL933 and >6.0-log reduction for *Salmonella* Typhimurium LT2 within 24 h ($P<0.05$). Interactions of EHEC-EDL933 and *Salmonella* Typhimurium LT2 with cultured host cells were altered significantly ($P<0.05$); combined treatment reduced adhesion and invasion into human intestinal epithelial cells (>3.8 and >3.4 log for EHEC-EDL933; >4.5 and >4.4 log for *Salmonella* Typhimurium LT2) and chicken macrophage cells (>3.1 and >3.2 log for EHEC-EDL933; >4.5 and >4.7 log for *Salmonella* Typhimurium LT2). Further, combined treatment significantly ($P<0.05$) down-regulated expression of multiple virulent genes such as *espA/B/D, eaeA, ler, tir* in EHEC-EDL933 by 1.8- to 4.8-fold and *hilA/C/D, invA/G, sipA* in *Salmonella* Typhimurium LT2 by 1.1- to 62.5-fold.

Significance: BPEs enhance effect of LC-CLA in reduction of both enteric bacterial pathogens' survival ability, colonization, and virulent gene expression, which may be able to prevent foodborne infections with EHEC and *Salmonella*.

T10-05 A Meta-Analysis on the Effectiveness of Electrolyzed Water Treatments in Reducing and Inactivating Foodborne Pathogens on Different Foods

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❖ ***Developing Scientist Competitor***

Introduction: Various challenge studies over recent years have proven the effectiveness of electrolyzed (EO) water treatments to reduce pathogens on foods; therefore, vast amounts of data currently exist to help estimate the precise efficacy of this treatment on different foods.

Purpose: The aim of this meta-analysis was to develop a global estimate of the pathogen reductions achieved when food products are treated with EO water.

Methods: An extensive literature search and systematic review was conducted to identify EO water intervention studies to reduce *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on poultry, eggs, meat, fish, and produce. Studies meeting the inclusion criteria were selected and the pathogen reduction data with treatment conditions were utilized in the estimation of effect sizes (forest plots with mean log reductions) and the assessment of heterogeneity between studies (I^2).

Results: Twenty-eight studies with 300 observations were obtained and the sanitizers consistently used as controls were water and sodium hypochlorite. Reductions of *Salmonella* and *E. coli* O157:H7 on lettuce were approximately 2.3 log CFU/g, while reduction of pathogens on fish, poultry, and meat ranged between 0.6 and 1.25 log. Reductions of pathogens were greatest in EO water treatments of tomatoes and eggs, with estimates of greater

than 4-log reductions. The reductions among different pathogens in the same food group were generally similar, except for tomatoes, where *Salmonella* reductions were 1.3 log CFU/tomato greater than *E. coli* O157:H7. I^2 values ranged between 60 and 99%, indicating high heterogeneity between studies. Meta-regression analysis found that free chlorine concentration, time, and temperature were significant predictors (P<0.05) in estimating the effectiveness of EO water as a sanitizer.

Significance: This analysis provides a precise estimate of the inactivation effect of EO water on different foods that can be applied in quantitative risk assessments to ensure food safety.

T10-06 Efficacy of Bacteriophages Alone or as a Co-Treatment in Reducing *Listeria monocytogenes* Contamination of Non-food Contact Surfaces

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Introduction: Listeria monocytogenes is a foodborne pathogen that is able to flourish in different food processing environments. Bacteriophages are viruses that target bacteria and may be effective in controlling *L. monocytogenes* contamination.

Purpose: In this study, we evaluated the efficacy of bacteriophages in controlling *L. monocytogenes* growth on non-food contact surfaces under different conditions.

Methods: Stainless steel coupons were inoculated with *L. monocytogenes* (LM94 or LM-GFP) and a cocktail of five *Listeria* bacteriophages under conditions that could limit or enhance phage treatment efficacy: pre-treatment of coupons with phage, protection of phages in oil, presence of competing organic matter, and presence of competing bacteria. In each case, coupons were incubated for 18 h at 21°C and *L. monocytogenes* concentrations were compared between phage-treated and untreated samples. Data were compared using two sample *t* tests and WMW tests based on data distributions. Differences were considered statistically significant at P<0.05.

Results: Final *L. monocytogenes* concentrations were significantly lower (P<0.05) in phage-treated coupons (1.4 log CFU/ml) versus untreated coupons (5.3 log CFU/ml). Phage treatment remained effective in the presence of soil, fat, and competing (non-*Listeria*) bacteria by significantly reducing bacterial concentrations by 1.2, 1.9, and 5.1 log CFU/ml, respectively, compared to untreated coupons (P<0.05). Phage treatment was not effective in reducing bacterial concentrations in the presence of blood or when used as a preventative, i.e., phages applied to coupons 4 h prior to *Listeria* challenge. Protecting phages in oil prior to application did not significantly improve efficacy of the treatment.

Significance: Listeria phages may be effective in reducing *L. monocytogenes* contamination of non-food contact surfaces in food processing environments. Under some conditions, however, phages may need some form of protection to ensure their efficacy (e.g., to prevent desiccation and inactivation).

T10-07 Enzyme-based Control of *Vibrio parahaemolyticus* by the Marine Bacterium *Pseudoalteromonas piscicida*

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Introduction: Vibrio parahaemolyticus is a naturally occurring marine bacterium and a common cause of illness among consumers of raw or lightly cooked oysters and other seafood. *Pseudoalteromonas piscicida* is an antagonist of *V. parahaemolyticus*, capable of inhibiting its growth through the production of proteolytic enzymes and other compounds. It also serves as a predator by directly transferring vesicles containing digestive enzymes to the surface of the *Vibrio* and feeding off of the nutrients released through holes formed in *Vibrio*'s cell wall. Direct substrate-based enzyme analyses have helped classify some of these enzymes, while *in silico* analyses of the *P. piscicida* genome has led to further insights into the types of potential *Vibrio*-digesting enzymes present.

Purpose: This study identifies *P. piscicida* enzymes that are associated with inhibition, digestion, and death of *V. parahaemolyticus* and other gram-negative foodborne pathogens.

Methods: Secreted and vesicle-associated proteolytic enzymes were identified in *P. piscicida* using synthetic fluorogenic substrates containing 7-amino-4-trifluoromethyl-coumarin. An *in silico* genomic search for additional proteolytic enzymes was also performed after *P. piscicida* sequencing using the PacBio sequencing platform. Scanning electron microscopy (SEM) was performed to confirm enzyme-based degradation of *V. parahaemolyticus*.

Results: SEM showed that *P. piscicida* transfers vesicles from its outer surface to the surface of *V. parahaemolyticus*. These vesicles contain enzymes that digest holes in the *Vibrio*'s cell wall. Vesicle-associated and secreted enzymes detected included a repertoire of serine proteases, cysteine proteases, and aminopeptidases. Genomic analyses of *P. piscicida* revealed the presence of genes for 11 serine proteases, five metalloproteases, 14 aminopeptidases, and four carboxypeptidases.

Significance: This study demonstrates the effectiveness of *P. piscicida* enzymes in controlling *V. parahaemolyticus* in the marine environment and the potential to use *Pseudoalteromonas* to reduce *Vibrio* in aquaculture.

T10-08 Biocontrol of Shiga Toxin-producing *Escherichia coli* on Fresh Produce Using Bacteriophages

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Introduction: Consumption of produce contaminated with Shiga toxin-producing *Escherichia coli* (STEC) has resulted in numerous cases of foodborne illness. Even after implementation of modern practices and technology in the food industry, control of these pathogens remains a challenge. It is, therefore, crucial to develop effective control measures. Bacteriophage-based treatments could provide an attractive alternative to control STEC in foods.

Purpose: Evaluate biocontrol capabilities of STEC-specific bacteriophages on fresh produce.

Methods: Bacteriophages, isolated from cattle operations, showing lytic activity towards diverse STEC isolates were tested for their inhibition capabilities on fresh produce (baby spinach, romaine lettuce, and cucumbers). Spinach and romaine leaves (5 by 3.5 cm pieces) and whole cucumber (1 by 6 cm marked surface) were prepared by washing with sterile distilled water and drying for 30 min. Prepared produce was spot-inoculated with individual or multi-serotype cocktail (6 log CFU/ml) of STEC (O157, O26, O45, O103, O111, O121, or O145) isolates and treated with respective (individual or multi-isolate cocktail) phage-treatment (9 log PFU/ml) or phosphate-buffered saline (PBS) control using an airbrush and stored at 4°C. Surviving bacterial

population was enumerated on days 0, 1, and 3 and visually analyzed under scanning electron microscopy (SEM). Data was analyzed using one-way analysis of variance (P<0.05).

Results: All bacteriophage treatments significantly (P<0.05) reduced their host STEC populations on fresh produce compared to the positive or PBS control. On spinach and lettuce, individual phage treatment reduced STEC O157, O111, O121, and O145 populations by 2.0 log CFU/cm^2 and reduced O26, O45, and O103 populations to undetectable levels after initial application. Multi-isolate phage cocktail reduced STEC populations by 1.4 los CFU/cm^2 on spinach and romaine, and by 1.7 log CFU/cm^2 on cucumbers by day 3. The SEM images revealed high bacterial counts, surrounded by extracellular matrix, with positive control. However, phage-treated produce surfaces had fewer STEC cells that were scattered sporadically and showed cellular damage, indicating that phage treatments were effective.

Significance: Bacteriophages could potentially be utilized as a biocontrol against STEC on fresh produce.

T10-09 Development and Evaluation of a Novel Assay to Identify Prophage Inducers as a New Class of Antimicrobials in Foods

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Introduction: Prophage induction and subsequent bacterial lysis represents an emerging approach to bacterial control. Natural prophage inducers could be used as antimicrobials to control growth of foodborne pathogens.

Purpose: The objective of this study was to identify food-grade prophage inducers using a high-throughput luminescent screening approach.

Methods: *Escherichia coli* K12 (BR513) carries a *lacZ*-prophage lambda gene fusion and produces beta-galactosidase upon induction of phage lambda. Prophage inducers were evaluated by incubating *E. coli* BR513 and a control *E. coli* K12 strain with 15 compounds (teas, coffee, spices, food compounds, and antibiotics) at various concentrations for 24 h at 37°C in comparison to uninoculated control BR513 and K12 cultures, followed 24 h later by measurement of beta-galactosidase production by luminescence. Luminescent results from triplicate cultures of the BR513 and K12 strains were evaluated by dividing the average BR513 relative light units (RLUs) by the average K12 RLUs, and compounds with a ratio greater than the threshold (determined by dividing the average RLUs of the uninoculated BR513 by uninoculated K12), were considered prophage inducers, where a greater difference corresponded to a stronger inducer.

Results: Rosemary and gallic acid (a component of tea) were potent prophage inducers, with RLU ratios that were 23 and 8.5 times greater than the threshold. In comparison, norfloxacin, a common antibiotic inducer, had a ratio of 5. Japanese green tea, black tea, and a green/black tea blend all induced prophages with ratios of 4.3, 3.9, and 3.9. Sodium citrate and cranberry juice (pH adjusted to 6.5) did not induce prophages. A 10% weight/volume solution of rosemary reduced the concentration of *Salmonella* Enteritidis by 1 log when incubated for 24 h in broth culture.

Significance: These results demonstrate that common foods cause prophage induction and are therefore potentially useful as antimicrobials to control the growth of foodborne pathogens.

T10-10 Transferability of IS*26*-Class 1 Integron-IncHI2 Plasmid in Antimicrobial-resistant *Salmonella* Typhimurium

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Introduction: *Salmonella* Typhimurium is globally recognized as an important foodborne pathogen, and multidrug-resistant *Salmonella* Typhimurium poses a serious threat to public health. The dissemination and evolution of diverse antimicrobial resistance genes has been largely manipulated by mobile genetic elements such as plasmids, insertion sequences, and integrons via horizontal gene transfer. Our previous study reveals that the IncHI2 plasmid is predominant in multidrug-resistant *Salmonella* Typhimurium, and the arrangements of complex IS*26*-Class 1 Integron are very common on these multidrug resistance-associated IncHI2 plasmids.

Purpose: The purpose of this study was to evaluate the transferability of complex IS*26*-Class 1 Integron arrangements on IncHI2 plasmid in *Salmonella* Typhimurium.

Methods: A total of 145 *Salmonella* Typhimurium isolates from food and clinic settings were detected for the presence of IncHI2 plasmid, IS*26*, and Class 1 Integron by conventional PCR. The resultant all-positive isolates harboring the three mobile genetic elements were screened for their antimicrobial susceptibility against 17 antimicrobials involved in the U.S. Food and Drug Administration's National Antimicrobial Resistance Monitoring System. They were then used in the conjugation assay via liquid mating. The conjugation experiment was performed using rifampin-resistant *Escherichia coli* C600 as the recipient strain (rifampin-resistant). Transconjugants were selected on LB agar plates supplemented with rifampin (200 µg/ml) and another appropriate antimicrobial (64 µg/ml ampicillin or 32 µg/ml nalidixic acid, according to their antimicrobial susceptibility).

Results: Thirty-five isolates were detected as all-positive simultaneously harboring IncHI2 plasmid, IS*26*, and Class 1 Integron. Among these isolates, 34 (97.14%) were confirmed as multidrug-resistant. The most prevalent was sulfisoxazole (100%, 35 of 35), ampicillin (94.29%, 33 of 35), and trimethoprim-sulfamethoxazole (88.57%, 31 of 35). No strains were found against amikacin, cefoxitin, azithromycin, and ceftiofur. Twenty-four transconjugants were obtained and the conjugation rate was 68.57%. Twelve complex IS*26*-Class 1 integron arrangements on the IncHI2 plasmid from donors were transferred to the recipient *E. coli* C600 along with the transfer of the donor's antimicrobial phenotype.

Significance: This study suggests that complex IS*26*-Class 1 integron arrangements on IncHI2 plasmid can undergo frequent interspecies transfer to exacerbate the antimicrobial resistance of *Salmonella* Typhimurium.

T10-11 Prevalence and Mobility of Antibiotic-resistance in *Salmonella* under Conventional or Organic Farm Environments

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Introduction: Microbial horizontal gene transfer is a continuous process that shapes bacterial genomic adaptation and composition of con-current microbial ecology. The role of human employed synthetic antibiotics and chemicals in genomic adaptation and their influence on evolution of biological networks is critical. Though attention has been paid to contributions of antibiotic utilization in farm animal production to overall antibiotic resistance, the mechanism behind the evolution is not yet fully understood.

Purpose: The purpose of this study is to investigate the prevalence and mechanisms of sub-therapeutic/therapeutic dosages of synthetic antibiotics that foster the exchange of genetic materials in microbial communities in simulated farming environments.

Methods: Antibiotic resistance in *Salmonella enterica* serovar Typhimurium isolates from conventional and organic farming systems were evaluated. Both resistant and sensitive isolates were inoculated in natural soil environment containing with/without 4 mg/kg tetracycline in triplicate to evaluate genetic adaptation. Tetracycline MIC of *Salmonella* Typhimurium isolates was determined by standard agar dilution method. Quantitative PCR was performed to confirm genetic adaption, and the genetic configuration relatedness among *Salmonella* Typhimurium isolates was investigated by genome-wide single nucleotide polymorphism comparisons.

Results: Antibiotic resistance was detected in 80% and 66.67% of *Salmonella* Typhimurium isolates from conventional and organic farm systems, respectively. The tetracycline-resistant *Salmonella* Typhimurium retained their resistance (MIC>64 µg/ml) over six months, whereas sensitive *Salmonella* Typhimurium gradually acquired higher MICs and evolved tetracycline-resistance (MIC>64 µg/ml). Differential expression of antibiotic-resistance genes in *Salmonella* Typhimurium artificially inoculated under antibiotic pressure with 12 passages was detected. Specifically, *tetA* and *vapB* were up-regulated by 650 to 3,300 and two- to five-fold. Besides, based on genome sequences, antibiotic-resistant *Salmonella* Typhimurium were clustered with pathogenic *Salmonella* Typhimurium, indicating a positive relevancy between drug resistance and pathogenicity.

Significance: Tracking and understanding the phenotypical transition of antibiotic resistance in microbial ecosystems offers us directed strategies in combating against *Salmonella* antibiotic resistance and minimization of their virulence.

T10-12 Molecular Characterization of Non-O157 Shiga Toxin-producing *Escherichia coli* Isolated from Sustainable Farming Systems Using Whole Genome Sequencing

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Introduction: Sustainable farming systems that do not use antimicrobials have emerged as an alternative to commercial productions systems that use antimicrobials and disinfectants. These farms often grow fresh produce in very close proximity to their livestock, increasing the risk of transmission of antimicrobial resistant (AMR) Shiga-toxin producing *Escherichia coli* (STEC).

Purpose: The purpose of this study is to determine if AMR STEC from livestock is transferring to produce on operating sustainable farming systems in Tennessee (TN) and North Carolina (NC).

Methods: Presumptive STEC samples were collected from multiple sources (manure, environment, and produce) from farms in TN and NC. The Illumina Miseq and CLC workbench were used to sequence, map, assemble, and analyze all 257 presumptive STEC isolates. The ResFinder and SeroTypeFinder databases were used to find AMR gene matches and identify the O and H antigens, respectively. Broth microdilution was used to determine antimicrobial susceptibility against a panel of fifteen antimicrobials.

Results: Results show that transmission of presumptive AMR STEC can occur within a distance of 400 ft. Overall, two of the top six non-O157 serotypes that are known to cause outbreaks were detected (O45 and O111) among the isolates. The presence of bla_{CMY} and bla_{ACT} genes was confirmed. All isolates possessed genes for β-lactamases (bla_{CMY} and bla_{TEM}). In addition, 56% out of 209 isolates possessed resistant genes to at least three antibiotic classes. One isolate, for example, was resistant to eight classes of the antimicrobials tested (FOX, AZI, CHL, TET, AXO, AUG2, GEN, XNL, FIS, SXT, AMP, and STR). Furthermore, 55.3% of 257 isolates were resistant to at least three or more antimicrobial classes according to sensititre data.

Significance: The results of this study shows that presumptive STEC can act as a reservoir of AMR genes, increasing the risk of outbreaks of AMR foodborne pathogens.

T11-01 Blockchain: Accelerating Traceback Investigations in Food Poisoning Outbreaks

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Introduction: The size of international food supply chains makes tracing contaminated foods back to their source difficult and time-consuming. Investigators must first retrace its journey backwards from the retailer through one or more distributors to its original manufacturer, farm, or ranch.

Purpose: Traceback investigations take a long time because: i) there is no single database for tracking food products through supply chains from their original source to their ultimate consumers; ii) not all supply chain information is digital and critical supply chain information exists only in paper documents; iii) paper documents must be physically gathered to reviewed by investigators; iv) food supply chains are now international, with food products routinely shipped from farms in one country to markets in another country/continent; v) investigators often live in the market country, not the country of the product's origin; vi) there may be language or interpretation barriers; and vii) the food supply chain has grown so complex that some retailers have great difficulty guaranteeing the provenance of the food products they sell.

Methods: A blockchain is a continuously growing list of records. The data cannot be changed without also changing all subsequent blocks in the chain. Investigators can then rapidly trace contaminated food products backwards from fork to farm and then forwards from farm to fork. Traceback investigations that currently take weeks or months could be completed in minutes. By accelerating traceback investigations, blockchain can lead to more rapid product recalls, reduce the spread of outbreaks, and ultimately result in fewer consumers being injured by contaminated food products.

Results: In May 2017, Walmart reported the results of its collaboration with IBM on blockchain technology. Using blockchain, Walmart traced Chinese pork and mangoes from the United States back to their original sources. In the past, this process would take two weeks. But with blockchain technology, the traceback took 2.2 seconds. Walmart, Nestlé, and Unilever are currently collaborating with IBM to apply blockchain to the global food supply.

Significance: We could find and detect outbreak sources faster and reduce the number/spread of foodborne illness outbreaks.

T11-02 Evaluating Trends in Foodborne Outbreaks and Outbreak-associated Illnesses for Various Pathogen Food Category Pairs from 1998 to 2015

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Introduction: The Interagency Food Safety Analytics Collaboration (IFSAC), established by the Centers for Disease Control and Prevention, the U.S. Food and Drug Administration, and the United States (U.S.) Department of Agriculture's Food Safety and Inspection Service, works to improve U.S. foodborne illness source attribution estimates. Evaluating trends in sources of foodborne illness is useful for developing food safety policies, but quantifying changes over time in the relative importance of those sources is challenging.

Purpose: IFSAC's goal was to develop a model to estimate changes in count data (outbreaks and outbreak-associated illnesses) over time for specific pathogen-food category pairs.

Methods: We developed a Bayesian negative binomial regression model using thin plate splines to estimate the number of foodborne outbreaks and confirmed outbreak-associated illnesses caused by *Salmonella, Escherichia coli* O157, *Listeria monocytogenes*, and *Campylobacter* from 1998 to 2015 attributed to 17 food categories of the IFSAC Food Categorization Scheme.

Results: Overall, our model is versatile and portable to various levels of food categorization granularity or other time series analyses of foodborne outbreak data. Our use of thin plate splines adequately follows changes over time while remaining resistant to outliers. The Bayesian approach allows hypothesis testing between relevant time periods. We found several significant changes in outbreaks and outbreak-associated illnesses over time, including a decrease between 2013 and 2015 (compared to 2010 to 2012) in the number of *Salmonella* outbreaks associated with eggs (median: 1.2 fewer outbreaks/year; 95% confidence interval: 0.2 to 2.0) and *E. coli* O157 outbreaks associated with land animals (median: 3.7 fewer outbreaks/year; 95% confidence interval: 0.1 to 8.6).

Significance: Our model can be used to estimate changes in the number of foodborne outbreaks and outbreak-associated illnesses over time for food category-pathogen pairs. It may be applicable in evaluating trends in other types of foodborne illness or time series data.

T11-03 Dysbiosis of Commensal Microbes and Its Correlation with Increased Systemic Dissemination and Gastrointestinal Pathology during Listeriosis

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Introduction: Invasive foodborne *Listeria monocytogenes* causes septicemia, meningitis, chorioamnionitis and is associated with high case-fatality rates in the elderly. We developed a geriatric murine model as a human surrogate for listeriosis and previously reported increased systemic infection in old mice due to imbalances in protective immune responses.

Purpose: Listeriosis-induced perturbation of gut microbiota and pathological changes in gastrointestinal tissues were compared between young and old mice.

Methods: Young and old C57BL/6 mice were dosed intragastrically for two consecutive days with ~10^6 CFU/mouse *L. monocytogenes* (EGD-Lmo-*InlA*^{m}). Spleen, liver, and gastric tissues were collected seven days post-infection. Spleen and liver tissues were cultured for viable *L. monocytogenes*. Gastric tissues were H&E stained. Intestinal tissues were analyzed for cytokine mRNA by real-time reverse transcription quantitative PCR. Fecal pellets were collected pre- and post-infection for microbiome analysis via shotgun metagenomics sequencing using Illumina's MiSeq platform.

Results: Metagenomics analysis of uninfected old mice showed a significant ($P \leq 0.05$; *t* test: significance) reduction in *Clostridiaceae* and *Lactobacillaceae* families compared to young mice. Older mice had significantly higher systemic *L. monocytogenes* counts in liver ($P=0.03$) and spleen ($P=0.05$). *Porphyromonadaceae* and *Prevotellaceae* were increased in infected young mice, while members of the *Ruminococcaceae* and *Lachnospiraceae* families were significantly increased in old mice after infection. Genera *Blautia* and *Alistipes* were abundant in uninfected young and old mice, respectively, but significantly ($P=0.027$, $P=0.032$) reduced post-*L. monocytogenes* infection. Immune-modulating bacteria *Pseudoflavonifractor* and *Faecalibacterium* were significantly ($P=0.014$; $P=0.043$) increased only in the old infected mice, correlating with increased inflammatory response and gastritis. IFN-γ and IL-10 mRNA was up-regulated in intestinal tissues from old mice. Histologic analysis of gastric tissues showed extensive lesions in the *L. monocytogenes*-infected old mice, more so in the non-glandular region and fundus than in the pylorus.

Significance: Aging changes composition of gut microbiota and may compromise resistance to *L. monocytogenes* infection. *Listeria* infection of old mice increases butyrate-producing inflammatory members of the *Ruminococcaceae*/*Lachnospiraceae* families.

T11-04 Seasonal Prevalence of *Salmonella* Typhimurium and Its Monophasic Variant Serovar I 4,[5],12:I:-, in United States Feed Mills

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Introduction: Salmonella *enterica* serovar Typhimurium and its monophasic variant 4,[5],12:i:- have been responsible for an increased number of outbreaks related to meat products in Europe and the United States. S*almonella* can be present along the entire food production chain from farm to fork, and recent studies reported the isolation of *Salmonella* Typhimurium and its monophasic variant in feed and feed ingredients. The occurrence of these pathogens in pre-harvest environments can translate to microbial contamination of the human food chain.

Purpose: The objective of this study was to evaluate the seasonal prevalence and distribution of *Salmonella* spp., *Salmonella* Typhimurium, and its monophasic variant in different feed mills across United States (U.S.).

Methods: Eleven feed mills were selected for this study and 12 environmental samples were collected within each facility and season. Visits were conducted during fall 2016, early spring 2017, and summer 2017. Samples were analyzed following the United States Department of Agriculture Food Safety and Inspection Service guidelines and *Salmonella* presumptive positive samples were confirmed by PCR. A multiplex real-time PCR was used to differentiate *Salmonella* Typhimurium and its monophasic variant from other serotypes. Associations between environmental, demographic, and management factors and prevalence of *Salmonella* were analyzed using generalized linear mixed effects models in SAS software v. 9.4.

Results: From all samples collected, 8.8 % were confirmed *Salmonella* spp. Among these isolates, 3.1% were identified as *Salmonella* Typhimurium and 4.6% as its monophasic variant. Geographical location and sampling season were significantly associated with the prevalence of *Salmonella* spp. in

U.S. feed mill environments (P<0.05). The highest number of *Salmonella* spp. isolates were collected in the Midwest region, and pathogen presence was determined to be higher during fall and summer, as compared to the spring season (P<0.05).

Significance: The data demonstrates the seasonal presence of *Salmonella* spp., *Salmonella* Typhimurium, and its monophasic variant in feed mills located in different regions of United States and could be used as a tool to implement mitigation strategies for food safety.

T11-05 Transcriptional Response of *Salmonella enterica* Serovar Enteritidis to Ethanol Treatment

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Introduction: Salmonella *enterica* serovar Enteritidis can adapt to environmental stresses (i.e., disinfectants, pH, temperature) encountered in food-related environments. Adaptation is usually achieved by coordinate regulation of gene expression in response to such stimuli.

Purpose: This work aimed to understand the transcriptional response of stress tolerance-related genes of *Salmonella* Enteritidis following ethanol adaptation.

Methods: Reverse transcription quantitative PCR was employed to determine the expression profiles of 78 selected genes of *Salmonella* Enteritidis after exposure to 5% ethanol for one hour. A gene was considered to be differentially expressed when its relative expression level compared to the control (i.e., *Salmonella* Enteritidis cells without the ethanol treatment) is over two-fold. One-way analysis of variance and Duncan's test were used for statistical analysis.

Results: Out of the 78 assessed genes, a total of 12 genes were significantly (P<0.05) differentially expressed in response to ethanol adaptation in *Salmonella* Enteritidis, with 11 genes down-regulated (*tolC*, *ompL*, *trkD*, *yehU*, *yfhK*, *cpxA*, *cspA*, *uspA*, *fliA*, *rpoN*, and *katG*) and one gene up-regulated (*rpoS*). These genes were mainly responsible for universal, cold, and oxidative stress responses. Moreover, metabolic pathways responsible for two-component systems, bacterial secretion systems, and cationic antimicrobial peptide resistance were found to be involved in the ethanol stress response.

Significance: These results provide an initial insight into the mechanisms that *Salmonella* Enteritidis utilizes to respond to ethanol adaptation. Alterations in expression of stress tolerance-related genes suggests that ethanol adaptation might provide some protection to *Salmonella* Enteritidis against environmental stresses.

T11-06 Genetic and Virulent Difference between Pigmented and Non-pigmented *Staphylococcus aureus*

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Introduction: It has been suggested that staphyloxanthin (STX), a golden carotenoid pigment produced by *Staphylococcus aureus*, can act as an important virulence factor due to its antioxidant properties. Restraining biosynthesis of STX was considered as an indicator of virulence decline in pigmented *S. aureus* isolates. However, it is not clear whether natural non-pigmented *S. aureus* isolates have less virulence than pigmented ones.

Purpose: In this study, it is aimed to compare the pigmented and non-pigmented isolates to clarify the association between the biosynthesis of STX and the virulence of *S. aureus*.

Methods: A total of 132 *S. aureus* isolates were divided into two phenotype groups depending on the absorbance (OD_{450}) of the extracted carotenoids. Then, all isolates were subjected to *spa* typing and multilocus sequence typing (MLST), and then the presence detection of 30 virulence factors and the gene integrity of *crtN* and *crtM*. Furthermore, 24 typical *S. aureus* isolates and four *S. argenteus* strains were selected for the murine infection assay of *in vivo* virulence, in which the histological observation and enumeration of CFUs were carried out.

Results: These isolates were distributed in 26 sequence types (STs) and 49 *spa* types. The pigmented isolates were scattered in 25 STs, while the non-pigmented isolates were more centralized, which mainly belonged to ST 20 (59.09%) and ST 25 (12.96%). Among the 54 non-pigmented isolates, about 20.37% carried intact *crtN* and *crtM* genes. The *in vivo* assay suggested that comparing with pigmented *S. aureus*, non-pigmented *S. aureus* and *S. argenteus* strains did not show a reduced virulence in murine sepsis models.

Significance: Between the two phenotype groups, there was difference in the genetic background; however, there was no difference in the virulence genotype and toxicity *in vivo*. Therefore, it suggested that STX is not essential for the infection of *S. aureus*.

T11-07 Identification of a *Pseudomonas* Locus Associated with Color Defect in Fluid Milk Using Comparative Genomics

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Introduction: Spoilage of high-temperature short-time (HTST) pasteurized milk is a major concern for the dairy industry. *Pseudomonas* species represent a major milk spoilage group that can grow at refrigeration temperatures and may result in flavor, odor, and texture defects in fluid milk. Recently, one *Pseudomonas* isolate (FSL E2-0548) was isolated from HTST-pasteurized fluid milk with gray coloration.

Purpose: Identify through comparative genomics a genetic mechanism in *Pseudomonas* leading to fluid milk color defects.

Methods: Isolate FSL E2-0548 was inoculated in full and half-full containers with HTST-pasteurized fluid milk and incubated at 6°C. After 14 days, milk in half-full (but not full) containers turned gray. In this study, FSL E2-0548 and four other *Pseudomonas* isolates associated or not associated with distinct color defects in different foods were whole-genome sequenced. Comparative genomics was used to identify genes putatively associated with the gray color defect.

Results: A locus encoding several proteins involved in tryptophan biosynthesis was identified as being present in FSL E2-0548 and another isolate (FSL W5-0203) associated with blue color defect in cheese. Further phenotypic characterization showed that FSL W5-0203 also results in the gray color defect after inoculation in half-full containers with HTST-pasteurized fluid milk, similarl to FSL E2-0548. The tryptophan biosynthesis pathway is oxygen-dependent, which may explain why only half-full pasteurized milk containers showed the color defect.

Significance: Milk spoilage represents a major burden to the dairy industry. Identification of the mechanisms involved in color defect spoilage may be used in the development of genetic markers to trace potential spoilers in a sample. Moreover, this study shows that comparative genomics can be a useful tool for identification of putative genetic features associated with food spoilage.

T11-08 Spatiotemporal Variability in Microbial Quality of Western Agricultural Water Supplies: A Multistate Study

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❖ ***Developing Scientist Competitor***

Introduction: In the United States (U.S.), foodborne illness outbreaks associated with consumption of raw produce have increased more than 6% over a 20-year period from the mid-1970s to the mid-1990s, coincident with nearly a 25.5% increase in the consumption of fruits and vegetables. Although there have been an abundance of studies to model bacterial survival kinetics, transfer dynamics, and preharvest risk factors linked to raw produce consumption, relatively little is known about the potential risk inherent in agricultural water supplies.

Purpose: The purpose of our study was to establish baseline microbial water quality profiles using agricultural water criteria from the U.S. Food and Drug Administration's Food Safety Modernization Act for irrigation districts utilizing surface water systems. We wanted to better understand the association of spatial-temporal factors that influence microbial water quality variability and how optimization of sampling metrics would create a robust statistical investigation of western irrigation systems.

Methods: This baseline study was conducted during the agricultural growing season of 2015 in Northern California (CA) and Central Washington State (WA). Water sampling sites (CA, n=42; WA, n=61) were strategically chosen to represent the spatial extent of each district. Small-volume (1 L) and large-volume (10 L) water samples were collected monthly at ~25% of the sites. Large volumes were processed using ultrafiltration and analyzed for bacterial pathogens (*Salmonella* spp., *Escherichia coli* O157, and non-O157 Shiga toxin-producing *E. coli*); small volume samples were analyzed for both bacterial pathogens and indicators.

Results: Samples collected in the rivers/creeks upstream of district control (source water) had significantly (P<0.05) lower concentrations of log *E. coli* (μ±sd=1.16±0.59 CFU/100ml) than samples collected from within the constructed canals of irrigation districts (μ±sd=1.46±0.59 CFU/100ml). The odds of detecting *Salmonella* improved more than 250% (OR=2.57) with an increase in volume from 1 to 10 L in paired samples.

Significance: Our work will help to decrease knowledge gaps regarding microbial water quality of irrigation water on large spatial scales. This will inform regulations.

T12-01 A 3D Cell-based Assay to Detect Shiga Toxin-producing

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❖ ***Developing Scientist Competitor***

Introduction: Detecting viable Shiga toxin-producing *Escherichia coli* (STEC) has been challenging because current culture methods are lengthy and assays that are commercially available can be non-specific, labor-intensive, and expensive.

Purpose: Our goal was to develop a mammalian cell-based assay for rapid high-throughput detection (CARD) of STEC using Vero monkey kidney epithelial cells or THP-1 human monocytic cells.

Methods: Mammalian cells were immobilized in a collagen gel matrix to create a three-dimension platform, which was evaluated to determine the specificity, limit of detection (LOD), and optimal time (2 to 16 h) required to obtain a positive response against STEC bacteria or crude toxin preparations. Cytotoxicity was assayed by measuring the lactate dehydrogenase (LDH) release, trypan blue uptake, and cryo-SEM. CARD was tested with artificially contaminated ground beef samples (n=27, 4 CFU/g), which was further verified by *stx1*- and *stx2*-specific multiplex PCR and by plating on sorbitol MacConkey agar (SMAC).

Results: Analysis of cytotoxicity of viable STEC cells on 3D Vero gave the highest LDH release and the LOD was estimated to be $10^{7\text{-}8}$ CFU/ml or 31.25 ng toxin/ml in 6 h with a cut off value of 12 to 15%. 3D-CARD was highly specific for STEC cells and did not yield any positive response from *Salmonella enterica*, *Listeria monocytogenes*, *Citrobacter freundii*, *Hafnia alvei*, *Serratia marcescens*, and non-pathogenic *E. coli*. 3D-CARD successfully detected STEC from artificially contaminated ground beef samples following a 15-h enrichment (~10^8 CFU/ml) in modified tryptic soy broth (mTSB) and 6-h cytotoxicity assay, with confirmation of STEC from positive food samples on SMAC plate and *stx1*- and *stx2*-specific PCR.

Significance: The results demonstrated that 3D Vero cell-based CARD is suitable for detection of STEC or Stx after a 15-h enrichment in mTSB followed by a 6-h cytotoxicity assay providing results much faster than the traditional Vero cell assay that requires 72 h or more.

T12-02 Comparison of Real-time PCR Results from *Listeria monocytogenes*-spiked Food Samples Grown in Rapid Media and Half-Fraser Broth: An Interlaboratory Study

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Introduction: Rapid methods have been developed to shorten the time from sample to result for *Listeria monocytogenes* detection in food. Time needed for enrichment of the bacteria prior to DNA extraction is crucial in order to detect *L. monocytogenes* by real-time PCR.

Purpose: To determine the reliability and effectiveness of a rapid enrichment media compared to Half-Fraser broth in an interlaboratory study using food samples spiked with *L. monocytogenes.*

Methods: Eighteen different laboratories evaluated the rapid media **food**proof *Listeria* StarBroth (SB) against Half-Fraser broth using two different matrices (cheese and sausage) in duplicate, spiked and unspiked. Samples were spiked to a concentration of 20 CFU of *L. monocytogenes* per 25 g food and incubated 22±2 h at 37°C in SB or 48±2 h at 30°C in Half-Fraser broth. DNA extraction was done with the StarPrep Two Kit using Procedure A (sensitive protocol) or StarPrep Two 8-Strip Kit. Real-time PCR results with the **food**proof *Listeria monocytogenes* Detection LyoKit were then compared for presence/absence. Crossing point (Ct) values of positive results were compared to a standard reference also present in each real-time PCR run.

Results: A 100% correlation for spiked and a 98% correlation for unspiked cheese and sausage samples grown in both rapid media and Half-Fraser was observed regardless of DNA extraction kit or lab used. Moreover, when results from positive samples were compared to the standard reference, a statistically significant shift forward in sample Ct values of approximately two log was found when enriching in SB. This forward shift in Ct value indicates a faster growth rate may be possible using the rapid media.

Significance: Interlaboratory study real-time PCR results demonstrated enrichment of *L. monocytogenes* spiked food samples in rapid enrichment media was as effective as or better than enrichment in Half-Fraser broth.

T12-03 Development of a Molecular *Listeria* Pattern Recognition Assay, a Novel Rapid Method for Identifying Resident *Listeria*

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Introduction: Outbreak investigations have highlighted the importance of identifying and remediating resident *Listeria* strains in manufacturing facilities. Resident strains increase the risk of finished product contamination. Hurdles to using existing identification methods (including pulsed-field gel electrophoresis and whole genome sequencing) include cost, time to results, and the need for an isolate. The *Listeria* Pattern Recognition Assay (LiPRA) allows direct analysis from an enriched sample 5 hours after a presumptive positive result. The fully automated multiplexed assay enables users to rapidly and cost-effectively determine whether a *Listeria* pattern has been observed previously, without requiring species identification.

Purpose: The study characterizes the performance of several successive LiPRA prototypes with increasing discriminatory power for characterizing *Listeria* strains.

Methods: To test the discriminatory power of the assay, studies were conducted using DNA isolated from *Listeria* strains (*n*=48 strains, 38 were *L. monocytogenes*). Three prototypes were evaluated. Prototypes 1, 2, and 3 utilize 6, 17, and 22 probes, respectively.

Results: Prototypes 1, 2, and 3 divided 48 strains of *Listeria* spp. into 11, 18, and 27 different groups, respectively. Individual groups resulting from the second prototype ranged from 2.1 to 25% of the total isolates, with an average group size of 2.7%. The third prototype split the largest group from prototype 2 into seven new patterns.

Significance: Current methods used for distinguishing resident from transient *Listeria* strains are time-consuming, and require isolation in pure culture following a positive screen. The LiPRA enables pattern recognition directly from a positive enrichment. This method allows users to identify recurring patterns of *Listeria* rapidly following a presumptive positive screen and quickly modify sanitation protocols, thereby reducing the risk of finished product contamination and recalls.

T12-04 Comparison in the Recovery of *Salmonella* from Poultry Slaughter Establishments Using Buffered Peptone Water with and without Neutralizers to Address Antimicrobial Carryover

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Introduction: Concerns about whether antimicrobials used during poultry slaughter could be transferred from the surface of the carcass into the sampling rinsate have led to the development of a new formulation of buffered peptone water. This new formulation contains additives to neutralize the antimicrobials commonly used during poultry slaughter and processing. This neutralizing buffered peptone water (nBPW) replaced the original formulation for all chicken carcass and chicken parts sampling programs run by the United States federal government beginning in July 2016.

Purpose: This study assesses the changes in the proportion test-positive rinse samples for Salmonella in the 12-month period following implementation and trends observed therein.

Methods: The analysis utilized data from United States Department of Agriculture Food Safety and Inspection Service poultry carcass sampling in the 13 months before the transition to nBPW and the 12 months following. The carcass dataset represents sample collected from 210 establishments and consists of 18,555 test results. A total of 9,447 samples were collected before implementation of nBPW and 9,108 after. The analysis begins with an assessment of the temporal change in the monthly proportion of positive samples. A penalized B-spline regression model was fitted to the data using a second-order difference penalty.

Results: The monthly proportion of *Salmonella*-positive samples increased from 0.019 to 0.057 following the implementation of nBPW, though most of the increase can be attributed to a subset of slaughter establishments. The statistically significant (P=1.86×10^{-6}) difference in proportion positive pre- and post-nBPW suggests that antimicrobial carry-over had been causing false-negative testing results prior to implementing the nBPW buffer.

Significance: An analysis of the effect of the new rinsate on large corporations demonstrated that changes in the percent-positive rates differed across the corporations, with some being unaffected while others saw dramatic changes. The results confirm earlier concerns that antimicrobial contamination of rinse samples was causing false negative *Salmonella* testing results.

T12-05 Detection and Characterization of Environmental Samples Naturally Contaminated with *Salmonella enterica*

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Introduction: Field-ready methods for on-site, rapid detection and characterization of *Salmonella* directly from food and/or environmental samples could have a significant influence on the economic impact of foodborne illnesses and reduce the sample-to-answer turn-around times typical for current foodborne pathogen surveillance efforts. To address this challenge, we demonstrated the use of field-based protocols and systems such as the Biomeme two3 qPCR system and Oxford Nanopore Technologies MinION sequencing platform for on-site detection and sequence-based characterization of *Salmonella* in water and sediment.

Purpose: The objective of this study was to assess the ability to rapidly test field samples and serotype *Salmonella* in under 24 hours.

Methods: Twenty-four water and sediment samples originating from the Virginia Tech Eastern Shore Agriculture Research and Extension Center (AREC) and surrounding areas were analyzed for the presence of *Salmonella.* Detection methods included a modified culture-based *Salmonella* U.S. Food and Drug Administration Bacteriological Analytical Manual method and a *Salmonella*-specific quantitative PCR assay ran on the portable Biomeme two3 system, with final confirmation on the Vitek MS.

Results: Of the 24 samples tested, 10 were detected as positive for *Salmonella* by all three methods, with the first qPCR detection occurring within 30 hours of collection. Select qPCR positive samples were also subjected to metagenomic shotgun sequencing on the portable MinION sequencing platform for further characterization.

Significance: The successful demonstration of these methods is an important step towards developing and validating field-ready methods aimed at shortening the sample-to-answer timelines for routine foodborne pathogen surveillance and outbreak investigations.

T12-06 Using the Isotopic Composition of Water to Detect Honey Adulteration

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Introduction: Though it's not a new problem, adulteration of honey is still a very important topic for the honey industry. Many methods exist to detect adulteration but they are often only effective for a couple of months, after which fraudsters adjust their mode of adulteration to prevent detection of their actions. For example, AOAC method 998.12 works very well when C4-sugars (e.g., corn syrup) are added but fraudsters changed to the addition of C3-sugars (e.g., rice syrup), which cannot be detected with that method.

Purpose: Consequently, there is a constant need to develop new methods to detect adulteration of honey, which was the aim of this study.

Methods: Instead of analyzing carbon isotopes, we were targeting the isotopic composition of water in honey. More than 40 water samples from rivers, lakes, groundwater, and water taps worldwide and more than 100 honey water samples were obtained and hydrogen as well as oxygen isotopes (^{2}H and ^{18}O) analyzed by means of isotopic ratio mass spectrometry.

Results: The correlation between the ^{2}H and ^{18}O isotopes in water samples from rivers, lakes, groundwater, and water taps is very clear. Honey water results indicate that the isotopic composition of water in honey is significantly different from that of water samples from rivers, lakes, and the other mentioned sources.

Significance: This finding could be used to detect the addition of water to honey, as water addition would lead to a shift of isotopic composition of the water contained in honey towards the one found in other sources.

T12-07 Determining the Impact on Varying Methods for Acid Adaptation on Thermal Resistance of Shiga Toxigenic *Escherichia coli* (STEC), *Listeria monocytogenes*, and *Salmonella enterica* in Orange Juice

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC), *Salmonella enterica*, and *Listeria monocytogenes* have been common targets when validating juice pasteurization treatments for juice Hazard Analysis and Critical Control Points (HACCP). While alternative processes are utilized by industry, heat remains the primary mechanism of pasteurization. It has been observed that foodborne pathogens which are adapted to an acidic environment may have greater thermal resistance; however, a common approach to acidification for process validation has not been agreed upon.

Purpose: The purpose of this study was to determine *D*- and *z*-values for acid-adapted cells using two common approaches (glucose or hydrochloric acid enriched media) compared to unadapted STEC, *L. monocytogenes*, and *S. enterica* to better understand how the mechanism of acid adaptation impacts thermal resistance in orange juice.

Methods: Cocktails of STEC, *L. monocytogenes*, and *Salmonella* were individually exposed to one of three growth treatments (two acid adaptation mechanisms and one control). Acid adaptation was evaluated by growing cells in tryptic soy broth (TSB) supplemented with 1N HCl to a pH of 5.0 or growth in TSB supplemented with 1% glucose. Cells were harvested from growth conditions, inoculated into orange juice, and exposed to three different heat treatments (50, 60, and 70°C). Each temperature and acid adaptation combination was evaluated along a series of six time points to calculate *D*- and *z*-values. All experiments were independently replicated (*n*=6).

Results: STEC, *L. monocytogenes*, and *S. enterica* all demonstrated an increase in thermal resistance with at least one of the acid treatments (P<0.05). STEC adapted in glucose at 60°C demonstrated the largest difference from non-acid adapted STEC with a *D*-value of 19.24 seconds, while STEC with no acid adaptation and treated at 60°C had a *D*-value of 8.96 seconds.

Significance: Outcomes from this work will help to provide scientific data to inform protocols utilized for preparing foodborne pathogens when validating a thermal process in an acidic environment.

T12-08 Study of the Microbiomes of Catfish Treated with Natural Preservatives Using 16S Metagenomics

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Introduction: The North American catfish (*Ictalurus punctatus*) is a lean protein source and one of the most consumed freshwater fish in the United States. Catfish spoil quickly even at refrigerated temperatures due to microbial activity, namely specific spoilage organisms (SSOs). Using natural preservatives on foods as antimicrobials have had some success, but results with catfish have been limited.

Purpose: The purpose of this study was to compare microbiomes of commercial catfish treated with different natural preservatives that could control certain SSOs and assess effectiveness of each preservative in prolonging shelf life.

Methods: Catfish from a local retail source were marinated in natural preservatives and stored in the refrigerator for two weeks. Every third day, CFU were enumerated to investigate bacterial growth for each preservative. Bacteria were also retrieved during storage period for metagenomics analysis. A DNA library was prepared according to the 16S metagenomics library guide. Samples were sequenced using Illumina platform and bacterial communities and α/β-diversities were assessed using QIIME and BaseSpace. SAV software showing index, cluster density, passing filter, and Q30 score were used for statistical analysis of sequencing quality.

Results: There was a considerable difference between total bacteria counts of natural preservatives and the control, as well as among natural preservatives. The data classified 10 core families; however, there are distinct differences in bacterial taxonomy at genus level and in α-/β-diversity with the different natural preservatives. Overall, *Janthinobacterium* spp., *Flavobacterium* spp., etc. were significant in the early phase, but effectively decreased with treatments.

Significance: Natural preservatives used on catfish as antimicrobials would offer an alternative to traditional preservatives. The results suggest that by controlling bacterial communities on catfish (primarily SSOs), natural preservatives could contribute to enhancing the safety and shelf life of catfish.

Poster Abstracts

P1-01 Influence of High-pressure Processing on the Microbiological Safety in Coffee Beans

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Introduction: Coffee is among the three major beverage crops in the world, with an annual global production of more than six million tons. Ochratoxin A (OTA) is a naturally occurring mycotoxin and a secondary metabolite of mould belonging to the genera *Aspergillus*. It is also the most common microbiological hazard that occurs in coffee and coffee-related products.

Purpose: The purpose of this study was to investigate the effectiveness of high-pressure processing (HPP) in inhibiting the growth of *Aspergillus fresenii* and reducing OTA content in coffee beans.

Methods: The aim of this study was to investigated the influence of HPP on the growth of *A. fresenii* and accumulated ochratoxin A content in coffee beans.

Results: The results indicated that investigation of the influence of HPP on *A. fresenii* growth on coffee beans showed that application of ≥400 MPa reduced *A. fresenii* concentrations to <1 log. Furthermore, during a 50-day storage period, we observed that a processing pressure of 600 MPa completely inhibited *A. fresenii* growth, and on day 50, the OTA content of coffee beans subjected to processing pressures of 600 MPa was 0.0066 µg/g, which was significantly lower than the OTA content of 0.1143 µg/g in the control group.

Significance: This study shows that HPP treatment can inhibit the growth of *A. fresenii*, thereby effectively reducing the production and accumulation of OTA and maintaining the microbiological safety of coffee beans.

P1-02 MC-Media-Pad: AOAC- and Microval-approved Culture Media Method for Rapid and Convenient Detection and Enumeration of Food Spoilage Microorganisms

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Introduction: The food and beverage industry needs methods to reduce workload and provide reliable, faster results compared to traditional culture media. Convenient culture medium devices have been developed for rapid and improved detection of total aerobic count, *Escherichia coli*, coliforms, yeasts, and molds. Devices comprise dried culture medium coated onto a pad protected by a transparent lid. Each 1-ml food sample is inoculated and rehydrated, according to reference method sample preparation procedures. Enumeration follows 24 to 72 h incubation. Color indicators and coding enable easy differentiation and improved read out.

Purpose: To pre-evaluate convenient media device performance for microbial monitoring in two key applications: dairy and processed food. Total aerobic count, coliforms, *E. coli*/coliforms, and yeasts/molds were assessed.

Methods: A total of seven microorganisms were artificially inoculated into two food matrice, pasteurized chocolate milk and canned tomato, at 10 to 100 CFU per test and in triplicate. Growth performance and enumeration were evaluated against ISO reference methods. Sample preparation followed the appropriate reference method, except inoculation volume, which was 1 ml.

Results: *Cronobacter sakazakii* and *Lactobacillus plantarum* were detected respectively within 24 and 48 h at 35°C in both matrices,with the total aerobic count device. Enumeration was comparable to the ISO 4832 and ISO 4833 reference methods and counting was facilitated by the universal red color of colonies. *E. coli*, *Enterobacter cloacae*, and a mixture of both were detected by coliform and *E.coli*/coliform devices within 24 h at 35°C in both matrices. The *E.coli*/coliform device differentiated by color *E. coli* colonies (red-purple) from coliform (blue). *Candida albicans*, *Saccharomyces cerevisiae*, and *Aspergillus brasiliensis* were detected in 48 h at 25°C with the yeast/mold, device with comparable results to the ISO 6611:2004 reference method.

Significance: Convenient culture media devices are a reliable alternative method to reduce time and workload in food spoilage detection.

P1-03 Molecular Characterization, Biofilm Formation, and Spoilage Potential of *Bacillus* Isolates from Different Milk Samples

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❖ ***Developing Scientist Competitor***

Introduction: An emerging food safety challenge in the dairy industry is that of the development of biofilm on food contact surfaces, plants, and equipment. Due to their highly resistant nature, biofilms defy all measures, such as the use of sanitizers and high temperature, commonly adopted to control spoilage and pathogenic contaminants in processing plants. Biofilm contaminations result in shelf life reduction of processed foods and are potential sources of pathogen outbreaks and food spoilage.

Purpose: The aim of the study is to characterise *Bacillus* isolates from raw, pasteurised, and packaged extended shelf life milk samples and determine their biofilm and spoilage potential.

Methods: The isolates were characterised using multi-locus sequence typing (MLST). Seven housekeeping genes were selected for the MLST. The universal primers used were designed from the coding region of each gene with the highest number of polymorphic sites. The proteolytic activity of the biofilms on stainless steel was done by using 5.0 g L^{-1} sulphanilamide azocasein solution in 50 mM 3-(N-morpholino) propansulfonic acid (MOPS, pH 6.7) containing 1 mM $CaCl_2$ with the absorbance measured at 450 nm. The lipase activity was also determined by using fluorimetric method of 4-methylumbelliferyl ester assay. The cells within the biofilms were visualised using fluorescence and Confocal Laser Scanning Microscopy. All experiments were performed three times and the data were analysed using multifactor analysis of variance.

Results: Fisher's Least Significant Difference was used to determine significant differences between the treatments at $P \leq 0.05$. Statistical analysis shows that there are significant differences in the proteolysis and lipolysis produced within biofilms of the isolates. Phylogenetic cluster analysis of the MLST sequences reveals six major clusters for all isolates.

Significance: The study presents the significance of biofilm-induced proteolysis and lipolysis in dairy processing plants and the spoilage of extended shelf life by isolates of the *Bacillus subtilis* complex group.

P1-04 Contamination Profile of Lactic Acid Bacteria in Production Environments of Sausage and Mayonnaise Factories

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Introduction: Lactic Acid Bacteria (LAB) have been identified as a significant spoilage microorganism in vacuum packed products (e.g., cooked sausage) and acidified foods (e.g., mayonnaise). To prevent LAB contamination in food products, the rapid and convenient enumeration of LAB is needed to isolate the sources of contamination.

Purpose: This study aims to establish the LAB contamination profile in raw material (RM), environmental surfaces, and finished products (FP) of sausage, salami, and mayonnaise production facilities.

Methods: Samples of RM, FP, and environmental swabs of Zones 1, 2, and 3 from three factories were obtained. RM and FP samples were prepared by homogenizing 10 g with 90 ml Butterfield's phosphate buffer, then serially diluting. 1 ml of diluted sample (or swab diluent) was inoculated onto 3M Petrifilm Lactic Acid Bacteria Count Plate (Petrifilm LAB), followed by plate incubation at 37±1°C for 48±3 hours.

Results: A total of 268 samples were obtained from three factories: 238 environmental swabs, 25 RMs, and 5 FPs. In Factory A (sausage), LAB contamination was found in RMs and the production environment. In each zone, the percentage of contaminated sites were: Zone 1=11.71% (1.00 to 2.38 $\log_{10}$ CFU/swab area); Zone 2=16.22% (1.00 to 4.43 $\log_{10}$ CFU/swab area); and Zone 3=18.92% (1.48 to 4.68 $\log_{10}$ CFU/swab area). After heat treatment, LAB contamination was not found in FPs. In Factory B (sausage), the percentage of contaminated sites in each zone were: Zone 1=19.35% (1.00 to 7.25 $\log_{10}$ CFU/swab area); Zone 2=11.29% (1.00 to 3.96 $\log_{10}$ CFU/swab area); and Zone 3=22.58% (1.00 to 2.48 $\log_{10}$ CFU/swab area). And finally, in Factory C (mayonnaise), LAB contamination was found in RMs and the production environment. Environmental contamination (2.69 to 5.99 $\log_{10}$ CFU/swab area) was found in pipelines, joints, blowers, valves, pumps, and T-shape tubes, which are often difficult to clean.

Significance: LAB is a significant spoilage microorganism in various food products. Petrifilm LAB can be used as a tool to assess sources of contamination to enable corrective and preventive actions for food factories.

P1-05 Evaluation of Commercial Cultured Food Ingredients Used to Maintain the Quality of Fresh Refrigerated Soup

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Introduction: Growing consumer preference for natural, freshly prepared and refrigerated foods, has led to an increase in the types and varieties of products in the retail space. As part of this trend, refrigerated soups have grown in popularity and availability. Although these are thermally treated during production, additional ingredients are often needed to help maintain an acceptable shelf life during distribution.

Purpose: The aim of the study was to evaluate the microbial succession of refrigerated soup when treated with different cultured food ingredients.

Methods: Two commercially available cultured ingredients were evaluated for their impact on the outgrowth of bacteria during the shelf life of refrigerated chicken noodle soup compared to a commercial nisin preparation. The treatments included an untreated control (UC), nisin preparation (N) at 0.04% (percent based on total formulation weight), and two cultured dextrose products (CD1 and CD2) with CD1 tested at 0.15% and 0.3% and CD2 tested at 0.3%. Chicken noodle soup was produced in a commercial kitchen and treatments were added before cooking was completed. Finished soups were packaged in individual cups then shipped on ice overnight and stored refrigerated (~7 to 10°C) for the duration of the study. Individual cups were enumerated in duplicate for lactic acid bacteria (LAB) and total aerobic plate count (TPC) on days 1, 9, 17, 27, and 36 post production. In addition, the tested soup material was pelleted down and stored at -20C for future microbial analysis.

Results: No differences were seen in LAB levels until day 17, at which time there were distinct differences between the untreated control soup (>1×10^5 CFU/ml) and all other treated soups (<1×10^3 CFU/ml) and continued through day 36. Microbial enumeration of TPC revealed that the soup treated with CD1 at 0.3% was able to maintain a lower total bacterial count compared to all other treated soups.

Significance: Understanding how these natural solutions function will help food formulators build robust and effective systems.

P1-06 Antimicrobial Ability of Modified Bacterial Cellulose Film against Spoilage Microorganisms

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Introduction: Bacterial cellulose (BC) as a highly crystalline linear polymer has high water-holding capacity and great tensile strength, but poor processability. Incorporating antimicrobial agents may improve its function and applicability as a packaging material.

Purpose: The aim of this study was to develop a flexible and antimicrobial BC film via the incorporation of polyvinyl alcohol (PVA) and silver nanoparticles (AgNPs).

Methods: BC was synthesized by growing *Gluconobacter xylinus* in Hestrin and Schramm medium at 26°C for 7 days. The BC slurry was mixed with a 3% PVA solution to improve its flexibility. AgNPs were incorporated by immersing the BC in 0.01 M $AgNO_3$ solution and 0.02 M $NaBH_4$ solution (R film), or exposing the $AgNO_3$ solution to a 365-nm wavelength ultraviolet light for 1 h (UV film). Films were used to wrap raw beef and potato pieces, which were then stored at 4°C. The pour plate method was conducted at different times over 14 days to determine the antimicrobial activity of the films.

Results: The films showed no inhibitory effect on non-pathogenic *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, and *Bifidobacterium animalis*, but effectively inhibited the pathogenic bacteria, *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Staphylococcus aureus*, and *Listeria monocytogenes*. Clear zones of around 1.5 mm were observed in the diffusion test of the UV film against all four tested pathogens. In a beef shelf-life test, a 1-log and 3-log CFU/cm^3 reduction of total bacterial count were conferred by the R and UV films, respectively, after 10 days of refrigerated storage. A 4-log CFU/cm^3 reduction of *E. coli* O157:H7 was conferred by the UV film after 4 days of refrigerated storage.

Significance: Development of novel flexible antimicrobial BC films may enable its application as a method to control microbial contaminants in the food industry.

P1-07 Withdrawn

P1-08 Innovative High-throughput Automated Membrane-based Real-time PCR Detection of *Salmonella*

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Introduction: Salmonella causes high incidences of infections worldwide due to food poisoning in humans which is associated with contaminated food products of animal origin. The need of the hour is to have a rapid, cost-effective and automated diagnosis for *Salmonella* in food.

Purpose: The objective of this study was to evaluate the performance of automated nucleic acid extraction system coupled with Real Time Probe based PCR assay.

Methods: A pre-enrichment and pre-extraction sample preparation protocol was done as per ISO guidelines. Enrichment was done on selective media followed by DNA extraction-purification using automated extraction system without any cross contamination while processing. Real Time Probe based PCR assay was performed with an in-house developed Salmonella Detection Kit using Real time PCR platform. An internal amplification control (IAC) was also included in the assay that was non-specific to *Salmonella*. Eight different food samples (solid and liquid) were spiked with *Salmonella typhimurium* standard culture dilutions which included media and non-spiked controls. The spiked dilutions were sampled in triplicates.

Results: Spiked dilutions of eight samples were found to be positive while media and non-spiked controls were found to be negative. The diagnostic accuracy of Salmonella detection was shown to be 100% compared to the traditional culture method. A minimum of 1 cell was identified using the in-house probe based *Salmonella* Detection PCR platform.

Significance: The current methodology of automated extraction with real time PCR offers low carryover contamination, and acceptance by easily accessible and user-friendly protocols for its application and interpretation. The overall analysis time was approximately 18 h, in contrast to 4 to 5 days of analysis time for the traditional culture methods, thereby significantly reducing the reporting time over the traditional culture methods. The current methodology can be replicated to identify other food borne pathogens which requires further studies.

P1-09 Association of Fungal Genera with Processed Foods and Production Failures

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Introduction: Fungal spoilage in commercially processed foods causes consumer dissatisfaction and contributes to food waste. Many fungi are tolerant to the conventional controls used in food production. However, these processing conditions and physiochemical properties can create niches which support the growth of a limited number of spoilage fungi.

Purpose: This study was designed to evaluate the influence of intrinsic and extrinsic food product variables on the identity of the specific spoilage fungi isolated from commercially produced foods.

Methods: The associated spoilage fungi were identified from 127 spoiled commercial products through amplification and sequencing of the internal transcribed spacer region. The prevalence and diversity of the identified spoilage fungi were evaluated in relationship to product-specific attributes (processing conditions, physicochemical properties) using various descriptive statistics and a bipartite network analysis. Additionally, recursive partitioning was used to generate a classification tree with the outcomes and genera of the spoilage isolates with at least four observations, divided into increasingly homogenous subgroups.

Results: All of the isolated fungi belonged to the *Ascomycete* phylum, except for four mucoralian isolates. The occurrence of filamentous fungi repeatedly isolated ranged from 2% (*Phoma*) to 18% (*Penicillium*). Around half of isolates came from low-pH products (57%) and high-aw (65%) products. In order of decreasing contribution to subgroup homogeneity, the split rules for the classification tree were based on process, water activity, food matrix category, and pH. Fungal representation in the terminal nodes indicated that production failures, in addition to product-specific attributes, were responsible for determination of the most probable specific spoilage organism.

Significance: Identification of specific spoilage organisms, and the processing failures associated with their introduction and proliferation, can help processors develop better quality control strategies.

P1-10 Metagenomic Analysis of Microbial Communities in Commercial Catfish Treated with Grapefruit Seed Extract

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Introduction: Consumers have become concerned with the use of synthetic treatments to preserve and extend the shelf life of foods. Natural antimicrobials are an alternative solution that can prevent microbial spoilage. Catfish (*Ictalurus Punctatus*) are one of the most commonly raised freshwater fish in the United States but have a short shelf life due to microbial activity, namely specific spoilage organisms (SSOs). High-throughput sequencing technology is applicable for metagenomic study to investigate microbial communities, including SSOs and their response to treatment with natural preservatives.

Purpose: The purpose was to examine bacterial diversities associated with catfish spoilage and effectiveness of controlling SSOs with grapefruit seed extract (GFS) treatment.

Methods: A commercial catfish was treated with GFS and stored in the refrigerator for 15 days with non-treated catfish. During the storage period, bacteria counts were taken every other day from both catfish samples and compared. DNA was extracted for PCR amplifications of hypervariable regions using 16S universal primers. DNA libraries were prepared in accordance with the Illumina metagenomics guide. Samples were sequenced using the Illumina MiSeq and sequencing run statistics were analyzed through SAV. Bioinformatics pipelines, BaseSpace, and QIIME, were used for metagenomics analysis.

Results: The treated catfish showed decreased CFUs compared to the control. The DNA sequencing read totals of 230,702 and 148,203 genera before and after treatment. During overall storage period, the *Shewanella* spp. responsible for fish spoilage significantly decreased among bacterial communities on treated catfish compared with non-treated catfish. Treatment samples showed less Shannon diversity, as well as further hierarchical clustering distance, contrasting with control samples.

Significance: The results suggest that grapefruit seed extract could be used to control bacterial communities, especially to decrease SSOs, and therefore be useful in fishery industries to extend the shelf life of catfish.

P1-1 Withdrawn

P1-12 Edible Nano-Coating for Extending Shelf Life and Improving Food Safety of Blueberries

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Developing Scientist Competitor

Introduction: Blueberries, one of the most commonly consumed berries in the United States, are well-known for their healthy attributes, including the production of various forms of bioactive, beneficial compounds. While the demand for berries is stable year-round, the growing season is short and berries are highly susceptible to fungal spoilage and a short shelf life. Also, possible contamination of blueberries can occur during agronomic practices, and postharvest handling leads to human food safety issues.

Purpose: Extending the shelf life of berries by minimizing postharvest losses due to spoilage through the use of an edible nano-coating (ENC) containing the anti-microbial agent limonene without compromising berry flavor or nutrition.

Methods: The antimicrobial efficacy of prepared nano-material was examined *in vivo* by testing against bacteria (gram positive and negative) and selected spoilage fungus. Freshly harvested blueberries were coated with developed nano-encapsulation and subjected to shelf-life study. At each sampling time of 0, 1, 7, 14, 21, 28, 35, 49, and 70 days, berries were analyzed for decay incidence, weight loss (WL), yeast and mold count, total phenolic content (TPC), total anthocyanin (TA), and antioxidant capacity.

Results: The antibacterial activity against *Escherichia coli* showed 1.7-$\log_{10}$ reductions in CFU ml^{-1} and higher efficacy of 3.4-$\log_{10}$ reductions exhibited for the gram positive bacterium (*Listeria monocytogenes*) within 48 h. Germination of *Botrytis cinerea* conidia was completely suppressed over 48 h. ENC reduced fruit loss, as well as showed significantly lower mold deterioration, WL, and higher firmness ($F_{3,8}$; P<0.05) throughout storage. The coating did not show a significant effect regarding titratable acidity, pH, and soluble solid, TPC, or TA of berries. Scan electron microscopy and fluorescent images depicted better integrity of ENC.

Significance: As an edible coating, liposome encapsulated limonene could be an excellent alternative postharvest treatment for extending the shelf life of short-season berries.

P1-13 *Pseudomonas* Spoilage Leading to a Lack of Foam Stability in Fluid Milk

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Introduction: Coffee shops represent a significant market for fluid milk producers in the United States. Foam stability is a critical quality for fluid milk used in lattes and cappuccinos. Recently, a fluid milk processor has been experiencing returns from coffee shop clients due to a lack of foam stability during steaming. No other quality defects (odor, color) were observed. The processor suspected microbial contamination to be the cause of this quality deterioration.

Purpose: The purpose of this study was to quantify microbial contamination of fluid milk and to verify the impact of microbial contamination on a lack of foam stability.

Methods: Milk samples suspected to have foaming problems were enumerated on spirit blue agar following incubation at 25°C for 72 h. Eight colonies displaying strong lipase activity were selected for inoculating 2% ultra-high temperature pasteurized milk. Inoculated milk samples (~3 log CFU/ml) were stored at 7°C for 4 to 8 days. Microbial growth was determined by serial dilution and plating on tryptic soy agar after incubation at 25°C for 72 h. Milk samples were foamed using an electric frother (Starbucks Verismo) and foam volume was measured at several time points (immediately after foaming, 15 min, and 30 min). Isolates of interest were identified by 16S rDNA sequencing.

Results: Three isolates were identified to cause a 20 to 30% reduction in foam volume that was correlated with a cell density of >7 log CFU/ml. Genetic analysis identified these isolates to be *Pseudomonas fluorescens*.

Significance: This research demonstrates that microbial contamination of fluid milk with *Pseudomonas* spp. can lead to a loss of foam stability. *Pseudomonas* spp. contamination is most likely a post-pasteurization contamination and emphasizes the need for increased sanitation and hygiene in commercial processing environments.

P1-14 *Salmonella* Survival in Pan-fried and Flash-fried Chicken Livers

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Introduction: Charcuterie has experienced a resurgence, and many restaurants and caterers in the Pacific Northwest are offering charcuterie boards on their regular menus. Undercooked poultry liver products, particularly chicken liver pâtés, have been implicated in recent *Campylobacter* and *Salmonella* outbreaks. Unlike meat tissues, *Campylobacter* and *Salmonella* have the ability to colonize the bile duct and become internalized in the parenchyma of the liver. Little data is available on the efficacy of cooking methods to inactivate foodborne pathogens in poultry liver.

Purpose: The objective of this study was to determine the efficacy of two common cooking procedures (pan-frying and flash frying) to reduce *Salmonella* in chicken livers.

Methods: Raw chicken livers were purchased from a local grocery and inoculated with a *Salmonella* cocktail (>6 log CFU/g). Individual livers were cooked by pan-frying (medium heat; 60 sec/side) or by flash-frying (240 to 260°C; 30 to 60 sec). *Salmonella* survivors were enumerated on Hektoen enteric agar following incubation at 37°C for 48 h.

Results: Inoculated, raw chicken livers contained 6.15 log CFU/g *Salmonella.* Pan-frying for 60 sec per side resulted in an average reduction of 1.30 log CFU/g with a characteristic and desirable "rare" appearance. Flash-frying for 30 sec achieved an average reduction of 2.06 log CFU/g. Increasing the time of flash-frying to 60 sec resulted in an average *Salmonella* reduction of 4.52 log CFU/g; however, *Salmonella* reduction in individual livers was as little as 1.70 log CFU/g. Efficacy of flash-frying was inversely correlated with the mass of individual chicken livers.

Significance: This research demonstrates the likelihood of *Salmonella* to survive cooking practices that are commonly used by restaurants in the preparation of poultry liver dishes. Culinary professionals need to be educated about risks associated with internal contamination of poultry liver products.

P1-15 Relative Thermal Tolerance of Isolates Responsible for Off-flavor Development and Spoilage of Fat-free Chocolate Milk

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Introduction: Pre-mature shelf-life failure due to the development of off-flavors in fat-free chocolate milk is a common problem reported by fluid milk processors. The rate of spoilage for chocolate milk is faster than that of white milk, possibly due to the extra inclusions (chocolate, sucrose, and vitamins). Previous research on spoiled chocolate milk has identified isolates that are associated with characteristic off-odor development ("Band-aid", fishy, bready); however, the source of these isolates and point of contamination has not yet been identified.

Purpose: The objective of this study was to determine the thermal resistance of microbial isolates associated with off-odor development in fat-free chocolate milk. This information will assist determining the likelihood of pre- or post-pasteurization contamination.

Methods: Five isolates that were previously verified to cause spoilage of fat-free chocolate milk were inoculated into ultra-high temperature pasteurized chocolate milk (>7 log CFU/ml). Aliquots (1 ml) of inoculated chocolate milk were pasteurized at 63°C for up to 30 min. Survivors were enumerated by spread-plating on tryptic soy agar following incubation at 25°C for 48 h.

Results: Spoilage isolates differed significantly in their thermal resistance in chocolate milk. Isolate 3 (*Paenibacillus* spp.) was the most resistant, experiencing an average reduction of 1.53 log CFU/ml at 63°C for 30 min. Isolate 4 (*Pichia* spp.) was also fairly heat-resistant, with an average reduction of 2.24 log CFU/ml under the same conditions. Isolates 1 (*Bacillus* spp.), 2 (*Bacillus* spp.), and 5 *(Acinetobacter* spp.) were the most sensitive to thermal treatment, with average reductions of 3.47, 4.30, and 3.73 log CFU/ml, respectively.

Significance: Microorganisms associated with off-odor development in chocolate milk differ in their thermotolerance. Heat-sensitive strains are likely to be associated with post-pasteurization contamination, whereas heat-resistant strains could be contaminating strains either pre- or post-pasteurization.

P1-16 Inactivation of Natural Spoilage Microflora in Refrigerated Raw Pineapple Juice with Added Isoeugenol

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Introduction: Growing public concern over loss of heat-labile nutrients during heat treatment of juices has led to an increase in the consumption of raw fruit and vegetable juices. Without a food preservation intervention, fruit juice spoilage microbes such as yeasts and molds can shorten the shelf life of the juice. Natural antimicrobials are gaining widespread consumer acceptance and may be effective at extending the shelf life of raw fruit juices while sparing heat-labile nutrients.

Purpose: A study was performed to evaluate the effectiveness of iso-eugenol (ISO-EU) for control of the natural spoilage microflora in refrigerated (4°C) raw pineapple juice with added yucca extract (YEX).

Methods: Raw pineapple juice (pH 3.5) with 0.5% YEX containing 0 (control), 0.50, 0.75, 1.0, or 1.5 µl/ml of ISO-EU was stored at 4°C. Raw juice without YEX and ISO-EU served as another control. At set intervals, survivors were determined by plating diluted (10-fold) samples on appropriate agar and counting microbial colonies after incubation for set times.

Results: Initial viable yeast and mold count increased in juice without added ISO-EU and YEX and reached 5.45 log CFU/ml in 24 days; however, no growth of this group occurred in juices with added ISO-EU. At 4°C, 1.0 and 1.5 µl/ml ISO-EU reduced the initial viable yeast and mold count in juice by ~1.31 and 2.60 log CFU/ml, respectively, after 24 hours. ISO-EU (1.5 µl/ml) completely inactivated yeasts and molds after 24 days. ISO-EU at 0.75, 1.0, or 1.5 µl/ml eliminated *Enterobacteriaceae* after just 4 hours (P<0.05), and ISO-EU at 1.0 and 1.5 µl/ml reduced the initial viable aerobic plate count in juice by ~1.85 and 1.94 log CFU/ml, respectively, after 4 hours.

Significance: ISO-EU in combination with yucca extract may be an effective natural preservative in extending microbial shelf life of refrigerated (4°C) pineapple juice.

P1-17 *Staphylococcus aureus* Growth in Egg Roll Filling at Different Storage Temperatures

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Undergraduate Student Award Competitor

Introduction: *Staphylococcus aureus* is a pathogen that potentially derives from human handling and produces a heat-stable toxin that thrives in protein-enriched environments. A product's holding time will influence the need for further heat treatment; thus, bacterial growth should be limited to less than 1 log. This preventative measure ensures that all heat-treated products are safe and pathogen-free.

Purpose: The purpose of this study was to determine the amount of time for 1 log of *S. aureus* growth in inoculated meat and vegetable fillings while held at various temperatures.

Methods: Through five trials, a cocktail of *S. aureus* ATCC 29213 and ATCC 6538 was used to separately inoculate meat and vegetable egg roll fillings, which were tested at two separate storage temperatures, 7 and 25°C, for a period of 12 hours at 2 hour increments. Positive and negative controls were done and the inoculated samples were plated on Express 3M Staph plates. The amount of *S. aureus* was plotted and the growth rate was recorded and compared using R software.

Results: The growth of S. *aureus* in refrigerated versus ambient storage temperatures was statistically significant *(P<0.05)* for both the meat and vegetable fillings, with a quicker growth rate for those stored at room temperature. The meat fillings at 25°C exhibited growth at 2.3±1.1 hours for 1 log of growth to appear, while the vegetable filling did not show significant growth. The meat filling at 7°C exhibited growth at 5.1±0.54 hours for 1 log of growth of the *S. aureus*, whereas growth in the vegetable filling never reached 1 log.

Significance: These data suggest that if stored at refrigerated or ambient temperatures, meat and vegetable egg roll fillings can be held for an extended amount of time before any significant amount of *S. aureus* growth occurs, which limits the need for processing.

P1-18 Evaluation of the Survival and Growth of *Listeria monocytogenes* and Lactic Acid Bacteria in Mango (*Mangifera indica*), Custard Apple (*Annona muricata*) and Blackberry (*Rubus ursinus*) Pulps from Costa Rica

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Introduction: Fruit consumption is a practice that has increased lately because of the health benefits associated. Fruit pulps represent the most basic food product obtained from fresh fruit processing, and tropical fruits' pulp possess enormous commercial potential, nevertheless they can represent a risk for the health of consumers

Purpose: The aim of this work is to evaluate the survival and growth of different *Listeria monocytogenes* concentrations inoculated into mango, custard apple and blackberry pulps through storage time. Also the behavior of lactic acid bacteria as quality indicators.

Methods: Three different fruit pulps were selected, in order to evaluate low, medium and high acidity levels. These were inoculated also with three different concentrations of *L. monocytogenes* (10^3, 10^5 and 10^7 UFC/ml) and incubated at 5°C for 15 days in order to evaluate its growth and survival. Lactic acid bacteria count was also evaluated Counts were performed at 0,3,6,9,12 and 15 days for each pulp and by triplicate.

Results: Blackberry pulp showed no survival of the bacteria analyzed. The other two pulps showed survival and even growth both of *L. monocytogenes* and lactic acid bacteria. For custard apple pulp, medium and high concentrations of *L. monocytogenes* showed growth up to day 3, and for mango pulp, same concentrations showed growth up to day 12 of storage.

Significance: Fruit pulps maintained at refrigeration temperatures may represent a risk for health of consumer, further research shall be done in order to offer safe products to consumers.

P1-19 Evaluation of an ATP Bioluminescence Detection-based Technology for Testing Microbial Contamination in Commercially Sterile Dairy UHT Products

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Introduction: Global demand for commercially sterile beverages is growing by about 9% annually, and rapid release of ultra-high temperature (UHT) products for commercialization may significantly impact inventory volume. Detection of adenosine triphosphate (ATP) through bioluminescence enables rapid product release by detecting microbial contamination in UHT products. Determining the appropriate enrichment conditions to achieve a microbial level for detection of microbial ATP methods is necessary for an appropriate quality release method implementation.

Purpose: To determine the level of bacteria required after UHT product enrichment to detect microbial ATP with a bioluminescent technology.

Methods: Two dairy UHT products (milk and chocolate milk) were inoculated in triplicate (n=360) with 1 ml of each of *Bacillus subtilis*, *B. licheniformis*, *Escherichia coli*, *Enterococcus faecalis*, and *Geobacillus stearothermophilus* using five microbial concentrations (10^3 to 10^9 CFU/container), with 10^9 being the largest inoculum. Additionally, 10 samples of UHT product were used as negative control. After inoculation, products were held for 3 h at 37°C and microbial contamination was determined by ATP bioluminescence. The samples were streaked on agar and total plate counts were also determined.

Results: Microbial detection in 349 of the inoculated samples was achieved by microbial ATP bioluminescence and agar. The lowest population of microorganisms to achieve 100% detection of microbial ATP after enrichment varied by bacterial strain: *B. subtilis* at $10^{5.3}$ CFU/container, *B. licheniformis* at $10^{4.72}$ CFU/container, *E. coli* at $10^{6.15}$ CFU/container, and *E. faecalis* at $10^{4.77}$ CFU/container. *G. stearothermophilus* was not detected by either method. All the negative controls yielded a negative result.

Significance: ATP bioluminescence enables rapid detection of microbial contamination in UHT products. Understanding what microorganisms are relevant to a specific UHT products and how their growth is affected by specific enrichment (incubation) conditions is essential for accurate microbial detection. Determining the lowest population required to achieve detection may be used as a criterion to optimize enrichment conditions for accurate detection and rapid product release.

P1-20 Growth and Survival of *Escherichia coli* O157:H7 in Model Vegetable Fermentations under Varying Salt Conditions

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Introduction: Shiga toxin-producing *Escherichia coli* strains (STEC) are the most acid-resistant pathogens of concern for vegetable fermentations. The safety of model fermentations was compared under three industrially relevant salt conditions: 2% sodium chloride (NaCl) representative of cabbage fermentations (kimchi and sauerkraut); 6% NaCl representative of cucumber pickle fermentations; and 1.1% calcium chloride ($CaCl_2$) representative of commercial cucumber fermentations currently being used to reduce NaCl waste.

Purpose: Our purpose was to determine how salt treatment affected the growth and death of STEC in competition with lactic acid bacteria in a model vegetable fermentation medium.

Methods: Sterile cucumber juice containing no known natural inhibitory compounds was used to create triplicate pure and mixed culture fermentations. Initial cell concentrations were 10^4 CFU/ml for *Escherichia coli* (O157:H7) B200 and a commercial vegetable fermentation isolate, *Lactobacillus pentosus* MOP3. Cells were selectively enumerated using LB agar at 37°C (B200) and MRS agar at pH 5 and 30°C (MOP3). Fermentations were carried out at 30°C in 20-ml vacutainer tubes and sampled by syringe to maintain anaerobic conditions.

Results: We found that regardless of salt condition, B200 grew to approximately 10^8 CFU/ml but was below the limit of detection (10^2 CFU/ml) by 48 h in competition with MOP3. MOP3 grew similarly but maintained 10^9 CFU/ml for up to 72 h in pure and mixed culture. Despite similar changes in pH, fermentations with $CaCl_2$ had approximately half the lactic acid (19.9±0.9 mM) or acetic acid (9.4±0.2 mM, produced by *E. coli*) cultures in NaCl. The results show that regardless of salt type or concentration, pH (rather than organic acid concentration) was an important predictor of fermentation safety.

Significance: These data may be used to help industry and regulatory agencies define factors affecting the safety of low- and alternative-salt vegetable fermentations.

Poster

P1-21 Persistence of *Salmonella* on Different Dry Tea Types and Fate under a Range of Brewing Processes

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Introduction: Dried tea contaminated with *Salmonella* has led to several product recalls. Previous studies have not considered the potential for *Salmonella* to enter a dormant state on tea, thereby leading to an over-estimation of die-off rates. In addition, there is a need to consider if *Salmonella* survives the increasing trend of cold or low-temperature brewing.

Purpose: To determine the survival of *Salmonella* serovars on different dried teas stored under a range of conditions, and fate during the brew process.

Methods: Salmonella (Javiana, Montevideo, Enteritidis, Typhimurium, and Newport) were introduced onto different dry tea blends and stored at 4, 10, or 25°C. Samples were withdrawn over the course of the 120-day storage period and *Salmonella* enumerated following a resuscitation step. The thermal inactivation kinetics of *Salmonella*, pre-adapted to tea, were determined using different brewing temperatures.

Results: In all cases, there was a multi-phasic decrease of *Salmonella* levels on tea with evidence of recovery from sub-lethal injury. The lowest persistence of *Salmonella* was encountered on black, green, or ginger tea, with peppermint and chamomile supporting extended survival. Low storage temperature (4°C) promoted survival of *Salmonella* with higher temperatures (25°C) making the pathogen more sensitive. *Salmonella* Newport was found to be the most stress-tolerant on tea compared to the other serotypes tested. In the majority of cases, *Salmonella* could still be recovered on all tea blends at the end of the 120-day storage period. The D_{60} values for *Salmonella* varied between 1.07 and 2.15 min (z=4 to 10°C) on the different tea types.

Significance: Salmonella can persist on dried tea over extended periods in a dormant state and can survive brewing if performed at <60°C. Consequently, *Salmonella* needs to be considered a risk in cold or low-temperature brewing.

P1-22 Concentration of *Lactobacillus brevis* from Experimentally Infected American Lager Beer by Innovaprep's Concentrating Pipette and Be Flat Degassing Jar

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Introduction: Beer spoilage organisms present a major risk for the brewing industry. To date, most laboratories still use time-consuming conventional cultivation methods, which require three to five days for beer to be released to market. Rapid microbiological analytical methods offer hope of increased reliability and reduced hold times; however, small analysis volumes currently limit their usefulness. Sample concentration techniques can enable detection of low microbial concentrations in beer, but their development has been outpaced by that of rapid detection methods.

Purpose: The purpose of this study was to determine if degassing and rapid concentration of experimentally infected American-style lagers offers a rapid alternative to current cultivation methods.

Methods: For experimentally infected beer, an average of 100 CFU of *Lactobacillus brevis* (WLP 672) was inoculated into 12 ounces of room-temperature beer, allowed to incubate in the degassing jar at 4°C for up to 20 minutes, and then concentrated. Concentrated samples were plated onto De Man, Rogosa and Sharpe agar at 30°C for up to 48 hours and CFU were enumerated.

Results: Experimentally infected beer (n=10) from two breweries was concentrated and plated to determine concentration efficiency. The average time to concentrate 355 ml of degassed beer from brewery 1 was 6.31 minutes (±0.73) and from brewery 2 was 3.89 minutes (±0.13), with average concentration elution volumes of 292 µL (±34.2) and 621 µL (±0.07), respectively. The average concentration efficiency for brewery 1 was 71% (±6.69) and brewery 2 was 73% (±10).

Significance: These data suggest degassing and concentration of beer can dramatically reduce the time to detection for quality control microbiological testing of finished beer.

P1-23 Modeling the Survival of *Salmonella* in Soy Sauce-based Products Stored at Two Different Temperatures

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Introduction: Soy sauce-based Asian-style condiments are growing in popularity in the United States, and many of them fall into the category of acidified foods. However, they may not receive a full thermal treatment due to convenience packaging. These products must rely on acid alone to ensure the destruction of pathogens. Predictive modeling can be used to identify the intrinsic and extrinsic factors that will ensure safety.

Purpose: A predictive model for the survival of Salmonella in soy sauce-based products was generated to assist manufacturers of acidified Asian-style sauces in determining hold times and temperatures for products that do not received a full thermal treatment.

Methods: Three levels each of pH (3.0, 4.0, and 5.0), soy sauce (0, 50, and 100%) and salt (2, 7, and 14%) served as inputs for the model. Eighteen combinations were stored at two different temperatures 18.3°C (65°F) and 23.8°C (75°F), which bracket normal warehouse temperatures. The inoculum level was above 8.0 log CFU/g. For each combination, Salmonella concentration was plotted versus time, fitting the data to the Baranyi and Robert model. For the secondary model, the maximum death rate (log CFU/g/h) of Salmonella under different conditions estimated from the primary models were fit to a quadratic equation, expressed as a function of temperature, pH, salt, and soy sauce percentages and their interactions.

Results: Temperature and pH were statistically significant to the death rate of Salmonella (P < 0.05), as were combinations of pH and soy sauce. The maximum death rate was 5.1 log CFU/g/ for the combination of pH 3.0, 100% soy sauce and 14% salt stored at 23.8°C (75°F). The model successfully predicted the response of Salmonella with an acceptable prediction performance of 83%.

Significance: This study represents the first known predictive model for the survival of Salmonella in Asian-style soy sauce products. These data suggest that the model can provide useful quantitative data for the development of safer acidified food products and processes.

P1-24 Growth Inhibitory Effect of D-Tryptophan on *Vibrio* spp. in Broth Culture, Seawater, and Live Oysters

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Introduction: The current postharvest technology for fresh oysters to control *Vibrio* spp., such as high-pressure processing, has limited application considering potential adverse effects on production, facility limitations, and high cost. Small amounts of D-Tryptophan (D-Trp) could exhibit growth inhibitory effect on some foodborne pathogenic bacteria in high-salt environments. Therefore, utilizing exogenous D-Trp in seawater would be regarded as a novel strategy for controlling *Vibrio* in live oysters.

Poster

Purpose: To determine the growth inhibitory effects of D-Trp on *Vibrio* spp. in live and shucked oysters in seawater. To confirm the working characteristics of D-Trp, we clarify the relationship between salt (NaCl) and D-Trp concentrations on growth inhibitory effects of *Vibrio* spp.

Methods: We examined the growth inhibitory effects of D-Trp on *V. vulnificus* and *V. parahaemolyticus* in liquid media at 25°C under various NaCl concentrations. The viable cell counts were determined by direct plating on triptic soy agar plates. *Vibrio*-inoculated shucked and live oysters were immersed in a seawater with or without D-Trp at 25°C. *Vibrio* were enumerated by plating on selective media (CHROM Agar). In parallel, the total viable bacteria count (TVC) in shucked oyster culture with or without D-Trp at 4°C were determined.

Results: The treatment of 40 mM D-Trp with NaCl significantly reduced *Vibrio* levels in broth culture at 25°C. The effectiveness was found to be greater at higher NaCl conditions (>4.0 weight/volume). Compared with *V. parahaemolyticus*, *V. vulnificus* were more sensitive to D-Trp. Similar results were also observed in artificial seawater and whole shell live oysters at 25°C. In addition, the growth of TVC in shucked oyster culture at 4°C was greatly inhibited by adding 40 mM D-Trp.

Significance: Utilizing exogenous D-Trp will be an alternative strategy for controlling *Vibrio* in oysters, even at ambient temperatures, and extending the shelf life of raw oysters at refrigerated temperatures.

P1-25 Comparison of *Listeria monocytogenes* Inactivation on Cellulose Filter Membranes during Hot-air Roasting

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❖ ***Developing Scientist Competitor***

Introduction: Listeria monocytogenes has been implicated in recent recalls of low-moisture, hot-air roasted foods. Guidelines for conducting challenge studies to validate processes such as roasting as kill steps suggest screening several strains to determine which has the greatest resistance. Currently, information regarding the heat resistance of desiccated *Listeria* strains is lacking.

Purpose: The purpose of this study was to compare the thermal resistance of different desiccated *Listeria monocytogenes* strains subjected to hot-air roasting.

Methods: Cellulose filter discs (0.22 µm pore size) were inoculated individually with one of seven *Listeria monocytogenes* strains (Scott A, ATCC 13932, ATCC 49594, NZRM 4242, NZRM 4237, FRRB 02542, and LM-004) at a level of 9.5 log CFU/filter. Strains were chosen based on their association with foodborne recalls and/or outbreaks. The inoculated membranes were dried for 24 h and then subjected to hot-air roasting at 129°C (265°F) for 0, 15, 30, or 45 min. Each study was conducted in triplicate. The filter membranes were vortexed in buffered peptone water to recover *L. monocytogenes* from the membrane surface. Viable organisms were enumerated on tryptic soy agar with 0.6% yeast extract and incubated at 37°C for 48 h. Colonies were confirmed as *Listeria* via streaking on Modified Oxford Medium.

Results: By pairwise comparison, the thermal inactivation curve for LM-004 was statistically different from curves of all other strains (P<0.05). With 15-min roasting, LM-004 reached the limit of detection (2 log CFU/mL) with a >6.86-log CFU/mL reduction, whereas an average 4.68±1.45-log reduction was achieved for the other six strains. There were no significant differences in inactivation rates among the other strains based on the same pairwise comparison. However, the effect of strain was detected with analysis of covariance.

Significance: These results indicate that thermal resistance of *Listeria* is strain-dependent. Screening *L. monocytogenes* strains for thermal resistance should be completed prior to conducting a challenge study.

P1-26 Water Activity Limits High-pressure Processing Efficacy to Control Fungi in Apple Juice Concentrate

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❖ ***Developing Scientist Competitor***

Introduction: High Pressure Processing (HPP) is a method to extend shelf life of foods by subjecting products in their final packaging to extreme pressure. This leads to protein degradation in microbial cells, membrane degradation, and eventual cell death. Survival of spoilage organisms, especially spore-formers, has not been closely considered in regards to the effect of water activity (a_w).

Purpose: Determine the effect of a_w on HPP apple juice to prevent fungal spoilage.

Methods: Apple juice concentrate was adjusted to a_w 0.94 (41.0°Brix), 0.96 (32.0°Brix), 0.98 (19.8°Brix), or 1.00 (6.5°Brix) and pH 4.6. Polyethylene terephthalate bottles were filled with concentrate and inoculated with *Penicillium, Aspergillus niger, Byssochlamys spectabilis, Byssochlamys nivea, Aspergillus pseudoglaucus,* or *Aspergillus fischeri*. Samples were HPP treated at 450 MPa for 1.5 min and for pressure-resistant species, at 600 MPa for 15 min to resemble industrial applications.

Results: Fungi were more resistant to HPP at lower water activities. Known heat resistant species *A. pseudoglaucus, B. nivea,* and *A. fischeri* were also more resistant to HPP than the other organisms. *A. pseudoglaucus* log reductions after 15 min at 600 MPa were 1.11±0.19, 1.11±0.32, 1.56±0.20 and 3.96±0.47 at 0.94, 0.96, 0.98 and 1.00 a_w respectively. *B. nivea* populations increased by 0.87±0.51, 1.64±0.85, 1.69±0.66 and 1.72±0.77 log. *A. fischeri* also increased by 0.34±0.25, 0.21±0.38, 0.16±0.25, 0.06±0.11 respectively. Typical spoilage fungi, *B. spectabilis, A. niger* and *Penicillum* experienced a 4.8- or greater log reduction at a_w of 0.98 and above. Their reductions ranged from 2.5 to 4.9 log reduction at 0.94 a_w and 3.3 to 5.5 log reduction at 0.96 a_w.

Significance: To reduce risk of fungal spoilage, HPP products should be at or above 0.98 a_w. When spoilage due to heat resistant organisms is known to occur, HPP may need to be combined with other hurdle methods to provide better shelf-life extension.

P1-27 Effects of High-pressure Processing and Hot Water Pasteurization on Inactivation of *Listeria monocytogenes* in Cooked Sausages Stored at 4 and 10˚C

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Introduction: In-package pasteurization treatments are widely used to control foodborne pathogens in RTE foods. These treatments include thermal pasteurization, such as hot water (HW) or steam, and non-thermal treatments, such as high-pressure processing (HPP) or ultraviolet light (UV) radiation.

Purpose: Evaluate the combined effects of cooking followed by HPP and HW pasteurization on inactivation of *Listeria monocytogenes* in cooked sausages stored at 4 and 10°C.

Methods: Sausages containing a four-strain cocktail of *L. monocytogenes* at 10^8 CFU/g were manufactured and cooked to an internal temperature of 72°C, cooled, vacuum packaged, and processed as follows: control (unpasteurized), heat-treated (HW pasteurization, 75°C for 15 min), and pressurized (HPP, 600 MPa for 180 s) and then stored at 4 and 10°C for 35 days. Changes in inoculated *L. monocytogenes*, natural populations of lactic acid bacteria, *Pseudomonas* spp., and coliforms were tested.

Results: Cooking of sausages resulted in >6-log reduction in inoculated *L. monocytogenes* numbers, and storage at 4°C resulted in no significant difference in *L. monocytogenes* numbers in both HPP and HW-pasteurized sausages compared to unpasteurized control. However, at 10°C, *L. monocytogenes* numbers in unpasteurized control sausages increased to about 7 log CFU/g by day 35, while in HPP treatment, numbers remained below the detection limit for up to 21 days and then increased to 4.5 log CFU/g by day 35. HW pasteurization, on the other hand, resulted in inhibition of *L. monocytogenes* to below the detection limit throughout the 35-day storage at 10°C.

Significance: HPP and HW pasteurization following cooking could be used to successfully enhance the safety and shelf life of cooked sausages. HW pasteurization was more effective as an in-package pasteurization treatment in controlling *L. monocytogenes* than HPP, especially when sausages are exposed to abusive temperature conditions (10°C).

P1-28 Impact of UV-C Irradiation on the Safety and Cytotoxicity of Cranberry-flavored Water Using a Novel Continuous Flow UV System

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Introduction: Dedicated microbial inactivation steps are essential to reduce the risk of foodborne infections in liquid food products such as flavored water. Ultraviolet-C (UV-C) irradiation is an effective means of inactivating many pathogenic organisms. It acts directly on the nucleic acids of the target microbe and impedes its replication.

Purpose: To assess the ability of UV irradiation to inactivate microorganisms in cranberry-flavored water (CFW), a highly opaque liquid food. To ensure that UV irradiation does not produce toxic by-products in irradiated CFW, cell cytotoxicity was evaluated using normal colon (CCD-18Co), colon cancer (HCT-116), and healthy mice liver cells (AML-12).

Methods: CFW inoculated with two bacteria (*Escherichia coli* ATCC 25922 and *Salmonella* Typhimurium ATCC 13311) was treated with UV-C irradiation. Biodosimetry techniques were used to calculate the reduction equivalent fluence (REF). CFW was irradiated using a Dean-flow reactor with the fluid pumped around a central low-pressure mercury UV lamp emitting at 254 nm wave-length. A series of known UV fluences (0 to 21 mJ/cm^{-2}) were delivered to the samples. Cell culture studies were conducted to assess the cytotoxicity of irradiated CFW. A balanced design with three replicates for each treatment was exposed to selected UV treatments.

Results: Inactivation levels of all organisms were proportional to REF. At the highest REF of 21 mJ/cm^{-2}, two pathogenic organisms were inactivated by more than 5 log. None of the treatments caused increased inhibition with respect to the viability of the cells in comparison to that of untreated CFW. These results suggest that UV treatments did not lead to the production of cytotoxic compounds.

Significance: This research study suggested that UV-C irradiation was successfully applied to inactivate microbial populations in formulated CFW without generating cytotoxic effects. These results suggest that this non-thermal technique can be an alternative to thermal pasteurization in producing high-quality beverages.

P1-29 Effect of Continuous Intense Pulsed Light on *Cronobacter sakazakii* Inoculated in Different Powder Samples

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Introduction: Intense pulsed light (IPL) represents an excellent alternative to conventional thermal or chemical disinfection of bacteria, thus ensuring safe foods with satisfactory nutritional and organoleptic qualities.

Purpose: Cronobacter sakazakii was recently associated with life-threatening infections in neonates. Contaminated powder samples such as milk powder, flour powder, and egg white powder were epidemiologically linked with these infections. Therefore, the present study aimed to improve the inactivation effect of *C. sakazakii* on these powder samples using IPL and by controlling environmental parameters.

Methods: These three powder samples were inoculated with *C. sakazakii* and treated by IPL for 18 to 28 s, with water activity levels from 0.20 to 0.35, initial temperatures from 25 to 35°C, and final temperatures controlled at ~57°C. Based on the optimal process, the inactivation effects of 1 to 4 passes of IPL were investigated and compared.

Results: The study showed the optimal IPL process conditions could be achieved at a final temperature of ~57°C, initial water activity of 0.25, and initial temperature at 30°C. Maximum inactivation occurred without significant change in the physical properties of powder particles (P< 0.05) at each pass. Adjustment of the water activity and temperature of the milk powder to maintain appropriate levels of water activity (~0.25 aw) and the initial temperature (~30°C) before each pass resulted in 5.27-log reduction for nonfat milk powder samples after 4 IPL passes; 3.97-log reduction for flour samples after 4 IPL passes; and 5.38-log reduction for egg white powder after 3 IPL passes.

Significance: The project takes a systematic approach to evaluate not only the engineering and microbiological aspects of the technology, but also the impacts of the process on the physical properties of the products being treated. IPL has a potential for providing an enhanced degree of microbiological safety for powdered foods.

P1-30 Reduction of Molds in Multi-grain Bread by Targeted Directional Microwave Technology

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Introduction: According to the United Nations' Food and Agriculture Organization, about one-third of the food produced for human consumption is wasted. Spoilage by molds, a large and diverse group of fungal species, is one of the various reasons for food loss, which can occur at any point in the supply chain.

Purpose: To assess the effects of targeted directional microwave (TDM) in mold reduction, quality, and shelf life of packaged bread loaves without added preservatives.

Methods: Preservative-free, multi-grain bread loaves were inoculated with 10^8 CFU/ml mold of *Penicillium* species. Two sets of inoculated loaves, 20 each, were individually subjected to two TDM treatments with varying amounts of microwave energy (T1:284 kJ and T2:244 kJ) and exposure time. Mold spores were enumerated by pour plate method using potato dextrose agar after incubation at 25˚C for 3 days. Non-inoculated loaves were also treated

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to measure moisture, analyze texture, and determine the shelf life, wherein loaves at room temperature were observed for signs of mold growth. Data were statistically analyzed by means of two-way analysis of variance.

Results: A significant reduction (P<0.05) of molds was observed on bread loaves treated with TDM. Treatment 1 with higher energy had only 2.14-log reduction compared to treatment 2 with 4.55-log CFU/50g mold reduction. There was significant difference (P<0.05) in crust moisture between the control and treatment 1 bread on all time points (days 1, 4, and 7). No significant difference (P>0.05) was noted in crumb moisture and texture (hardness and resilience) between the control and treated bread loaves. One of the loaves from treatment 2 appeared to have mold growth 23 days after production. There was absence of visible molds on the bread from treatment 1, even beyond 60 days post-production.

Significance: Results illustrate an effective intervention in TDM to control molds on bread, potentially improving bread shelf life and reducing waste.

P1-31 Effect of Processing Temperature on Pathogen Inactivation in Juice Using High-pressure Processing

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❖ ***Developing Scientist Competitor***

Introduction: High-pressure processing (HPP) is a non-thermal processing method used to inactivate pathogens and preserve the fresh qualities in certain food products. The juice industry has seen a shift towards functional and nutritional juices, which has led to an exploration of alternative non-thermal processes that ensure desirable qualities, as well as food safety. HPP is an important method in this trend, where the main factors that can be controlled, including time, pressure, and temperature, have yet to be systematically characterized.

Purpose: This study was carried out to investigate the effect of processing temperature on pathogen inactivation using HPP. Apple juice was chosen for the study and processed under two temperatures to determine the microbial reduction on key pathogens.

Methods: Five strains of each pathogen (*Escherichia coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes*) were grown and separately inoculated into apple juice (pH 4.5) at 5 and 20°C. Samples were processed by HPP using water at the holding temperature (5 or 20°C) and immediately plated on tryptic soy agar. Microbial counts were enumerated and log reductions were calculated and compared under the two conditions. Experimental trials were performed in triplicate.

Results: The results show a significant effect of temperature (P<0.05) on pathogen inactivation. At 5°C, the apple juice inoculated with *E. coli*, *Salmonella*, and *Listeria* showed a 1.58-, 4.02-, and 6.38-log reduction, respectively, whereas at 20°C, improved values of 3.48, 7.61, and 6.65 were respectively obtained.

Significance: The data has indicated that the processing temperature plays a critical role in pathogen inactivation by HPP. The outcome of the study may potentially impact how juice companies ensure food safety during processing operations.

P1-32 Withdrawn

P1-33 Fate and Decontamination of O157 and Non-O157 Serogroups of Shiga Toxin-producing *Escherichia coli,* including ATCC 43895, as Affected by Elevated Hydrostatic Pressure

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Introduction: Various serogroups of Shiga toxin-producing *Escherichia coli* (STEC), including O157, O26, O45, O103, O111, O121, and O145, have been involved in an array of recalls associated with meat products leading to 423 outbreaks from 1998 to 2016.

Purpose: This study investigated fate and effect of elevated hydrostatic pressure on reduction of wild-type phenotypes of various serogroups of STEC

Methods: A six-strain mixture of *E. coli* O157:H7, non-O157 *E. coli* (Centers for Disease Control and Prevention's "big six" serogroups), as well as ATCC 43895 (1992 to 1993 outbreak strain) were inoculated in 10% meat homogenate. In addition to a two-week aerobic storage trial, hydrostatic pressure at 380 MPa (55,000 PSI) was applied at various time intervals (0 to 10 min) for decontamination of the inoculated serogroups. Analysis of variance followed by LSD-based mean separations were conducted at type I error level of 5% using OpenEpi software. Experiments were conducted in two biologically independent repetitions, each as a blocking factor of a randomized complete block design.

Results: During the aerobic storage at 10°C, O157 *E. coli* strains were increased (P>0.05) from 5.55±0.1 to 7.67±0.2 log CFU/mL of inoculated meat homogenate on days 0 and 14, respectively. Similarly, non-O157 *E. coli* strains were increased (P>0.05) from 5.81±0.2 to 7.79±0.2 log CFU/mL on days 0 and 14, respectively. A treatment at 380 MPa for 10 minutes reduced (P<0.05) the pathogen counts by 1.74, 1.91, and 2.08 log CFU/ml for O157, non-O157, and ATCC 43895 samples, respectively. Treatments below three minutes showed low efficacy (P≥0.05) in decontamination of the pathogen.

Significance: In the vast majority of tested time and pressure combinations, O157 and non-O157 *E. coli* showed similar (P≥0.05) sensitivity and reduction patterns. The 1992 to 1993 *E. coli* O157:H7 outbreak strain also showed comparable sensitivity to high-pressure pasteurization.

P1-34 Assessing the Efficacy of Chemical Treatments to Control *Salmonella* Typhimurium in Rendered Chicken Fat Applied in Pet Foods

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Introduction: Rendered chicken fat is commonly used as a coating on dry pet foods to increase energy density and enhance palatability. It occurs after the established kill step. Residual water in bulk fat might be a source of *Salmonella* contamination in pet food.

Purpose: To evaluate the effect of sodium bisulfate (SBS), lactic acid (LA), and phosphoric acid (PA) on *Salmonella*-inoculated rendered chicken fat.

Methods: MICs of the three chemicals were determined using the broth microdilution method in tryptic soy broth. Efficacies of the chemicals at three different concentrations (0.5% weight/volume, 0.5% volume/volume, and 0.05% volume/volume, respectively) against *S.* Typhimurium (~10^6 log) at 45°C were tested in chicken fat for up to 24h. *Salmonella* counts in both fat and water phases were enumerated at different intervals (0, 2, 6, 12, and 24h) by plating on tryptone soya agar and xylose lysine agar and incubating at 37°C for 24h, all in triplicates.

Results: The MICs of SBS, LA, and PA were found to be 0.5, 0.5, and 0.25%, respectively. In the water phase, with 2.73-log reduction, 0.5% SBS was more (P < 0.05) effective than LA and PA at 0h. SBS and LA, respectively, were found to be more (P < 0.05) effective than PA with >4-log reductions at 2h and complete kill at 6h. After 24h, each of the chemicals were effective in complete elimination of *Salmonella*. However, in the fat phase, none of the

tested chemicals were effective against *Salmonella*. Unlike the initial >6 log in water phase, the fat phase achieved only ~4 log of inoculum, regardless of treatment. By 12h, *Salmonella* were not detected in any treatments in the fat phase.

Significance: This study suggests that inclusion of SBS or LA could potentially reduce *Salmonella* in rendered chicken fat contaminated by residual water encountered during storage and transport.

P1-35 Cold Plasma Treatment of Valencia Oranges Reduces Persistence of *Salmonella*

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Introduction: Fresh fruits such as oranges are an increasingly popular food, consumed directly or as ingredients in "raw" juice blends prepared at home or commercially at the point of consumption. However, because of past outbreaks associated with orange juice and the risk of cross-contamination during peeling and processing, there is a need for antimicrobial interventions which can effectively eliminate pathogens from fruit surfaces.

Purpose: To evaluate atmospheric pressure cold plasma as a means to inactivate *Salmonella* on peel-on oranges.

Methods: Valencia oranges (n=9) were lab-inoculated with *Salmonella* Anatum on the peel, in the stem scar, or in the blossom end, and allowed to air dry for 2 hours to promote adherence. The oranges were treated with air-based, atmospheric pressure cold plasma, created with high-voltage electrical discharge. During treatment, the site of inoculation on the oranges was passed in and out of the plasma plume to stimulate "tumbling" of oranges on a conveyor belt that are being exposed to cold plasma from above. The site of inoculation on the oranges (stem scar, blossom scar, and peel) were treated with 4 ft^3/min atmospheric cold plasma for 0 (control), 1, 3, or 5 minutes, at distances of 0 or 7.5 cm from the cold plasma emitter head.

Results: All treatments significantly (P<0.001) reduced *Salmonella* on oranges, on all surfaces tested. The 0-cm treatments yielded log reductions ranging from 0.94 to 2.09 (stem scar), 1.57 to 3.56 (blossom end), and 2.4 to 4.09 (peel), with longer treatment times yielding greater reductions. The 0-cm treatments were uniformly more effective than the 7.5-cm treatments, which yielded log reductions ranging from 0.15 to 1.57 (stem scar), 1.01 to 1.80 (blossom end), and 0.37 to 1.22 (peel). Temperature measurements confirm plasma treatment as a nonthermal process.

Significance: These results suggest cold plasma could be a waterless, chemical-free sanitation step for peel-on fruits such as Valencia oranges.

P1-36 Use of *Listeria innocua* and *Clostridium sporogenes* as Surrogate Organisms for In-plant Validation of a Sous Vide Process for Chicken Breasts Using Celery Nitrite

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❖ ***Developing Scientist Competitor***

Introduction: The availability of sous vide products are more prevalent than ever and have made inroads into commercially available products. Sous vide processing renders products more tender and palatable; however, products that are cooked ahead of time and stored may have issues with spore-forming pathogens because of heating and vacuum packaging.

Purpose: Our purpose was to validate a sous vide process for chicken breasts and compare the application of celery nitrite versus chemical nitrite in the prevention of *Clostridium* spore germination on RTE meat products.

Methods: Three strains of *Listeria innocua* (ATCC 33090-2, ATCC 33091-2, and ATCC 51742-2) were used along with a three-strain spore crop from *Clostridium sporogenes* (ATCC 3584, ATCC 19404, and ATCC BAA-2695). The sous vide process for chicken breasts consisted of a marinade step, a short-flame grilling process, vacuum packaging, and final processing in hot water. *L. innocua* were introduced by packet inoculation into the interior of the breast (entire process) or as a dip inoculation (flame grill only); *Clostridium* spores were added during marination and then subjected to flame grilling and processing in hot water.

Results: The flame grill process resulted in a 2-log reduction of *L. innocua*, whereas no effect was observed internally on the inoculum packets. The hot water process resulted in a more than 8-log reduction of *L. innocua* in the interior inoculum packets. However, we only observed a 0.5-log reduction when using celery nitrite. All trials were performed in quintuple replication and one-way analysis of variance was carried out to see significant differences (P<0.05) in the response to different treatments.

Significance: The thermal process in this study was more than adequate to inactivate 8 log of *Listeria* in the center of the breast, enclosed in envelope packets. Additional testing will examine improvement of inhibition of *Clostridium* spore germination.

P1-37 Applied Pre-Inoculation and Resistance Development of Lactic Acid Bacteria for Competitive Exclusion of Environmental Pathogens in a RTE Frozen Food Processing Environment

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Introduction: Competitive exclusion of environmental pathogens by the application of lactic acid bacteria (LAB) has been applied in a number of food processing industries. Ready-to-eat individually quick frozen fruit is a primary component of "smoothies" in today's fast-paced food consumption. Processing RTE foods requires an extensive sanitation and environmental monitoring program (EMP) to prevent the formation of resident pathogen biofilms of *Listeria Monocytogenes* and *Salmonella* spp., which are primary pathogens of concern.

Purpose: Our goal is to competitively exclude environmental pathogens *L. monocytogenes* and *Salmonella* in order to prevent contamination of fresh fruit processed in season by introducing probiotics to the environment of frozen fruit processing facilities.

Methods: A six-month evaluation, commencing with pre-season inoculation of the processing environment to known historical pathogen niches with a of strains of a pre-biotic formulation of LAB. The pre-biotic LAB cocktail received incremental sanitation chemical application to develop resistance anticipated for seasonal processing. Seasonal processing was a +90-day duration of LAB application and monitoring. To validate the presence and learn the influence of sanitation chemicals on LAB population, before- and after-sanitation samples were collected for LAB, environmental, and general indicator microbes. The viability of microbes was tested using the spread-plating method, where the viable plate count was calculated in CFU/ml. Standard sanitation operating procedures were maintained, supported by the EMP.

Results: Results confirm LAB resistance development, where after-sanitation samples showed >330 CFU/ml of LAB and aerobic plate count (APC); hence, results proportionally showed <10 CFU/ml of coliforms, yeast, and mold. All environmental samples showed negative for *L. monocytogenes* and *Salmonella* spp. LAB survival increases the APC, decreases the counts of coliforms by consumption, and finally decreases counts of yeast and mold.

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Significance: Implemented a potentially new food plant sanitation discipline, other than standard chemistry, to mitigate the risk of environmental pathogens by inoculating and developing a resistant LAB population, occupying the microbial niches favored by pathogens. Goal is to create a microbiome with beneficial resident microbial community, outcompeting the introduced transient pathogens, preventing resident biofilm formation.

P1-38 Changes in Concentrations of AMP, ADP, and ATP over Time in Bovine and Porcine Muscle Tissue

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Introduction: Adenosine triphosphate (ATP) hygiene monitoring tests are rapid and convenient methods to detect the presence of surface contamination by meat and microbial sources. Novel hygiene tests based on responses to the cumulative concentrations of ATP, adenosine diphosphate (ADP), and adenosine monophosphate (AMP) have been developed on the premise that ATP concentrations will be substantially depleted due to dephosphorylase activities as a function of time and physiological state. However, there is little information in the applied literature describing the concentrations of these adenylate isomers for meat tissue contaminants.

Purpose: Determine concentrations of ATP, ADP, and AMP at slaughter to the time of fabrication in bovine and porcine tissues.

Methods: Concentrations of adenylate isomers were calculated by the measurement of ATP, ATP+AMP, and ATP+ADP+AMP based on luciferin-luciferase using a luminometer. Concentrations were calculated against standard curves of authentic adenylate species. Muscle tissue samples from bovine and porcine carcasses (minimally five time periods, each done in duplicate) were measured for adenylate concentrations from the time of slaughter to fabrication.

Results: ATP had the highest relative concentration of adenylate compounds at slaughter but displayed an approximately 2-log reduction in concentration over 48 h. Over the time course studied, ADP became the dominate adenylate compound with relatively minor concentrations of AMP. Total adenylate concentrations in bovine samples decreased from an average of 3.0×10^{-7} mol/g at slaughter to 3.8×10^{-8} mol/g over 48 h. Adenylate concentrations in porcine tissues displayed similar trends.

Significance: ATP levels dropped significantly at slaughter to time of fabrication in bovine and porcine tissues. Hygiene tests that measure the cumulative concentrations of ATP, ADP, and AMP may provide improved sensitivity and accuracy for the assessment of surface contamination in meat processing facilities.

P1-39 Cleaning Tools and Utensils – Everything You Need to Know about GFSI Audit Scheme Compliance Requirements

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Introduction: Food industry cleaning tools and utensils have long been identified as a major source and vector of cross-contamination. In 1990 a study[1], funded by the UK government to establish food industry guidance on microbiological sampling, showed that 47% of cleaning tools tested positive for *Listeria monocytogenes*. In 2017[2], Schäfer determined that 67% of equipment and utensils used in a poultry processing plant were contaminated with *L. mono,* even after cleaning. Nevertheless, cleaning tools and utensils are rarely considered in relation to food safety. Fortunately, GFSI approved food safety schemes, including those operated by the British Retail Consortium[3]; the International Standard Organisation[4] and Safe Quality Food[5], now drawn attention to them specifically.

Purpose: To review GFSI food safety schemes with regard to the selection and maintenance of cleaning tools and utensils, to summarise this information, and to share it with those involved with food safety.

Methods: GFSI scheme standards were reviewed and key points relating to cleaning tool and utensil selection and maintenance were summarised. Using the information obtained various articles, and training and information presentations have been produced in order to share this valuable food safety knowledge.

Results: Articles on the hygienic design and food contact compliance of cleaning tools and utensils have been published[6,7,8]. Two training and information presentations[9,10] have been developed, and a White Paper[11] and an information booklet[12] have been produced. The presentations, White Paper and booklet have been made available at various food industry exhibitions and food safety conferences. The White Paper is also available via the Vikan website, has been published on-line[13] and will be printed in the June edition of New Foods Hygiene supplement, distributed via their email database throughout US and Europe, and handed out in their hygiene supplement at IAFP Utah.

Significance: The use of cleaning tools and utensils is ubiquitous in the food industry. Given their proven role as a major source and vector of contamination, sharing knowledge of ways in which they can be controlled is essential in order to promote food safety.

P1-40 The Comparison of Detection Sensitivities for Allergens in Foods between the ATP+ADP+AMP (A3) Test and the Protein Swab Test

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Introduction: ATP rapid hygiene monitoring tests are useful for the implementation of HACCP and HARPC programs, including the prevention of allergen contamination. Recently, the ATP+ADP+AMP (A3) test was shown to be a powerful tool to reveal improper cleaning and the presence of contamination that conventional ATP tests miss due to the degradation of ATP to ADP and AMP. Hence, the A3 test may be valuable to screen for food residues that may contain residual allergens.

Purpose: The detection limits of the A3 test for foods that are known as allergens were compared to those of the protein swab test.

Methods: Various foods that are regulated as allergens in United States (milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat, and soybean), Canada (e.g., sesame, mustard), and Japan (27 items, e.g., shrimp, crab, wheat, buckwheat, egg, milk, and peanuts) were homogenized and serially diluted with water. The samples (100 ml) were applied to the A3 test (LuciPac A3 Surface/Lumitester PD-30, Kikkoman Biochemifa) and the commercially available protein swab test, and the detection limits were evaluated (n=3). The measurement output of the A3 test was relative light units (RLU). As references, protein concentrations were also determined using Bradford assay.

Results: The detection sensitivities of the A3 test were shown to be superior to those of the protein swab test for all foods except gelatin. Protein determinations suggested that the A3 test can detect many food residues, such as seafood, meat, grains, nuts, fruits, and vegetables at equivalent to 10 ppm protein levels if 100 RLU is judged as positive.

Significance: The A3 tests appeared to be useful for screening the presence of contamination after the use of allergenic foods. Since immunological detection tests for some specific allergens are not available, the A3 tests may be a candidate for a complementary approach.

P1-41 Development of a Laboratory Method Using Stainless Steel Coupons to Determine the Efficacy of Surface Sampling Devices

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Introduction: Environmental monitoring is a foundational element of a program to manage pathogen and spoilage organisms in food production facilities. Unfortunately, little information is available to guide those responsible for food safety in selecting an environmental sampling device when residues of sanitizing agents are anticipated, larger surface areas need to be sampled, and when it is necessary to hold the collected sample for several days until it can be processed in the laboratory.

Purpose: Development of a laboratory method to create a robust and reproducible challenge environment in which differences in performance between sampling devices can be discerned.

Methods: In this method, 25 µl of a diluted *Listeria innocua* culture was inoculated onto an isolated portion of the surface of a stainless steel coupon and dried under ambient conditions for up to 72 h. Sanitizers were also dried on the coupon around the inoculation spot of some samples to simulate a production environment, in which injured harborage organisms are present in a concentrated area and sanitizer residues are on the surface. Samples were collected with polyurethane foam sponges hydrated with HiCap Neutralizing Broth and tested in accordance with procedures from the U.S. Food and Drug Administration's Bacteriological Analytical Manual.

Results: Greater cell die-off was noted in spots dried for 48 and 72 h when compared to spots dried for 1 h. About 100 times more cells were needed in the 48- and 72-h spots than in the 1-h spots in order to produce culture positive results. Extensive cell injury was seen in the 72-h spots, with some culture positives obtained only after 48-h broth enrichment. HiCap was able to effectively neutralize quaternary ammonium sanitizer that was applied on the coupons at use concentrations.

Significance: The developed protocol more closely represents real-world conditions in the food processing environment and allows for better determination of the true performance of a sampling device.

P1-42 Strain-specific Differences in Response of Human Noroviruses to pH Challenge

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Introduction: Disinfection is an important tool for controlling norovirus outbreaks. Marketed disinfectants against human norovirus are formulated across a wide pH range, from highly acidic to highly alkaline. Because most studies on the impact of disinfectants against human norovirus use cultivable surrogates and some surrogates are more sensitive to extremes in pH, a comprehensive analysis of the effect of pH on common human norovirus strains can better inform surface disinfectant formulations for outbreak control.

Purpose: To determine the pH values of solutions most effective at facilitating reduction of representative human noroviruses GII.4 Sydney and summer seasonal human norovirus GI.6.

Methods: Clarified 20% suspensions of human feces confirmed positive for GII.4 Sydney or GI.6 were used either directly or after chloroform extraction for additional clarification. Aliquots were exposed to buffered solutions (ranging from pH 3 to 13) for contact times of 30 min or 2 h. Following neutralization, the suspensions were subjected to RNA extraction (with or without RNAse pre-treatment) and subsequent RT-qPCR. Log reduction was calculated based on genomic copies, and all experiments were repeated in triplicate.

Results: Neither GII.4 Sydney nor GI.6 demonstrated significant genome copy number log reduction at acidic or neutral pH at an extended 2-h contact time in suspension. Solutions with alkaline pH values had variable efficacy; GI.6 showed between 1.5- and 3.5-log reduction for solutions at pH 10 and 12 to 13 at a 30-min contact time, and GII.4 Sydney showed lesser degrees of reduction (0.4 to 1.5 log) at pH 12 to 13 and none at pH 10.

Significance: Disinfectant formulations made at pH 12 or above may provide synergistic anti-noroviral activity from an added pH effect, which could reduce necessary disinfectant concentrations or contact times. Tailoring pH values to specific virus strains may enhance this effect.

P1-43 Ease of Biofilm Accumulation and Efficacy of Sanitizing Treatments in Removing the Biofilms Formed on Selected Abiotic Surfaces

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❖ ***Developing Scientist Competitor***

Introduction: Biofilms formed by bacteria on food contact surfaces can increase the risks of product contamination.

Purpose: This study assessed 1) the formation of biofilms by fecal coliforms, isolated from different blueberry packing lines on selected abiotic surfaces, and 2) the efficacy of different sanitizing treatments in removing the biofilms.

Methods: Biofilm-forming ability of six bi-strain mixtures of fecal coliforms was assessed at 10°C for seven days on coupons made of materials commonly found on blueberry packing lines, including high-density polyethylene, stainless steel, rubber, polyvinyl chloride, polypropylene, polyurethane, and recycled polyethylene. Surface coupons with developed biofilms were treated for 1 min with sanitizers used by blueberry packers, including 5 ppm active chlorine dioxide, 3 ppm ozonated water, 200 ppm quaternary ammonium, or 200 ppm sodium hypochlorite. Residual biofilms on treated coupons were quantified using the crystal violet binding assay.

Results: The amount of biofilms accumulated on polypropylene were significantly higher (P<0.05) than on polyurethane coupons. Biofilms formed on polyvinyl chloride and rubber coupons were statisically similar, but were significantly lower than those on polypropylene and polyurethane coupons and significantly higher than those on high-density polyethylene coupons. Recycled polyethylene and stainless steel coupons had similar amounts of biofilm mass, but were significantly lower than those on other coupons. Ozonated water had significantly higher efficiency in biofilm removal than quaternary ammonium compound, which had significantly higher efficiency than chlorine dioxide, followed by sodium hypochlorite. Significantly more residual biofilm mass was found on rubber coupons. Residual biofilm mass on polypropylene, polyvinyl chloride, and high-density polyethylene coupons was statistically similar, but they were significantly higher than those on polyurethane and stainless steel coupons.

Significance: The evaluated sanitizers had different efficacies in biofilm control. The type of coupons and fecal coliform isolates involved in the study played a significant role in biofilm accumulation and removal from the selected abiotic surfaces.

Poster

P1-44 Evaluation of Surface Cleanliness in Seafood Production Lines by ATP Bioluminescence Application

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Introduction: Microbiological hygiene in seafood production is crucial for the safety and quality of food products. Effectiveness of sanitation procedures on production surfaces has traditionally been evaluated using methods like contact plates and swabs. The food industry has adopted rapid methods, including adenosine triphosphate (ATP) bioluminescence, to assess surface cleanliness in real time.

Purpose: This study aimed to evaluate surface cleanliness of two ready-to-eat seafood production lines for sushi squid (SQ) and boiled shrimp (BSh) using traditional microbiological methods and rapid-ATP bioluminescence.

Methods: Swab samples were collected from 92 surfaces of SQ and 63 surfaces of BSh production lines during two visits. Each swab sample was collected from a 100 cm^2 stainless steel (SS), polyvinyl chloride (PVC), or nylon surface after pre-operational cleaning, and followed by traditional microbiological analysis using 3M Petrifilm Aerobic Count Plate. The same surfaces were swabbed for testing with 3M Clean-Trace for ATP measurement.

Results: Overall, 85.7 and 88.4% of the samples from SQ production line and 84.6 and 40.5% of the samples from BSh production line from visit 1 and 2, respectively, showed aerobic plate counts acceptable for food contact surfaces. ATP bioluminescence showed that 71.4 and 100% of samples from SQ production line and 73.1 and 51.4% of the samples from BSh production line from visit 1 and 2, respectively, had acceptable results. Considering all surface types, concordance between results from the two methods was 84.8% for SQ and 79.4% for BSh production lines. Most differences might be due to high levels of ATP detected from organic debris left on certain surfaces like PVC of baskets and SS of working tables.

Significance: Verification of food contact surface cleanliness is important. ATP bioluminescence yields real-time results for regular and frequent monitoring of complex production lines. Data obtained from ATP bioluminescence provides information on the amount of organic and microbial contamination on production surfaces.

P1-45 Thermal and Chemical Inactivation of Human Norovirus: Impacts on Viral Capsid Integrity

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Introduction: Human norovirus is the leading cause of acute viral gastroenteritis and foodborne illness. Due to lack of a readily available cell culture system for human norovirus, virucidal efficacy of intervention methods has usually been determined either directly by evaluating viral genome and capsid integrity or indirectly by using cultivable surrogates.

Purpose: We investigated the direct impact of chemical and physical intervention techniques on human norovirus capsid integrity, i.e., receptor-binding and capsid structure.

Methods: Virus-like particles (VLPs) of human norovirus GII.4 Sydney were tested in suspension according to ASTM protocol E1052-11 in order to evaluate the impact of the inactivation treatments on viral capsid. VLPs were exposed for 2 min to either sodium hypochlorite (household bleach) at 0 to 1,000 ppm free available chlorine, or to heat at 21 to 95°C. Following treatments, capsid integrity was evaluated using an H Type 2 HBGA-receptor binding assay and Western blot analysis.

Results: For heat, human norovirus capsid lost receptor-binding capability at ≥72.5°C (P<0.05). No noticeable degradation of VLP polypeptide was observed at temperatures as high as 95°C; however, large polypeptides (>120 kDa) appeared at ≥45°C, indicating a cumulative aggregation of virus particles over increased temperature. Chlorine treatment resulted in loss of receptor-binding at concentrations as low as 1 ppm, as evidenced by 2.2 reduction in normalized absorbance at 450 nm (P<0.05). On the other hand, ≥50 ppm chlorine was required to observe significant degradation in norovirus capsid protein by Western blot.

Significance: The GII.4 Sydney major structural protein displayed resistance to thermal degradation but was susceptible to the oxidizing nature of sodium hypochlorite. Based on the method used in this study, loss in receptor-binding was observed before capsid protein degradation. The impact of these treatments on infectivity remains to be determined. This study provides an improved understanding of changes in norovirus structure and functionality when formulating inactivation strategies.

P1-46 Effect of Drying Conditions and Microbial Species on Biofilm Formation and Resulting Probability of Detection by Various Swab Types

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Introduction: The ability of bacteria to attach and form biofilms on contact surfaces is a well-known factor leading to persistent contamination of food. A biofilm is defined as a microbial structural community consisting of a singular or multiple species and contained within an extracellular polymeric substance (EPS) which results in altered gene expression, specialization of cells, and increased resistance to detachment, antimicrobials, and stress conditions. The ability of bacteria to colonize a surface is known to be dependent on factors such as temperature, hydrophobicity, and species present.

Purpose: Evaluate the probability of detection of microorganisms from stainless steel using various swab types (MSX foam, MS Rayon, and large sponge) as a function of drying method and species present.

Methods: Drying conditions were 4, 25 and 37°C for 1 h to simulate a quick spill, 8 h to simulate end-of-day sanitation, and 24 h for prolonged residue. Microbial species included: i) gram positive - *Listeria monocytogenes* and *Staphylococcus aureus*; ii) gram negative - *Escherichia coli* and *Salmonella enterica*; and mixed species - *L. monocytogenes* + *Enterococcus faecalis* and mixed coliforms. Prepare and overnight culture, and serially dilute to extinction in a low-protein diluent. Inoculate surface with series and allow to dry under specified conditions. Swabs were taken and perturbed into a liquid diluent for counting using tryptic soy agar, confirmations were run using BAX PCR and chromogenic agars for coliforms.

Results: The probability of detection decreases as the temperature of drying increases. Both *L. monocytogenes* and Enterococci were detected at 37°C when dried for 1 h at higher dilution series only. The 37°C did not recover the Enterobacteriaceae or the coliforms when dried at 37°C for any time. At 25°C, the survival of all bacteria was measured at various probabilities of detection.

Significance: Allow food manufacturers to make informed choices regarding sampling techniques appropriate for their specific needs.

Poster

P1-47 Hydrogen Peroxide and Hypochlorite Disinfectants are More Effective against *Pseudomonas aeruginosa* Biofilms Than Quaternary Ammonium Compounds

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✦ ***Undergraduate Student Award Competitor***

Introduction: Foodborne and opportunistic pathogen (e.g., *Pseudomonas aeruginosa*) biofilms are major challenges to the food and healthcare industries. Bacterial biofilms are more resistant to cleaning and disinfecting applications than planktonic cells. Limited data is available on antimicrobial efficacy against biofilms using new Environmental Protection Agency (EPA) methodology.

Purpose: The purpose of this study was to investigate the efficacy of seven disinfectants representing three chemistries against *P. aeruginosa* strain ATCC #15447 biofilms using EPA standard operating procedures (SOPs) MB-19 and MB-20.

Methods: We used EPA SOP MB-19 to stimulate the growth of *P. aeruginosa* strain ATCC #15447 biofilms on a borosilicate glass coupon using a CDC Biofilm Reactor to test the efficacy of eight disinfectants (EPA SOP MB-20; two quaternary ammonium compounds, one hyochlorite, and four hydrogen peroxide disinfectants). Efficacy was measured as log reduction of bacterial cells after exposure to disinfectant at label use time and concentration. The overall efficacy of each individual disinfectant, as well as differences among disinfectants, was determined by one-way analysis of variance; least squares means were calculated due to unbalanced experimental design (SAS software, v 9.4).

Results: Hydrogen peroxide and sodium hypochlorite disinfectants were significantly more effective against *P. aeruginosa* strain ATCC #15447 biofilms than quaternary ammonium compounds under label use conditions (P<0.0001). Hydrogen peroxide products and hypochlorite resulted in an average 8.6±0.5-log and 8.7±0.2-log reduction in *P. aeruginosa* strain ATCC #15447, respectively. Quaternary ammonium compounds reduced *P. aeruginosa* strain ATCC #15447 an average 0.7±0.3 log

Significance: These results indicate that disinfectants vary in their efficacy against *P. aeruginosa* strain ATCC #15447 biofilms, but hydrogen peroxide and sodium hypochlorite more effectively disrupt biofilms than quaternary ammonium-based disinfectants under these test conditions.

P1-48 Changes of Lethal Activities of Gaseous Chlorine Dioxide as Affected by Relative Humidity against *Escherichia coli* O157:H7 on Stainless Steel

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❖ ***Developing Scientist Competitor***

Introduction: Chlorine dioxide (ClO_2), available in aqueous and gaseous forms, has been widely used to inactivate foodborne pathogens present in food and food contact surfaces. Compared to aqueous ClO_2, gaseous ClO_2 has been known to leave less residue and to be more effective in inactivating foodborne pathogens in biofilms due to its superior penetration ability. However, environmental factors such as relative humidities (RHs) affecting the lethality of gaseous ClO_2 have not been intensively investigated.

Purpose: We investigated the influences of RHs on the lethality of gaseous ClO_2 against *Escherichia coli* O157:H7 on stainless steel.

Methods: E. coli O157:H7 was spot-inoculated on stainless steel coupons (SSCs; 5 by 2 cm) by 6 log CFU/coupon. The inoculated SSCs were placed in airtight containers (1.8 L) adjusted to 43, 85, or 100% RH and incubated with or without gaseous ClO_2 (peak concentration, ~1,000 ppm) at 25°C for 30 min. After incubation, the survived cells of *E. coli* O157:H7 on SSCs were determined.

Results: The populations of *E. coli* O157:H7 inoculated on SSCs were ~5.2 log CFU/coupon after drying for 1 h. Without gaseous ClO_2, the populations of *E. coli* O157:H7 reduced to 5.0, 3.8, and 3.9 log CFU/coupon during incubation at 43, 85, and 100% RH, respectively, for 30 min. Whereas, when treated with gaseous ClO_2, the populations of *E. coli* O157:H7 decreased to 3.9, <1.5, and <1.5 log CFU/coupon at 43, 85, and 100% RH, respectively, for 30 min (detection limit: 1.5 log CFU/coupon).

Significance: It was confirmed that the lethality of gaseous ClO_2 against *E. coli* O157:H7 on SSC was significantly (P≤0.05) enhanced as RHs were increased. These results would provide useful information to help develop strategies to inhibit foodborne pathogens on abiotic surfaces using gaseous ClO_2 in food production and processing environments.

P1-49 Optimization of the CDC Biofilm Reactor for Generation of *Listeria monocytogenes* Biofilms and Impact of Biofilm Age on the Efficacy of Chemical Sanitizers

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Introduction: Biofilms facilitate the persistence of microorganisms in the environment and on food contact surfaces. There is concern that over time, pathogens entrapped in biofilms may become resistant to disinfection. This issue is not well-studied, particularly in systems relevant to what occurs in the real world.

Purpose: Adapt the CDC Biofilm Reactor (CBR) to generate *Listeria monocytogenes* biofilms having cell density and stability appropriate for challenge testing purposes (7 log CFU/coupon) related to pathogen susceptibility to chemical disinfection.

Methods: L. monocytogenes biofilms were generated on 304 stainless steel coupons using a CBR in accordance with the ASTM E2871-13 standard, with minor adaptations aimed at increasing biofilm density. Once optimized, 1- and 4-day-old biofilms were exposed to household bleach and a commercial quaternary ammonium compound (QAC) at concentrations of 200 ppm with a 1-min contact time. Surviving *L. monocytogenes* populations were plated on solidified tryptic soy broth (TSB).

Results: Increasing the TSB concentration from 0.1 to 3 g/L during the CBR continuous phase increased *L. monocyogenes* cell counts from 5.4±0.1 to 7.0±0.2 log CFU/coupon, respectively (P<0.05). Cell counts were further increased to 7.6±0.4 after extending the CBR continuous phase to 3 days. Sodium hypochlorite (200 ppm, 1 min) produced a 4.4±0.3 and 4.5±1.3 log CFU/coupon reduction on biofilms aged 1 and 4 days, respectively (P>0.05). The QAC (200 ppm, 1 min) produced a 3.4±2.0 and 3.4±0.5 log CFU/coupon reduction of biofilms aged 1 and 4 days, respectively (P>0.05).

Significance: The CDC Biofilm Reactor can be adapted to generate high density *L. monocytogenes* monoculture biofilms. Results demonstrate sodium hypochlorite and a common QAC retained the same degree of efficacy to inactivate *L. monocytogenes* in biofilms up to four days old.

Poster

P1-50 Treat Water Like Glass – Sanitation's War on Water to Reduce Pathogen Risk

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Introduction: Uncontrolled water is potentially a significant contributor to the growth and persistence of pathogens in food processing environments. Just as glass and brittle plastic are physical hazards that require control programs, we are proposing that uncontrolled water should also require a specific control program.

Purpose: Creating a culture where water is infrequently used for cleaning will provide several advantages. One advantage will be a reduction in water available for microbial growth, especially in low water activity products produced in systems and in facilities with less-than-ideal sanitary design. Microbial niches in equipment and infrastructure caused by water and chemicals will also be reduced, as will risk to human safety related to the reduction of exposure to high temperatures and chemicals. Minimizing water usage will also potentially reduce the time and frequency of cleaning and improve equipment reliability. Original equipment manufacturers, contractors, and consumer packaged goods companies need to partner in the effort to revise standard cleaning practices.

Methods: We are proposing a sanitation model that prioritizes design coupled with cleaning methods. Preferred cleaning methods would rank in the following order: 1) dedication or redundancy of equipment to avoid and/or minimize cleaning; 2) pushing to remove soil adequately; 3) dry cleaning with methods such as scraping, brushing, or vacuuming; 4) dry cleaning with chemicals; 5) clean in place (CIP); 6) wet cleaning out of place; 7) manual CIP; and 8) flood cleaning.

Results: This design should not only reduce the food safety risk (both pathogen and allergen), but also decrease the total life cycle costs in managing the affected assets. Inherent to this initiative will be challenging and adjusting the success criteria of cleaning. Success criteria may include qualitative and quantitative measures. These changes will help prevent the use of water to get surfaces cleaned to an unnecessarily high standard.

Significance: Conversion to water-controlled methods has the potential to improve microbiological results during validation and verification of all sanitation tasks.

P1-51 Effects of Slightly Acidic Electrolyzed Water Treatment on Microbial Reduction in Salted Young Radish

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Introduction: After the foodborne outbreak related to insufficiently fermented kimchi, public health concern about microbiological safety has been increasing. For kimchi made through fermentation, it is necessary to develop a sanitizing means to eliminate potential microbial hazards during preparation steps.

Purpose: This study was to evaluate the effect of slightly acidic electrolyzed water (SlAEW) treatment on microbial reduction during washing and to design an efficient washing process for salted young radish.

Methods: Sodium hypochlorite (NaOCl) of 100 and 200 ppm, SlAEW of 30 and 80 ppm, and tap water were used to wash the radish with the mass ratio of solution to radish and treatment time while the combinations of pre- and post-rinsing were introduced to the washing process. The number of total aerobic mesophilic bacteria and coliforms in the radish were analyzed and the survival number of *Escherichia coli* O157:H7 was estimated.

Results: The reduction level of total aerobic bacteria was 0.37, 1.23, 1.26, 1.04, and 0.93 log CFU/g treated with tap water, 100 and 200 ppm of NaOCl, and 30 and 80 SlAEW for 5 min, respectively, showing no significant differences between NaOCl and SlAEW as well as between SlAEW concentrations and treatment time (5 and 10 min). The combined washing steps with pre- and post-rinsing resulted in more reduction (1.65 log CFU/g) than washing only with sanitizing solution. The reduction of *E. coli* O157:H7 was higher (1.15 log CFU/g) in ratio of 25:1 than 10:1 (0.81 log CFU/g).

Significance: These results suggest that the 30 ppm SlAEW solution could be used to reduce the microbial load of salted young radish. The combination of pre-rinsing for 1 min, SlAEW washing with ratio of 25:1 for 5 min, and post-rinsing for 1 min is proposed as an effective washing process.

P1-52 Comparison of Dual Enzyme Treatment with Alkaline Treatment for Removal and Sanitation of *Listeria innocua* Biofilm Components Attached to Stainless Steel Surfaces

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Introduction: Biofilms of *Listeria* species create food contamination risk due to their persistence in processing environments. Effective antimicrobial treatments do not ensure removal of the inactive biofilm, which may serve as a platform for new biofilm attachment and growth. It is desirable not only to sanitize the food contact surface, but also to eradicate the biofilm components.

Purpose: The objective of this study was to evaluate the effectiveness of an enzyme mix in removing *Listeria innocua* biofilms from stainless steel (SS) surfaces and compare it to alkaline treatment commonly used for cleaning in place of SS surfaces.

Methods: Eight SS coupons were immersed in 9×10^8 CFU/ml *Listeria innocua* in phosphate buffered saline at 25°C for 2 h, transferred to a 1/10 tryptic soy broth medium at 25°C for 24 h, then subjected to one of four treatment solutions for 15 min: 1) deionized water, 2) a mix of amylase and alcalase at 0.1% concentration, 3) a pH 13 NaOH solution, or 4) enzyme treatment followed by alkaline treatment. Duplicate coupons were either swabbed or imaged by fluorescent microscopy after staining. Solutions were neutralized and cultured. The experiment was replicated in triplicate on separate days using new inocula and treatment solutions.

Results: Compared to control, enzyme treatment removed >99.99% of viable cells from the coupon, though with low lethality. Alkaline treatment resulted in non-detectable levels of viable cells on the coupon, but with minimal removal of biofilm components. Enzyme treatment followed by alkaline treatment resulted in both removal of biofilm components and >99.99% lethality.

Significance: This study indicates that a dual-stage process of enzyme treatment followed by sanitation greatly improves the ability to both remove and disinfect biofilms from SS surfaces.

Poster

P1-53 Survival and Inactivation of Human Norovirus Gii. 4 Sydney on Airplane Plastic Tray Table Surfaces

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❖ ***Developing Scientist Competitor***

Introduction: Air travel outbreaks associated with norovirus, a leading cause of foodborne illness, have recently emerged. Contaminated surfaces play a key role in norovirus transmission, and adequate sanitation is needed to control transmission risk.

Purpose: To investigate the survival of human norovirus GII.4 Sydney on airplane plastic tray tables and the disinfection efficacy of selected Environmental Protection Agency (EPA) registered disinfectants against norovirus with and without additional organic load.

Methods: Plastic tray table coupons were inoculated with norovirus with and without additional organic load (simulated gastric fluid [pH 2.5] and phosphate buffered saline [pH 7.4], respectively). Coupons were then incubated in a controlled environmental chamber (21.8°C, relative humidity=26 %) and sampled for up to 30 days. Virucidal efficacy of Clorox HW (0.65% sodium hypochlorite), Clorox QS (1.4% hydrogen peroxide), and Clorox HS (quaternary ammonium formula) against norovirus were assessed in suspension and on the surface according to ASTM E1052-11 and E1053-11 methods, respectively. RT-qPCR with prior RNase treatment was performed, and log genomic copies per sample were calculated.

Results: Norovirus persisted for 30 days at concentrations of 5.29±0.08 and 5.17±0.21 log genomic copies per sample with and without additional organic load, respectively, with a significant effect from organic load ($P<0.05$). In suspension assay and under both tested conditions, sodium hypochlorite and quaternary ammonium fully inactivated norovirus (5.70±0.11- and 5.66±0.12-log reduction, respectively). In surface assay and with additional organic load, all three disinfectants failed to completely inactivate norovirus, with hydrogen peroxide-based disinfectant being the least efficient (0.80±0.17-log reduction).

Significance: Although tested products are EPA-registered against norovirus, they failed under soiling conditions to achieve the EPA standard of ≥4-log reduction for a product claiming virucidal activity. A prior cleaning step along with increasing disinfectant contact time may help attain maximum efficacy.

P1-54 Evaluating Environmental Monitoring Protocols for *Listeria* spp. and *Listeria monocytogenes* in Frozen Food Manufacturing Environments

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Introduction: Food processors face serious challenges due to *Listeria monocytogenes*. Environmental monitoring programs are appropriate for facilities that produce RTE products regulated by the U.S. Food and Drug Administration and the United States Department of Agriculture. It is important to develop awareness and practices related to environmental monitoring within the industry. While frozen food does not support the growth of *Listeria monocytogenes*, it has been subjected to recent recalls.

Purpose: The purpose of the study is to determine the current level of awareness and practices used in the frozen food industry related to environmental monitoring for *Listeria* spp. and *Listeria monocytogenes.*

Methods: A survey tool was created focusing on current environmental monitoring practices within the frozen food industry. Topics included cleaning and sanitizing applications and frequency, microbiological testing, and environmental areas of concern. The survey was examined by academic and industry experts with knowledge of microbiology and frozen food processing and was field tested by industry personnel with extensive knowledge of environmental monitoring. The survey was distributed and analyzed electronically via Qualtrics to 150 frozen food contacts. Data was gathered so respondents were unknown to the researchers.

Results: The response rate for participants was 31% (n=46). Overall, facilities are more likely to test for *Listeria* spp. in environmental monitoring zones 2 through 4 on a weekly basis. Few facilities test for *Listeria* in raw materials or finished products, with more testing focused on preventing contamination in the processing environment. The areas of concern in facilities for finding *Listeria* positive results are floors and drains. There is a greater focus in the industry to improve environmental monitoring as a component of a comprehensive food safety plan.

Significance: This data suggests there is an industry focus on current environmental monitoring programs to improve and develop extensive practices to reduce prevalence of *Listeria monocytogenes* in food processing environments. Additional guidance for smaller processors would be useful.

P1-55 Determination of an Effective Cleaning Regime for *Listeria* spp. for Squeegees Used in Condensation Mitigation Strategies

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Introduction: Food manufacturers often use squeegees to remove condensation from overhead pipes in processing environments. Common squeegee materials include rubber and double foam. These squeegees should be cleaned using standard procedures for equipment (e.g., detergent, scrubbing, rinse and sanitizer). However, this procedure is not always followed by food processing employees, which may lead to cross-contamination in processing facilities.

Purpose: The objectives of this experiment were (i) to assess the effectiveness of common sanitizers (peroxyacetic acid, chlorine, and quaternary ammonia) to remove bacterial contamination from different squeegee materials, and (ii) to understand the effectiveness of different cleaning regimes when these procedures were not performed as recommended.

Methods: To perform these experiments, both materials were inoculated with *Listeria innocua* ATCC 33090 (>6 log CFU/in^2). After inoculation and drying, materials were cleaned and sanitized using four cleaning regimes: (i) water rinse (1 min); (ii) exposure to sanitizers (1 min); (iii) detergent (10 min)+rinse+sanitizer (1 min); and (iv) foam detergent+scrubbing+rinse+sanitizer. For each treatment, three replicates with three sub-samples were analyzed for the survival of *Listeria* spp. using environmental *Listeria* petrifilm. Statistical analysis (analysis of variance) was performed to determine significant differences between cleaning regimes and squeegee materials.

Results: Results showed that sanitizers alone were able to remove 1 to 2 log CFU/in^2, depending on the material type. Extensive cleaning regimens recommended for the equipment were able to remove 3 to 4 log CFU/in^2. Overall, slightly higher reductions were observed on rubber than foam material, which suggests that rubber squeegees are more sanitary than foam for these types of applications.

Significance: This experiment highlights the importance of performing and completing the recommended cleaning regimes for these tools to avoid the likelihood of cross-contamination in the food processing environment.

Poster

P1-56 Survival of *Listeria* spp. on 3M Condensation Management Tape and Its Potential Application in the Food Industry

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Introduction: Condensation on overhead utilities represents a potential risk for pathogen contamination in food processing facilities when droplets detach over food contact surfaces. These facilities face a condensation issue every day, when different temperatures and high relative humidity are encountered. 3M has designed a potential solution consisting of a plastic-polymer tape that contains channels which allow water to spread and evaporate.

Purpose: The objectives of this research were (i) to evaluate the effectiveness of 3M Condensation Management Tape (3M-CMT) to evaporate condensed water against a control tape (CT), and (ii) to determine if this potential solution does not increase the risk for harborage of environmental pathogens.

Methods: Galvanized pipes with applied CMT and CT tapes were placed inside of a cooler (5.5°C) in which indirect steam was applied until saturation was achieved. Observations were made every 30 min for 24 h to evaluate the effectiveness of each tape on moisture evaporation. To determine if CMT tape could be a reservoir for *Listeria*, both tapes were inoculated (6 log CFU/in^2, *L. innocua* ATCC 33090) before the steaming protocol and enumerations were performed during five consecutive days on tryptic soy agar and Oxford agar, for three replications.

Results: 3M-CMT was able to evaporate the condensed water in about 3 to 4 hours, contrary to the control tape, which took up to 18 h. This increase in evaporation on CMT decreases the risk that condensed water will fall, thereby reducing potential contamination. Microbial analyses showed that survival of *Listeria* was the same on both tapes (6-log reduction over 48 h); therefore, this intervention does not provide a mobile reservoir for environmental pathogens.

Significance: To our knowledge, this study is the first to investigate this condensation management solution. This information is key in order for food processors to make informed decisions and to reduce the risk of potential contamination from condensation.

P1-57 Transfer of *Listeria* spp. to Water Droplets and Surfaces When Using a Squeegee as a Condensation Mitigation Strategy

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Introduction: Condensation on overhead utilities represents a potential risk for pathogen contamination in food processing facilities when drops de-attach over food contact surfaces. Food manufacturers often use squeegees to remove condensation. Therefore, it is unknown if this practice could lead to more contamination.

Purpose: The objectives of this experiment were to determine: (i) the concentration of *Listeria* sp. in the falling drops during its removal, (ii) its transfer rate across the surface of pipes, and (iii) the *Listeria* concentration present on the squeegee after removal of condensate.

Methods: Galvanized pipes were covered with aluminum tape to improve recovery of bacterial cells. Aluminum tape (1 in^2) was inoculated with approximately 6 log CFU/in of *Listeria innocua* (ATCC 33090). After inoculation and drying, pipes were placed inside a cooler (5.5°C) in which indirect steam was applied until saturation was achieved. By using a rubber squeegee, hanging drops were collected in sterile conical tubes at 4, 8, 12, 16, 24, and 36 inches away from the inoculated portion. For the second and third objectives, pieces of the aluminum tape (same length intervals) and the squeegee were collected and analyzed using EL petrifilm. Three replicates with three sub-samples per replicate were performed.

Results: The concentration of *Listeria* sp. in collected drops decreased from 5.1 to 1.8 log CFU/mL within the first 16 inches. Similarly, the concentration decreased on the surface of the aluminum tape, from 5.3 to <1 log CFU/in^2, within the same length interval. This suggests that contamination can spread across the first foot. After removal of condensed water, the squeegee had a *Listeria* concentration of 3.5 log CFU per squeegee, which suggests a potential for later transfer.

Significance: This research demonstrates the potential for cross-contamination due to the use of squeegees as a mitigation strategy and provides quantitative data to improve sanitation practices.

P1-58 Comparison of Swabbing Efficiency of Hygiena 1" Foam Swabs with Large Foam Swabs

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Introduction: It is often assumed that the size of the swab means less efficient pick-up from a surface. The aim of these experiments is to demonstrate swabbing efficiency, the ability of the device to remove bacteria from surfaces through the action of swabbing.

Purpose: Using two different swab sizes to remove dried bacteria from a surface using an agar overlay method to enumerate at low levels post-swabbing.

Methods: *Listeria monocytogenes* ATCC 19115 was grown overnight at 37°C in tryptic soy broth (TSB). This was serially diluted 1:10 using 10% TSB. A total of 90 µL were taken from dilutions -3 through -6 and laid on 150-mm petri dishes in a 3 by 3 by 3 matrix of even volume. A total of 100-µL spread plates were taken from dilutions -6 and -7. Five replicates were performed per dilution per collection device for each surface. Surfaces were then allowed to dry with plate covers on for 24 h at 20ºC. Plates were swabbed with 1-inch foam devices and sponges. Agar was then laid over swabbed surfaces and petri dishes were incubated at 37ºC. Colony counts were taken at 24 h and finalized after 48 h. Colonies remaining on the surface were then enumerated.

Results: The survival rate post-drying was 0.01% of the inoculated CFU. Colonies on the surface after swabbing with sponges or foam swabs were for 1-inch foam swab 57±53 CFU (12 to 148); for the large sponge, mean colonies post-swabbing was 40±37 CFU (13 to 104). The analysis of variance demonstrated no significant difference (P=0.57). This demonstrates that a similar CFU level dried under the same conditions can be successfully swabbed to a similar removal efficiency as a large sponge.

Significance: The work begins a debate that the swabber is more significant than the swab size and that inefficiencies will exist no matter the size of the swab.

Poster

P1-59 Evaluating the Hygiene Conditions and the Food Safety Level in Fresh Produce Wholesale Markets in Doha, Qatar

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Introduction: Preventing food contamination from harmful causes such as bacteria is difficult in many developing countries like Qatar, where the majority of food comes from countries with poor hygiene practices. Fresh produce is considered a healthy food choice and Qataris have started consuming such foods in their diet. The contamination of fresh produce can occur at any stage in the farm-to-table process, including when produce is handled by workers who may be infected with pathogens. In Qatar, most of the food workers come from Far East. The workers' hygiene in the wholesale market in Doha, Qatar is considered an important factor that affects the transfer of microorganisms to produce.

Purpose: This study was carried out to evaluate the hygiene conditions at the wholesale market in Doha, especially the hygiene of workers, who are in direct contact with produce.

Methods: A total of 120 workers were surveyed in the study. During the survey, hand-swab samples were collected and several produce samples were analyzed to determine their microbial quality.

Results: All respondents indicated that they did not receive any official training on food safety. The primary age interval of the workers was 31 to 40 years old (36.7%), and only 37.5% of the workers had a high school degree. More than 67% had been working in the area for more than five years. Pathogens identified as microbial hazards isolated from swabs of produce handlers' hands and produce included *Klebsiella* and *Enterococcus faecium.*

Significance: These results demonstrate inadequate hygiene practices of produce handlers, highlighting the need to train them on food safety and hygiene practices, and to adopt better control measures at the produce market to improve sanitary conditions.

P1-60 Evaluation of Disinfectants and Wiping Substrate Combinations to Inactivate *Staphylococcus aureus* on a Hard, Non-porous Surface

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❖ ***Developing Scientist Competitor***

Introduction: Improper cleaning and sanitizing of surfaces is a continual problem within the food service industry and in individual's homes. Proper cleaning and sanitizing helps to ensure risk to potential pathogens is minimized. Environmental Protection Agency (EPA) approved disinfectants are currently used United States, but there is limited information on the effectiveness of these when used with different wiping substrates (cloths).

Purpose: This study evaluates the effect of 4 disinfectants (quaternary ammonium, chlorine, hydrogen peroxide, and non-disinfectant) applied using three types of wiping substrates (microfiber, non-woven disposable cleaning wipes, and cotton) against *Staphylococcus aureus* on formica coupons.

Methods: Formica coupons (1×1 inches) were inoculated with *S. aureus*. Cloths were dipped into disinfectant and wiped across the square. Following the disinfectant contact time, cloths and squares were separately submerged in neutralizing buffer (NB). NB was serially diluted and plated on Tryptic Soy Agar and incubated for 48 hours at 36°C. Counts of *S. aureus* remaining on squares and cloth were separately compared using a one-way ANOVA

Results: Generally, hydrogen peroxide and chlorine were more effective than quaternary ammonium and non-disinfectant control at reducing populations of *S. aureus* on both cloth and square. It was clear wiping substrate impacted the disinfectant efficacy. Application of hydrogen peroxide disinfectant with microfiber cloths resulted in no detectable surviving *S. aureus* on the formica. Application of chlorine with cotton cloth also resulted in no detectable surviving *S. aureus* on the formica or cloth. Quaternary ammonium was still an effective disinfectant, and was significantly more effective when applied with microfiber cloth ($P < 0.05$) compared to other cloth types. Mechanical removal of bacteria from the squares' surface was less effective with disposable wiping cloths compared to cotton and microfiber (P <0.05).

Significance: Cloth type used for application of a disinfectant can significantly influence ability of the disinfectant to remove of *S. aureus* from formica.

P1-61 Surface Charge Studies of Cetylpyridinium Chloride on Sanitation of *Salmonella* Typhimurium in Poultry Processing

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Introduction: *Salmonella* Typhimurium is a foodborne pathogen and a major safety concern in the poultry industry. Cetylpyridinium chloride (CPC), a quaternary ammonium compound, has been used in poultry processing to reduce *Salmonella* Typhimurium attached to chicken skin. CPC has a positive surface charge and binds to negatively charged bacterial cell membrane surfaces. The CPC penetrates cell membranes and kills those cells. In this study, *Salmonella* Typhimurium (8.995 ± 0.029 log CFU/ mL) was exposed to CPC to understand changes in bacterial surface potential.

Purpose: We aim to understand the interaction between surface potentials of *Salmonella* Typhimurium and CPC, as well as the effect of this sanitizer on structure and size of the bacteria.

Methods: *Salmonella* Typhimurium suspensions were mixed with different concentrations of CPC (up to 0.8%) to clearly understand the effect of the sanitizer. While changes in surface charges of the *Salmonella* were measured by zeta potential analyzer, size distributions of treated bacteria were measured by dynamic light scattering. The effect of sanitizer on the bacterial structure and geometry was visualized via scanning electron microscopy. CPC-treated bacteria were plated on 3M™ Petrifilm™ Aerobic Count Plate films to test bacterial inhibition.

Results: The surface charge of bare *Salmonella* was measured to be -12.73 ± 1.31 mV. Application of the CPC turned their surface charge immediately from negative to positive, depending on the CPC concentration up to +16.63 ± 1.38 mV due to the net-positive charge of CPC. Cell structures of the CPC-treated microorganisms were completely disrupted while bare microorganisms maintained their original cell structure. It is apparent that there is a significant correlation between bacterial surface charges and plating results: while negative surface charges showed bacterial growth, positive surface charges showed no such result.

Significance: Our results suggest that understanding the variations in the surface charge of the *Salmonella* Typhimurium can provide significant information about the effectivity and mechanisms of sanitizer treatments.

Poster

P1-62 Fluid Milk and Milk Processing Environment Surveillance Using Amplicon Metagenomics

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Introduction: Microbial communities in food processing plants can potentially serve as indicators of food quality and safety. Hence, it is vital to understand the microbial communities in the food processing environment and how they overlap and contaminate the final product.

Purpose: The purpose of this study was to determine if there are overlaps between the microbial communities of the processing environment and the microbial communities of the product at different stages of processing, starting from the raw materials to the finished product.

Methods: The study was conducted in a fluid milk processing plant in the Midwest region of the United States, which is capable of producing 6,000 gallons of fluid milk per day. Samples consisted of fluid milk during various stages of processing and from the plant environment. Genomic DNA was isolated from all the samples and subjected to 16S rRNA gene amplicon sequencing to study the bacterial population in the complex microbiome.

Results: The microbiota of the raw milk and the processing environment were complex in contrast to the pasteurized and the final packed milk. Genera such as *Paenibacillus*, *Viribacillus, Lysinibacillus*, and *Carnobacterium* that can cause product spoilage in cold-stored products were found at higher relative abundances in the production environment, product contact surfaces, and in the final packed product. The raw product carried *Listeria* and *Salmonella*, which were eliminated by pasteurization. The microbial communities in the product were distinctly different from those in the processing environment. The processing environment had indigenous and product-related microbial communities. Product contact surfaces and final product had overlapping microbial communities.

Significance: Facility microbiome surveillance activities provide a valuable approach to help monitor the food processing facility and product microbial communities. These measures can support improved strategies in zoning decisions.

P1-63 Microplate Lethality Assay to Determine the Efficacy of Commercial Sanitizers for Inactivation of *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* spp. in Extended Biofilms

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❖ ***Developing Scientist Competitor***

Introduction: Biofilms enable the persistence of pathogens in food processing environments. In order to inactivate these microorganisms, sanitizing agents are needed that are effective against pathogens entrapped in biofilms, which are more difficult to inactivate than those recently displaced and found on equipment surfaces.

Purpose: Our objective was to use the most adherent strains identified from among *Listeria monocytogenes*, Shiga toxin-producing *Escherichia coli*, and *Salmonella* strains in our collection to form biofilms and examine the effectiveness of various commercial sanitizers after different time points on these enhanced biofilms.

Methods: Biofilm-forming strains of *L. monocytogenes*, *E.coli* O157:H7, and *Salmonella* serovars were detected using a microplate fluorescence assay (5,6-CFDA). Adherence levels were determined by differences in relative fluorescence units and confirmed by stripping bacteria from biofilms with enzyme treatment, followed by microbial plating. In sanitizer assays, microplate biofilms were washed and provided fresh sterile growth medium daily for seven days before use. We examined five different sanitizers (three of which were used at two different concentrations) for efficacy against the three pathogenic biofilms.

Results: Quat and chlorine-based sanitizers were the least effective, showing partial inhibition of the various biofilms within 2 h (1 to 2-log reduction). The best performing sanitizer across all three pathogens was a combination of modified quaternary ammonium chloride, hydrogen peroxide, and diacetin, which resulted in ~6 to 7-log reduction (below LOD) within 1 to 2.5 min. All treatments were performed in triplicate and repeated measures one-way analysis of variance was carried out to see significant differences (P<0.05) in response to sanitizer treatment over time.

Significance: Sanitizer effectiveness is best tested on extended growth biofilms that mimic worst case scenarios rather than 30-min bacterial attachments. Moreover, the application of enzymatic treatments to recover cells looks to be a promising treatment for the removal of both live and dead residual biofilm that could otherwise initiate new biofilm formation.

P1-64 Evaluating Food Safety Risk of *Toxoplasma gondii* in Naturally Infected Meat Animals in the United States

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Introduction: Toxoplasma gondii is a protozoan parasite that infects virtually all warm-blooded animals including humans, livestock, and marine mammals. Various surveys have found that 10 to 50% of the adult population has been exposed to this parasite. The main modes of *T. gondii* transmission are ingestion of food, soil, or water contaminated with oocysts, or eating raw or under-cooked meat containing tissue cysts. A substantial portion of human *T. gondii* infections are acquired through consumption of infected meats.

Purpose: Limited information is available on the presence of viable *T. gondii* in muscle tissues of meat animals. The goal of this study was to quantify viable *T. gondii* concentration in muscle tissues of naturally infected lambs and goats.

Methods: The muscle tissues of eight lambs and 20 goats, along with their hearts, were serologically tested for *T. gondii* antibodies through modified agglutination testing and then bio-assayed in mice in different amounts (5, 10, and 50 g) to observe its infection rate. Samples were also bio-assayed in cats to collect *T. gondii* oocysts. DNA was isolated from positive tissue samples and further genotyped with *T. gondii*-specific markers.

Results: The prevalence of viable *T. gondii* was calculated to be 30% in 5 g, 34% in 10 g, and 36% in 50 g of goat muscle samples based on mice-bioassay results. The relationship between muscle sample size and number of mice positive with *T. gondii* infection was significant at P<0.05. Detection of 529 base-pair bands in agarose gel confirmed *T. gondii* DNA in 1-g muscle samples and 1-ml pepsin digested samples.

Significance: This study indicated that the parasite can be present in naturally infected meat animals and could pose threat of foodborne illness to consumers.

Poster

P1-65 Rapid Bacterial Detection Using β-Cyclodextran and Surface Enhanced Raman Spectroscopy in Ground Beef

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Introduction: Fresh foods, including meats, produce, and cheeses, are the fastest growing market in the supermarket and the class of foods most likely to cause a bacterial foodborne illness. As the rate of consumption of perishable products increases, rapid detection of pathogens within the food supply becomes a critical issue. Current methods used for the detection of bacteria that cause foodborne illnesses are time-consuming, expensive, and often require selective enrichment.

Purpose: The development of a rapid methodology to detect bacteria within perishable foods is necessary to ensure the safety of the food supply.

Methods: In this study, we adapted a separation technique originally developed for PCR to extract bacteria from ground beef using β-cyclodextran (β-CD) and milk protein coated activated carbon as filtration agents. The extracted bacteria were then bound to a gold slide via a 3-mercaptophenylboronic acid (3-MPBA) sandwich assay and detected with surface enhanced Raman spectroscopy (SERS).

Results: The concentration of the β-CD used in the separation technique was optimized to 20 g/100 mL to yield a supernatant with the fewest SERS inhibitors. The 3-MPBA sandwich assay used in conjunction with the separation technique performed over 4 trials, with a new sample created for each trial was used to create 200 point replicate maps at varied bacterial concentrations. This allowed detection of Salmonella enterica Enteritidis (BAA-1045), separated from a ground beef matrix, as low as 1×10^2 CFU/g.

Significance: Detection at this level was accomplished in less than eight hours, significantly faster than plate count or enrichment methods that require multiple days. Previously, SERS has been used to detect bacteria within simple matrices; this is the first study to have utilized SERS bacterial detection in a complex matrix such as ground beef.

P1-66 Outbreak-associated *Salmonella* Heidelberg Isolates Have Higher Baseline Expression of Genes Encoding Heat Shock Proteins, Stress Tolerance Mechanisms, and Virulence Systems at 37°C

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Introduction: Salmonella Heidelberg caused a massive outbreak associated with chicken during 2013 to 2014. Two outbreak-associated food isolates (PUL R1-0006, R1-0007) had significantly greater survival at 56°C (hard scald) than the *Salmonella* Heidelberg reference strain (SL476). Examination of heat stress gene expression profiles of R1-0006, R1-0007, and SL476 did not correlate with enhanced heat tolerance.

Purpose: The purpose of this study was to determine if outbreak-associated isolates had higher baseline expression of genes associated with heat tolerance and virulence compared to SL476, thus potentially priming outbreak strains for survival and virulence.

Methods: RNA was extracted with Trizol (Ambion) from stationary-phase bacteria grown at 37°C from biological triplicates. RiboZero (Illumina) and TURBO DNAse (Thermo Fisher) were used to remove rRNA and DNA, respectively. cDNA libraries were constructed (Scriptseq v2 library kit; Illumina); these were sequenced on the Illumina HiSeq platform. Trimmed reads were aligned with the SL476 index (Tophat, v2.1.1), read counts were obtained (HTSeq, v.0.6.1), and differential expression between samples was determined (DESeq2, v.1.12.4). Differential expression was defined as 2 log fold change >1 and P_{adj}<0.05.

Results: Compared to SL476, heat shock proteins genes *hspQ*, *hslR*, *dnaJ*, *clpB*, and *clpX* had significantly higher expression in R1-0006. In R1-0007, *hslR* and *hslU* had significantly higher expression and two negative regulators of *sigE* had significantly lower expression than SL476. Compared to SL476, both isolates also showed higher expression of genes involved in osmotic stress, phage shock, and DNA repair. Multiple virulence genes, including genes from SPI-1, SPI-2, and SPI-5 were also expressed at significantly higher levels in R1-0006 and R1-0007.

Significance: Combined with phenotypic data, our results suggest that outbreak-associated isolates R1-0006 and R1-0007 may be uniquely primed to survive stress and cause illness in humans. This data will inform future risk assessments and studies of this particularly virulent serovar.

P1-67 Inactivation of *Escherichia coli* and *Enterococcus faecium* on Beef Surfaces Using Microwaves

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Introduction: Inactivation of pathogens on meat has been a significant problem for beef processors since the declaration of *Escherichia coli* O157 as an adulterant in the United States. No process has been able to reliably provide the necessary degree of pathogen inactivation and also maintain raw product that is acceptable for human consumption/

Purpose: Quantify the inactivation of pathogen surrogates (*E. coli* and *Enterococcus faecium*) on both lean and fat surfaces of beef by gyrotron-generated microwave treatment and determine whether the appearance was significantly changed.

Methods: The gyrotron-generated millimetric-length microwaves. Lean and fat surfaces of beef were inoculated with a culture of the surrogates to a level exceeding7 log per piece. High power, short time and low power, and long time (less than one second) microwaves were applied to heat the inoculated surfaces (five replicates of each combination). Meat pieces were rinsed in sterile Butterfield's buffer before plating onto MacConkey agar and KF Streptococcus agar and incubated at 35±2°C for 48 h. Some uninoculated pieces were wetted prior to treatment and appearance was assessed.

Results: The microwave treatment reduced *E. coli* on lean surfaces from an average 7.21 to 1.80 log for high-power treatment and 1.81 log for low-power treatment. *E. faecium* numbers were reduced from an average of 7.11 to 1.87 log for high-power treatment and an average of 1.92 log for low-power treatment. The results for fat surfaces were almost identical. No visual change to lean surfaces were noted.

Significance: Greater than 5-log reduction in pathogen surrogates on meat surfaces was achieved in less than one second through gyrotron-generated microwave treatment without significantly affecting the appearance of the meat surfaces.

P1-68 *Yersinia enterocolitica* in Tonsils and Heads of Swine Slaughtered in Minas Gerais, Brazil

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Introduction: The international trade of pork products requires adequate control of microbial contamination during different steps of production, from breeding to processing. *Yersinia enterocolitica* is a relevant foodborne pathogen associated with pork and demands a rigorous control during slaughtering.

Purpose: This study aimed to verify the presence of *Y. enterocolitica* in palatine tonsils and head cuts in swine slaughtered in Minas Gerais, Brazil.

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Methods: Samples from slaughtered animals (tonsils=100, head cut=17, palate=30) were obtained and subjected to *Y. enterocolitica* detection. The samples were enriched in peptone sorbitol bile broth treated with 0.5% potassium hydroxide (25°C for 72 h) streaked onto plates containing Yersinia Selective Agar (30°C for 48 h), and typical colonies were subjected to identification by PCR targeting *16S rRNA*, *inv*, and *ail*. Serotyping was conducted by PCR, targeting *per*, *wbbU*, *wbcA*, and *wzt*.

Results: Based on obtained results, 14 samples were positive for *Y. enterocolitica* (tonsils=10 of 100, head cuts=1 of 17, palate=3 of 30), confirming these as relevant points for monitoring this foodborne pathogen. A total of 28 isolates from positive samples were obtained and confirmed as *Y. enterocolitica* due to positive results of the target genes 16S rRNA, *inv*, and *ail*. *Y. enterocolitica*-confirmed isolates amplified PCR products for *wbbU*, related to O:3 serotype, which is often associated with yersiniosis cases.

Significance: The obtained results highlighted the relevance of swine as *Y. enterocolitica* reservoirs and demonstrated the tonsils, head cuts, and palates as relevant sources of contamination of this foodborne pathogen in the slaughtering and processing environment.

P1-69 A Statistical Overview of Hygiene Indicator Microorganisms on Slaughtered Cattle as a Function of Process Steps and Regions in Brazil

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Introduction: Cattle carcasses are subjected to different sources of contamination during slaughter, therefore demanding rigorous hygienic control in slaughterhouses. Hygiene indicators for microorganisms are relevant to monitoring, as they are used to assess the sanitary conditions of beef-processing facilities.

Purpose: To evaluate the sanitary conditions of cattle slaughter in Brazil and to establish a statistical model to compare the efficiency of process steps.

Methods: Eight slaughterhouses were selected for the study, located in three Brazilian states: Minas Gerais (MG, 3), Parana (PR, 3), and Rio Grande do Sul (RS, 2). Surface samples were obtained from 464 animals (MG=209, PR=105, RS=150) at different slaughtering steps (A: hide, before bleeding; B: after hide removal; C: after evisceration; D: after end washing) and subjected to enumeration of mesophilic aerobes (MA), *Enterobacteriacea* (EB), coliforms (CF) and *Escherichia coli* (EC) by using petrifilm. Counts were converted to log CFU/cm² and compared by significance analysis.

Results: Counts of MA ranged from 4.7 (step A, MG) to 2.0 (step C, PR), EB ranged from 3.6 (step A, RS) to 0.8 (step B, PR), CF ranged from 3.5 (step A, RS) to 0.9 (step D, PR), and EC ranged from 3.7 (step A, RS) to 0.5 (step D, PR) (results in log CFU/cm²). Microbial counts presented significant decreases over processing (A to D, $P<0.05$), considering all data and each state. Correlation indexes between steps A and B ranged from 0.16 (EC) to 0.43 (MA), between B and C ranged from 0.29 (EC) and 0.42 (EB), and between C and D ranged from 0.23 (EB) to 0.44 (MA), and all were significant ($P<0.05$). Similar trends were observed for each state, though with varying confidence measures.

Significance: The study demonstrated how the concentration of hygiene indicator microorganisms on carcasses change at different stages of cattle slaughter.

P1-70 Distribution and Virulence of *Listeria* spp. in a Pork Production Chain in Brazil

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Introduction: Due to their ubiquity, *Listeria* spp. can persist for long periods in pork-processing facilities. Thus, it is important to monitor them in order to control of *L. monocytogenes* contamination in end products.

Purpose: To assess the contamination by *Listeria* spp. in different steps of a pork production chain in Brazil.

Methods: Ten pig lots were sampled in different steps of pork production: finishing barns (20), slaughtering (pig carcasses before bleeding=100, after buckling=100, after evisceration=100, after final washing=100), processing (180), and end products (cuts=20, sausage=10). *Listeria* spp. were detected according ISO 11290 and obtained isolates were identified by biochemical tests. PCR was used to confirm identification and to identify serogroups and virulence genes. Isolates were subjected to *Apa*I and *Asc*I macrorestriction and PFGE.

Results: Only sausages presented *Listeria* spp. (n=4, 18 isolates). Isolates were identified as *L. monocytogenes* (10 isolates from two samples) and *L. innocua* (eight isolates from four samples). *L. monocytogenes* isolates were characterized as belonging to serogroups 1/2a or 3a (n=8) and 4b, 4d or 4e (n=2), and presented *hlyA*, *iap*, *plcA*, *actA*, *inlA*, *inlB*, *inlC* and *inlJ*. Isolates from different serogroups were obtained from a same sample, and isolates from a same serogroup presented PFGE profiles with high similarity (>90%). *L. innocua* isolates also presented the tested virulence genes, excepted for three isolates that were negative to *plcA, inlA*, *inlB*, *inlC* and *inlJ*. PFGE grouped all *L. innocua* isolates in a same cluster with high similarity (>90%).

Significance: Results demonstrated the low frequency of *Listeria* spp. contamination in the studied pork production chain, but the presence of virulent strains in an end product (sausage) poses a risk for consumers.

P1-71 Inhibition of *Listeria monocytogenes* by a Bacteriocinogenic Strain of *Lactobacillus curvatus* in a Fresh Sausage System

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Introduction: The control of *Listeria monocytogenes* contamination is a challenge in the food industry, especially in meat products. Alternative methodologies have been adopted in order to control the contamination and growth of *L. monocytogenes*, and bacteriocins produced by lactic acid bacteria (LAB) have been used for this purpose.

Purpose: To assess the interaction between a bacteriocinogenic *Lactobacillus curvatus* and *L. monocytogenes* in a fresh sausage system during storage.

Methods: Fresh sausage was prepared with pork, fat, and condiments. Cultures of *L. monocytogenes* (isolated from pork, BHI, 3 log CFU/mL), bacteriocinogenic *L. curvatus* (isolated from sausage, MRS, 6 log CFU/mL), and a non-bacteriocinogenic *Lactobacillus sakei* (ATCC 15521, MRS, 6 log CFU/mL) were inoculated in the sausage mix, resulting in six treatments: 1) control (no added cultures), 2) only *L. monocytogenes*, 3) only *L. curvatus*, 4) only *L. sakei*, 5) *L. curvatus* and *L. monocytogenes*, and 6) *L. sakei* and *L. monocytogenes*. Sausages were stored at 7°C up to 10 days, and LAB and *L. monocytogenes* populations were monitored in days 1, 4, 7, and 10. Sausages were produced in three independent repetitions, and counts were compared by analysis of variance ($P<0.05$).

Results: LAB populations increased in all treatments after seven days of storage ($P<0.05$), even in non-inoculated sausages (treatments 1 and 2). *L. monocytogenes* populations in treatments 2 and 6 ranged from 3.4 (day 1, both treatments) to 4.4 (day 10, treatment 2) and 4.6 log CFU/g (day 10, treatment 6), while treatment 5 ranged from 1.9 (day 1) to 3.2 log CFU/g (day 10). *L. monocytogenes* populations in treatment 5 were significantly lower when compared to treatments 2 and 6 ($P<0.05$).

Significance: The study demonstrated the potential of a bacteriocinogenic *L. curvatus* in controlling *L. monocytogenes* in a fresh sausage system.

P1-72 *Campylobacter* Multi-locus Sequence Typing Subtypes Detected on Chicken Livers Available at Retail

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Introduction: Foodborne campylobacteriosis has been traced to undercooked chicken liver. It is not known what prevalence of *Campylobacter* to expect on fresh chicken livers available at retail.

Purpose: The objectives of this study were to measure prevalence of *Campylobacter* associated with chicken livers at retail and determine which subtypes are detected on the surface and inner tissue of livers.

Methods: Fifteen packages of fresh chicken livers, each representing a unique combination of processing plant and sell by date, were collected at retail grocery stores. Three intact, undamaged livers per container (n=45) were selected and sampled, each by three methods: outside swab, inside swab accessed by pressing through a heat sterilized outer surface and whole liver by blending in enrichment broth. Each liver sample and one mL of exudate from each package was cultured for *Campylobacter* by plating on campy-cefex agar. The most prevalent *Campylobacter* colony type from each positive sample was subjected to whole genome sequencing and multi-locus sequence typing.

Results: Campylobacter was detected in at least one sample from every package. Surface swabs were positive for 29 of 45 livers; significantly fewer ($P<0.01$) swabs of internal tissue were positive at 14 of 45. *Campylobacter* was detected in 30 of 45 blended whole liver samples. A total of 15 MLST sequence types were detected representing 1 clonal complex of *C. coli* and 6 clonal complexes of *C. jejuni*. Multiple subtypes were detected on five livers. In two cases, a different subtype predominated on the surface than internally. On one liver, three different subtypes were detected.

Significance: A variety of subtypes of *Campylobacter* can be readily isolated from fresh retail chicken livers. Undercooked chicken livers pose a food safety risk.

P1-73 Relationship between Shopping Practices and Contamination by Meat Juice from Raw Poultry Packages

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Introduction: Raw poultry products are often contaminated with *Salmonella* and *Campylobacter*, which can be transmitted through meat juice on retail packages. There is little quantitative data of consumer exposure to the meat juice from poultry packages during shopping and subsequent transfer to other food items and kitchen surfaces.

Purpose: The objective of this study was to assess consumer shopping practices linked to meat-juice contamination from raw poultry packages through observational studies and meat-juice assay.

Methods: An observational study was conducted to assess consumer exposure to meat juice during shopping and to quantify the transmission of meat juice from poultry packages to hands and other surfaces. Ninety-six participants completed the shopping studies; 402 swabs were collected and analyzed for the presence of meat juice by an immunoassay.

Results: Overall, meat juices were detected on 61% of poultry package surfaces, 34% of shoppers' hands, 41% of grocery bags, 60% of kitchen surfaces, and 51% of food item surfaces. When the meat juice was detected on the purchased poultry packages, the chance of meat juice being on the shopper's hands, grocery bags, food items, and kitchen surfaces was significantly higher ($P<0.005$). Shoppers who had poultry wrapped separately during checkout had a significantly lower chance ($P<0.05$) of meat juice on the food items. However, using plastic bag and wrapping poultry separately did not significantly reduced the likelihood of meat juice on kitchen surface at home due to consumers' practices of repackaging before storage.

Significance: Results suggested that the transfer of meat juice through direct contact with the poultry packages is a major concern during shopping and should be prevented.

P1-74 Shiga Toxin-producing *Escherichia coli* O157:H7, Non-O157 STEC, and *Salmonella* spp. Occur in Raw Beef Product Samples Independently of Each Other

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Introduction: The Food Safety and Inspection Service (FSIS) tests raw beef samples for *E. coli* O157:H7 and *Salmonella* in raw ground beef, manufacturing trim, component and bench trim verification sampling projects (MT43, MT60, MT64 and MT65, respectively), and in four follow-up sampling projects. Beef manufacturing trim (MT60) samples and all follow-up samples are also tested separately for six non-O157 STEC (O26/O45/O103/O111/O121/O145).

Purpose: To determine the likelihood that a raw beef sample positive for a STEC adulterant will contain additional STEC strains or *Salmonella* in the same sample.

Methods: FSIS analyzed MTxx sampling data obtained between June 2012 - January 2017*.

Results: There were 10,233 MT60 samples tested for all seven STEC and *Salmonella*, with 255 positive for one or more pathogens (2.49%). However, only 11 samples were positive for both STEC and *Salmonella* (0.11%). One sample was positive for both *E. coli* O157:H7 and non-O157 STEC (0.01%). Of 2,399 follow-up samples tested and 93 (3.88%) positive, only five samples were positive for both STEC and *Salmonella* (0.21%). A total of 40,616 samples (40,212 verification, 404 follow-up) were tested for both *E. coli* O157:H7 and *Salmonella*, with 824 positive samples (2.02%). Only three of these samples (0.01%) were positive for both pathogens. Finally, of 5,381 MT60 samples plus 1,916 follow-up samples tested only for *E. coli* O157 and the six non-O157 STEC (prior to *Salmonella* testing, initiated in 2014), with 109 positive samples (1.49%), only two samples (0.03%) were positive for *E. coli* O157 plus a non-O157 STEC.

Significance: Most samples testing positive for an adulterant (STEC) or *Salmonella* were positive for only one specific pathogen. The data indicate that the presence of *E. coli* O157:H7 with other STEC or with *Salmonella* in raw beef samples is an uncommon occurrence.

*FSIS acknowledges the contributions of its field inspectors and lab personnel in sample collection and testing.

Poster

P1-75 Older Adults and Parents Have Different Handling Practices for Raw Poultry

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Introduction: Salmonella and *Campylobacter* cause an estimated 1.8 million foodborne infections each year in the United States. Most illnesses are associated with eating raw or undercooked poultry or cross-contamination. Young children and older adults are more likely to have severe infections.

Purpose: The purpose was to estimate adherence to recommended food safety practices among older adults and parents of young children when handling raw poultry and to compare the results of the two groups.

Methods: A Web-based survey of millennial parents (ages 21 to 40 years) in the United States with a child ≤5 years old living in household (*n*=1,957) and older adults (aged 65+, *n*=1,980) was conducted.

Results: The findings present adherence rates for 21 recommended food handling practices. Parents were significantly more likely to report following nine recommended food handling practices. Older adults were significantly more likely to report following seven recommended food handling practices. For the remaining five recommended food handling practices, no differences were found between the two groups. Parents were significantly more likely than older adults to report following recommended food handling practices related to cleaning and cooking. For example, parents (38.8%) were significantly more likely compared with older adults (30.8%) to report not rinsing or washing raw poultry (P<0.001). Older adults were significantly more likely than parents to report following food handling practices related to separating and chilling raw poulty. For example, older adults (86.6%) were significantly more likely compared with parents (68.5%) to report cooking, freezing, or discarding raw poultry within 1 to 2 days of purchase per United States Department of Agriculture cold storage recommendations (P<0.001).

Significance: To reduce foodborne illness caused by *Salmonella* and *Campylobacter*, education is needed to improve consumer handling of raw poultry. To motivate behavior change, food safety messages and materials must be targeted to specific at-risk populations, as their practices are different.

P1-76 Spoilage and Safety Impact Associated with Sodium Reduction in Cooked Ham

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Introduction: Nutritionally improved food products by means of sodium reduction minimise one of the primary antimicrobial hurdles, which may have an impact on the microbial quality and safety.

Purpose: The aim of the study was to assess the growth of spoilage lactic acid bacteria in comparison with *Listeria monocytogenes* in standard and sodium-reduced cooked ham from four different commercial brands.

Methods: The growth rate of *Lactobacillus sakei* CTC746 was determined at 7ºC through challenge tests in sliced, vacuum-packaged products. Additionally, hams were characterised for pH, aw, moisture, sodium, and organic acid concentrations, and the growth rate of psychrotolerant *Lactobacillus* spp. was predicted with the model available in the Food Spoilage and Safety Predictor (FSSP v4.0). Results were compared with those previously obtained with *L. monocytogenes*.

Results: The growth rate of *L. sakei* was from one and a half to five times higher in sodium-reduced cooked hams in comparison with the standard counterparts, with a consequent shortening of shelf life of the same magnitude. The FSSP predictions were close to those of the challenge test results in case of sodium-reduced cooked ham products (B_f=1.14), but overestimated the growth rate for standard products (B_f=0.59). The *L. sakei* growth rate was faster but more sensitive to salt concentration than that observed by *L. monocytogenes*. However, in general, the time taken by the lactic acid bacteria to achieve spoilage level (7 log CFU/g) was longer than the time taken by *L. monocytogenes* to achieve the critical level of 2 log CFU/g permitted by the European Union regulation. The difference was lower in the case of sodium-reduced cooked ham.

Significance: Consumer health and compliance with the current food safety microbiological criteria for *L. monocytogenes* in cooked ham before the spoilage occurs is at risk, though it is lower in sodium-reduced products.

P1-77 *Enterobacteriaceae* Levels and Pathogen Prevalence in Commercial Poultry Processing Facilities in Colombia

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❖ ***Developing Scientist Competitor***

Introduction: *Salmonella* and *Campylobacter* are well-known pathogens associated with poultry production. Despite isolated efforts to assess microbial levels during processing in Colombian poultry processing facilities, there is not an official microbial baseline or in-plant reference data available on the prevalence of major pathogens and levels of indicator microorganisms throughout the chicken processing chain. Establishments have the responsibility to demonstrate control levels based on their food safety systems.

Purpose: To evaluate the microbial profile throughout the chicken process to assess pathogen prevalence and indicator organism levels in three poultry processing facilities in representative poultry production regions of Colombia.

Methods: A total of 270 chicken rinse samples and 30 fecal samples were collected at different processing stages of three chicken processing plants in the regions of Antioquia, Cundinamarca, and Meta. Samples were transported to the Microbiology Department at Javeriana University for microbiological analysis. Rinses and fecal samples were processed for the detection of *Salmonella* and *Campylobacter* and enumeration of *Enterobacteriaceae* following United States Department of Agriculture Microbiology Laboratory Guidebook methodologies (MGL 4.09, 41.04, and 3.01). *Salmonella* and *Campylobacter* results were reported as prevalence percentages and significant differences were identified with chi-square test.

Results: The overall prevalence of *Salmonella* and *Campylobacter* in the three processing plants were: Plant A 77% (77 of 100; Confidence interval [CI]: 67 to 84%) and 74% (74 of 100; CI: 64 to 82%); Plant B 63% (57 of 90; CI: 52 to 73%) and 91% (82 of 90; CI: 82 to 96%); Plant C 82% (82 of 100; CI:72 to 89%) and 74% (74 of 100; CI: 64 to 82%). *Enterobacteriaceae* counts had an average level of 4.98, 5.62, and 6.71 log CFU/ml for Plants A, B, and C, respectively.

Significance: This project provides reference data to inform regulatory agencies, the scientific community, and poultry industry in Colombia. Data collected at different stages throughout the chicken processing chain will support the implementation of science-based risk management options focused on proven mitigation strategies for pathogen control in processing facilities.

Poster

P1-78 Antimicrobial Resistant Patterns and Pathogen WGS of Chicken Carcass Rinse Samples Collected during Processing

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Introduction: The pathogenic diversity and antimicrobial resistance (AMR) profiles of *Salmonella* spp. and *Campylobacter* spp. linked to poultry production systems and how they are affected by multiple antimicrobial interventions at different stages during processing are not fully understood. Significant research gaps in understanding the mechanisms and factors that contribute to microbial resistance diversity and transmission to animals and humans still exist.

Purpose: To assess the microbial profiles and pathogen prevalence of a poultry processing plant during a typical processing day and evaluate the AMR profiles of *Salmonella* spp. and the microbial community diversity from chicken samples collected as they move forward in the processing value chain.

Methods: A total of 110 rinses (95 whole chicken carcasses and 15 chicken parts) and 6 fecal samples were collected throughout processing in a poultry processing plant located in the Southeastern United States. Enumeration and detection was performance based on the Microbiology Laboratory Guidebooks 3.02 (indicator microorganisms), 4.09 (*Salmonella*), and 41.09 (*Campylobacter*). Antimicrobial susceptibility testing was performed with 58 *Salmonella* isolates following the National Antimicrobial Resistance Monitoring System protocol, and whole genome sequencing was conducted with isolated pathogens.

Results: The overall prevalence of *Salmonella* spp. was 55.17% (64 of 116; Confidence interval [CI]: 45.6 to 64.4%), and for *Campylobacter* spp., the prevalence was 12.93% (15 of 116; CI: 0.76 to 20.7%). The ARM patterns showed that 98% (57 of 58) of the *Salmonella* isolates presented resistance to at least one antimicrobial agent, and 1.7% (1 of 58; 0 to 10%) were pan-susceptible. Resistance to tetracycline 91.3% (52 of 57; CI: 80-97%), and streptomycin 77% (44 of 57; CI: 64-87%) represented the higher resistance patterns.

Significance: This project provides a more comprehensive understanding of the population dynamics and pathogen changes that occur in a poultry processing chain, as the process introduces a series of hurdles and potential selective pressures that modify the pathogenic profiles and antimicrobial resistance patterns of final poultry meat products.

P1-79 *Escherichia coli* O157:H7 and Non-O157 Shiga Toxin-producing *Escherichia coli* in Veal Samples Collected by the Food Safety and Inspection Service

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Introduction: Contamination of meat with *Escherichia coli* O157:H7 and non-O157 Shiga toxin-producing *Escherichia coli* (STEC) is a public health concern. In addition to beef, veal calves can carry pathogenic *E. coli*, which may contaminate veal products. Recent analysis has indicated that veal carcasses may be more of a concern for STEC contamination during the hide removal process.

Purpose: To evaluate United States Department of Agriculture Food Safety Inspection Service (FSIS) sampling data to determine O157:H7 and non-O157:H7 pathogens of concern in veal, based on various parameters including slaughter class, O antigen type, and region.

Methods: Data for samples collected from 166 FSIS-regulated veal establishments between 2010 and 2017 were analyzed. SAS software logistic regression was used to determine statistical significance (P< 0.05).

Results: Veal sampling data show that 36 of 2,929 veal samples (1.2%) tested positive for *E. coli* O157:H7, whereas there were 67 of 871 (7.7%) samples positive for non-O157:H7 STEC. This difference was statistically significant (P<0.0001). Of the STEC isolates, 37% were O103, 34% were O157:H7, and 13% were O111. Regionally, the Northern and Central regions of the United States had significantly higher non-O157 STEC samples compared with *E. coli* O157:H7 (P<0.0001). From 24 establishments with at least one STEC-positive sample, 44% of slaughtered animals were bob veal, 51% were formula-fed veal, and 5% were non-formula fed veal. However, slaughter data from establishments with no positive samples indicated a much higher percentage of non-formula fed veal (48%) and much lower percentage of formula-fed veal (7%), with no difference in bob veal (45%). This suggests a possible association between formula-fed veal and STEC contamination.

Significance: The results indicate that non-O157 STEC is a significant pathogen of concern in veal. Accordingly, veal establishments should be aware of this trend when implementing biological hazard controls in their Hazard Analysis and Critical Control Points plans. Further research is needed to determine why veal has a higher percentage of samples positive for non-O157:H7 STEC.

P1-80 Growth of Proteolytic *Clostridium botulinum* in Beef under Isothermal Conditions from 10 to 46°C

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Introduction: Since *Clostridium botulinum* spores occur naturally in soil and are potential contaminants in minimally processed foods, control measures are primarily directed against spore germination, outgrowth, and multiplication of vegetative cells to ensure safe foods. The main contributing factors leading to botulism outbreaks is inadequate cooling of cooked products or their storage at improper temperatures.

Purpose: To quantify growth from proteolytic *C. botulinum* spores in beef over the entire growth temperature range from 10 to 46°C.

Methods: Beef samples inoculated with approximately 2 log CFU/g spores were immersed in a programmable water bath and cooked in a linearly increasing temperature to 71°C in 1 h to kill vegetative cells and heat-shock the spores. The packages were removed, chilled to <10°C, and then transferred to incubators set at several isothermal temperatures between 10 and 46°C. At appropriate intervals for each isothermal growth temperature, *C. botulinum* populations were determined by plating on differential reinforced clostridial agar and incubating anaerobically for 48 h at 35°C. Two trials, each performed in triplicate, were performed.

Results: C. botulinum growth from spores was not observed at ≤10 or ≥46°C. The pathogen grew rapidly in precooked beef during storage at temperatures above 20°C. Total viable counts after 2 days at 20°C increased from 1.79 to 3.56 log CFU/g and were as high as 6 log CFU/g within 5 days. Increased storage temperature resulted in parallel increase in *C. botulinum* population densities. At 35 and 40°C (optimal temperature for growth), about 3 log increase was quantified within 12 h.

Significance: C. botulinum may grow to high levels if precooked beef is handled poorly. The behavior of surviving *C. botulinum* spores in cooked beef will assist the food industry in guarding against the hazards associated with the pathogen in minimally processed foods.

Poster

P1-81 Food Safety Practices of Consumers When Grilling Meat and Poultry Outdoors

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Introduction: Recent studies show that consumers are grilling more frequently than in the past. Substantial research on consumers' food handling practices in the home has been conducted; however, few researchers have explored consumers' reported food safety behaviors when preparing food, specifically meat and poultry, in an outside environment.

Purpose: This study conducted a comprehensive investigation of reported food safety practices of consumers when using an outdoor grill to prepare meat and poultry.

Methods: A nationwide survey of consumers who grill meat or poultry outdoors (n=1024) was conducted. Estimates of the percentage of grillers who practice detailed safe food handling and cooking procedures during were determined.

Results: The majority of grillers were males (73%) and Caucasian (77%). Sixty-three percent of respondents washed poultry before grilling; 58% reported washing meat, Only 22% reported washing utensils after turning or moving the poultry or meat on the grill before using them again, which can cause potential cross contamination; however > 90% placed cook meat on a clean plate. Consumers used a variety of techniques to determine the doneness of the meat or poultry, but only 25.6% used a thermometer. Women (28%) were more likely to use a thermometer for meat than men (20%).

Significance: Results of this survey indicate that food safety educators should focus on cross contamination and thermometer use when designing food safety messages for the grilling population.

P1-82 Rapid Detection of *Salmonella* in Poultry Farm Environmental Samples Using Real-time PCR Combined with Immunomagnetic Separation and Whole Genome Amplification

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Introduction: Nontyphoidal *Salmonella enterica* causes an estimated 1.4 million illnesses annually in the United States. A major cause of human salmonellosis is the consumption of contaminated poultry products. Most *Salmonella* serotypes do not typically cause mortality in poultry, and poultry farm environmental samples tend to indicate the presence of *Salmonella* in the flock. Therefore, there is a need for efficient and sensitive detection methods in poultry flocks.

Purpose: To evaluate rapid detection of *Salmonella* from poultry farm environmental samples using the combination of immunomagnetic separation (IMS), multiple displacement amplification (MDA) and real-time PCR.

Methods: The limits of detection (LOD) of IMS-MDA real-time PCR were determined in 25g samples of *Salmonella*-spiked litter after 0, 4, 6, and 8-h enrichment in buffered peptone water. In addition, a total of 174 environmental samples (boot swabs, drag swabs, and litter) from poultry farms in the University of Georgia poultry research center, were tested to assess the equivalence of this method with 8-h enrichment by comparing with real-time PCR and culture method.

Results: The LOD of IMS-MDA real-time PCR for detection of *Salmonella* in litter samples were 10 CFU/g, 1 CFU/g, and 0.1 CFU/g after 0, 4-6, and 8-h enrichment, respectively. The detection rate of IMS-MDA real-time PCR with 8-h enrichment was higher than that of real-time PCR and culture method by detecting 25, 24, and 19 out of 174 samples, respectively. However, there was no statistical difference ($P > 0.05$) between IMS-MDA real-time PCR and culture method, and Cohen's kappa index indicated strong concordance.

Significance: We demonstrated the potential of IMS-MDA real-time PCR as a rapid, sensitive and affordable method for detecting *Salmonella* in poultry farm environmental samples. The successful application of this method suggests that this technique may be used for other pathogens and types of environmental samples.

P1-83 Evaluation of Roka Atlas System for Detection of *Salmonella* in Egg Products in Comparison with Culture Method, PCR Assay, and Isothermal Amplification Methods

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Introduction: The Roka Atlas *Salmonella* SEN Detection Assay is a molecular method that uses ribosomal RNA as target for detection. Since each bacterial cell can have 500 to 10,000 copies of rRNA, the assay could theoretically be more sensitive than PCR or isothermal amplification method targeting DNA sequences of a single gene.

Purpose: The objective was to evaluate the effectiveness of the Altas assay for detecting *Salmonella* in egg products in comparison with culture method, PCR assay, and isothermal amplification methods.

Methods: The assay was compared with the U.S. Food and Drug Administration's Bacteriological Analytical Manual (FDA BAM) culture method, *Salmonella* kits of ABI 7500 Fast real-time PCR, 3M Molecular Detection System (MDS), ANSR Pathogen Detection System (PDS), and Genie III System. A total of 158 isolates (124 for inclusivity and 34 for exclusivity) and 200 egg products samples with the inoculation level of 1 to 5 CFU/25g were analyzed. The study also estimated the limit of detection of the molecular methods and illustrated their advantages and disadvantages.

Results: Results indicated that the assay was not significantly different from the FDA BAM culture method and other four molecular methods in detecting *Salmonella* in egg products. For the exclusivity test, all 34 non-*Salmonella* isolates were negative by all five molecular methods studied. For the inclusivity test, all 124 *Salmonella* isolates were positive by 3M MDS, ANSR PDS, and Genie III. However, the system and PCR results were negative for nine isolates of *Salmonella bongori*. Additionally, the detection limit of the five molecular methods ranged from 1 to 3 CFU/g, with the Atlas system being the most sensitive.

Significance: The results indicated that the assay was equally effective in detecting *Salmonella* from egg products as the FDA BAM culture method and other four isothermal amplification and PCR methods evaluated in the study, with higher sensitivity.

Poster

P1-84 Integrating Molecular Data into a Risk Assessment Framework for *Salmonella* spp. in Poultry

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❖ ***Developing Scientist Competitor***

Introduction: *Salmonella* spp. are a primary source of foodborne illness and a diverse group of pathogenic bacteria, with more than 2,500 named serovars. Despite the abundance of molecular information, research on risk assessment does not account for molecular diversity and heterogeneity, as molecular data cannot be translated into a modeling framework.

Purpose: The purpose of this study was to determine the relationship between meteorological effects of bacterial expression and *Salmonella* stress response and virulence gene expression in order to modulate bacterial prevalence estimates for applicability into a risk assessment framework.

Methods: The whole genome sequences of 140 *Salmonella* spp. and serovars (Enteritidis, Heidelberg, and Typhimurium) isolated from chicken by the U.S. Food and Drug Administration GenomeTrakr project were analyzed for the presence of genes associated with virulence (*lpfA*, *agfA*, *invA*, *hilA*, *avrA*, *sopE*, *sivH*, and *spvC*) and stress response (*rpoS* and *rpoH*). A hierarchical logistic regression model was developed using SAS software (v 9.4) to determine the relationship between the presence or absence of these genes and the frequency of gene expression against common meteorological effectors of bacterial expression, such as temperature and precipitation, obtained from the National Oceanic and Atmospheric Administration.

Results: Considerable inter- and intra-strain variations were observed in the virulence profiles of the *Salmonella* isolates. The samples were divided into different genetic profiles based on the expression of virulence genes: P1 (presence of all genes [82% prevalent]); P2 (negative for *sivH* [96% prevalent in serovar Heidelberg]); and P3 (negative for *lpfA*, *sopE*, and *hilA)*. The expression of the stress response gene *rpoS* was significantly correlated with temperature <10°C and precipitation index=3.18 (odds ratio=2.95, 95% confidence limits=1.97 and 4.41; $P<0.0001$). These virulence and stress response profiles can, in turn, be incorporated into existing *Salmonella* prevalence distributions to be applied to risk assessment.

Significance: This study demonstrates a way to bridge the gap between the application of molecular data and quantitative risk assessment.

P1-85 Chicken Liver-associated Outbreaks and Contamination in the United States, 2000 through 2017 — Opportunities for Outreach and Education

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Introduction: Chicken liver-associated outbreaks have been reported in the United States (U.S.) and other countries. In the U.S., limited data exist on the frequency and characteristics of such outbreaks. Additionally, little is known about pathogen contamination of commercial chicken liver in the U.S.

Purpose: To inform foodborne illness prevention, we described common factors among chicken liver-associated outbreaks and assessed pathogen contamination levels in chicken livers sampled by the United States Department of Agriculture Food Safety and Inspection Service (FSIS).

Methods: Chicken liver-associated outbreaks during 2000 to 2016 in the U.S. were identified by reviewing data reported to FSIS and the Centers for Disease Control and Prevention. Outbreak frequency and selected characteristics, including etiology, chicken liver dish, food preparation settings, and contributing factors, were described. During November 2016 to November 2017, chicken liver rinsate samples were aseptically collected from FSIS-regulated establishments. Livers were rinsed in 50 mL of buffer and analyzed for *Campylobacter* and *Salmonella*.

Results: During 2000 to 2016, 28 chicken liver-associated outbreaks of confirmed foodborne infection in the U.S. were reported (24 campylobacteriosis; 5 salmonellosis; and 2 caused by both pathogens); 18 (64.3%) occurred during 2014 to 2016. Blended chicken liver dishes (e.g., pâté) were implicated in 24 (85.7%), preparation in a foodservice setting was identified in 25 (89.3%), and chicken liver was reported to be undercooked in 26 (92.8%) of the 28 outbreaks. Among FSIS chicken liver samples collected during November 2016 to November 2017, 66 of 87 (75.9%) and 57 of 85 (67.1%) were positive for *Campylobacter* and *Salmonella*, respectively.

Significance: Chicken liver is an emerging source of U.S. outbreaks, and blended chicken liver dishes prepared in foodservice settings are a common source. Safe preparation methods for these dishes should be developed and promoted among food preparers and consumers. Guidance should be given to chicken liver-producing establishments to reduce pathogen contamination.

P1-86 Evaluate the Efficacy of Commercial Antimicrobials against Unstressed, Acid-, Starvation-, or Cold-Stress-adapted *Campylobacter jejuni* on Broiler Wings

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❖ ***Developing Scientist Competitor***

Introduction: Locally grown poultry products raise microbial safety concerns due to their exemption from the USDA Food Safety Inspection Service (FSIS) Poultry Products Inspection Act, and 40% of broilers processed at a small USDA-inspected facility were positive for *Campylobacter*.

Purpose: This study aims to compare the inactivation of stress-adapted and unstressed *Campylobacter jejuni* on broiler wings dipped in commercial antimicrobials.

Methods: Three overnight cultured (18 h) *C. jejuni* strains were unstressed or acid-shocked in pH 5.0 Bolton's broth (2 h), sub-cultured in 0.9% saline solution (2 h), and stored in Bolton's broth at 4°C (5 days) to prepare acid-, starvation-, and cold-stress-adapted cells, respectively. Unstressed or stress-adapted *C. jejuni* inoculated fresh wings were undipped or dipped into peroxyacetic acid (PAA; 1,000 ppm), lactic acid (LA; 5%), lactic/citric acid blend (LCA; 2.5%), sodium hypochlorite (SH; 70 ppm), and SaniDate 5.0 (SD; 0.25% [PAA and H_2O_2]) for 30 sec. Surviving bacteria were recovered onto Brucella agar under microaerophilic conditions. Data (2 repeats/3 to 4 samples/repeat) were analyzed using the SAS software mixed model.

Results: Initial acid-stressed *C. jejuni* counts were 2.67 log CFU/ml, lower ($P<0.05$) than the unstressed (4.71 log CFU/ml), starvation- (4.21 log CFU/ml), and cold-stress-adapted cells (4.44 $\log_{10}$CFU/ml). Unstressed and stressed bacteria behaved differently with antimicrobials, with a significant effect of antimicrobials ($P<0.05$), stress ($P<0.05$), and the interaction ($P<0.05$). Antimicrobials reduced ($P<0.05$) unstressed *C. jejuni* by 1.53 to 2.45 log CFU/ml compared to the control. Compared with unstressed cells, reductions of acid-, starvation- and cold-stress–adapted cells indicated cross-protection (0.15 to 0.36 log CFU/ml), cross-protection (0.86 to 1.81 log CFU/ml), and no effect (1.52 to 2.31 log CFU/ml), respectively, of pathogens during subsequent exposure to antimicrobials. Among all cultures, PAA- (0.34 to 2.45 log CFU/ml) and LA-treated samples (0.36 to 2.33 log CFU/ml) had the greatest ($P<0.05$) reductions, compared to LCA (0.28 to 1.89 log CFU/ml), SH (0.20 to 1.56 log CFU/ml), and SD (0.15 to 1.53 log CFU/ml).

Significance: Applying post-chilling antimicrobial treatments, especially PAA and LA, could effectively reduce *Campylobacter* on broiler parts. Challenge studies should include stress-adapted cells.

Poster

P1-87 Pork Juice is Hotbed for Biofilm Formation in *Listeria monocytogenes*

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Introduction: *Listeria monocytogenes* is one of the most important foodborne pathogens. It can commonly contaminate food, such as raw meat and RTE food. *L. monocytogenes* can attach to food contact surfaces to form biofilm, which leads to cross-contamination of food products, thereby introducing high risk to public health. Recent research on biofilm formation in *L. monocytogenes* is commonly focused on laboratory media, which cannot represent real conditions in food systems.

Purpose: In this study, different meat juices (pork, chicken, and beef) were used to simulate the real conditions in meat processing environments to compare the effect of different meat juices on biofilm formation of meat-borne *L. monocytogenes.*

Methods: Forty-seven *L. monocytogenes* isolates were separated and identified from 153 retail raw meat samples in Shanghai, China using the Chinese National Standard (GB 4789.30-2010) method. Using multiplex PCR, these isolates were divided into three serogroups: 1/2a-3a, 1/2b-3b-7, and 1/2c-3c. A Calgary Biofilm Device was introduced to cultivate biofilm, and crystal violet staining and viable cell enumeration were used to determine the quantity of bacterial cells in biofilm. A confocal laser scanning microscope (CLSM) was also used to observe the structure and measure the thickness of biofilm.

Results: It turned out that there was no significant difference in biofilm formation among the three serogroups; however, different meat juices had significant impacts on the biofilm formation for each single strain. *L. monocytogenes* produced the highest quantity of biofilm in pork juice, followed by chicken juice; there was little biofilm formed in beef juice. Being co-cultured in pork juice, the cell numbers of *Salmonella enterica*, *Staphylococcus aureus*, and *L. monocytogenes* had a significantly decreased tendency compared to mono-species biofilm, which revealed a competitive interaction among the three species. The decline of the thickness of biofilm in multispecies biofilm also proved the competitive interaction among the three species.

Significance: Meat juice was used as a simulation of real conditions in a meat processing environment, and the results suggest that the meat industry should keep a close eye on raw pork product.

P1-88 Thermal Inactivation of *Salmonella* spp. in Chicken Liver Pâté

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Introduction: Undercooked pâté prepared from chicken livers has been recognized as a vehicle for transmission of *Salmonella*. Thus, research is warranted to validate cooking practices to reduce the potential risk of salmonellosis associated with pâté.

Purpose: Evaluate the effect of heating times and temperatures on inactivation of *Salmonella* in chicken liver pâté.

Methods: Raw chicken liver was blended in a mixer with hard-boiled eggs (2 units) and with a mixture of sautéed onions (10%), salt (0.5%), black pepper (0.25%), and butter (11.2%). The tempered batter was inoculated with a nine-strain cocktail (approximately 6.5 log CFU/g) of *Salmonella* and approximately 25-g portions were aseptically transferred into sterile 50-mL conical tubes. The tubes were completely submerged in a thermostatically controlled, circulating water bath. One set of pâté was cooked to target instantaneous internal temperatures ranging from 60 to 73.8°C in a water bath set at 74.8°C, whereas an otherwise similar set of pâté was cooked at 60, 63, 65, 68, 71.1, or 73.8°C, with holding times of 3 to 30 min in a water bath set at 1°C above of each target cooking temperature. After cooking, the tubes containing pâté were removed from the heated water and immediately cooled in an ice-water bath for 30 min.

Results: Regardless of the process, when pâté was cooked to a target instantaneous internal temperature of 60 to 73.8°C, pathogen numbers decreased by approximately 1.9 to ≥6.4 log CFU/g, whereas additional reductions of approximately 0.8 to 1.3 log CFU/g in pathogen numbers were observed when pâté was cooked to a target internal temperature of 60 to 73.8°C and held for 3 to 30 min.

Significance: These findings may be useful for establishing cooking guidelines for pâté and, thus, for lowering the risk of illness if chicken livers are contaminated with *Salmonella* and the attendant batter is not handled or cooked properly.

P1-89 Inactivation of *Salmonella* spp. on the Surface of Chicken Livers and in Chicken Liver Pâté Using High-pressure Processing

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Introduction: Chicken livers may be internally and/or externally contaminated with foodborne pathogens. Consumption of undercooked livers or pâté was linked to outbreaks and recalls due to contamination of these products with *Salmonella* spp. Thus, interventions are needed to reduce the risk of salmonellosis attributed to chicken liver and pâté.

Purpose: Evaluate the effectiveness of high-pressure processing (HPP) to inactivate *Salmonella* spp. on the surface of raw chicken livers or in chicken liver pâté.

Methods: Raw chicken liver or chicken liver pâté were uniformly inoculated with a seven-strain cocktail (approximately 6.5 log CFU/g) of *Salmonella* spp. Products were separately double-packaged in nylon polyethylene bags and vacuum sealed to 950 mBar. Samples were subjected to 483 or 600 MPa for 0 to 5 min. In addition, chicken livers were subjected instantaneously to pressure of 100 or 600 MPa without a holding time.

Results: In general, the greater the time of treatment and/or the greater the level of pressure applied, the greater the reduction of *Salmonella*. Overall, similar reductions of *Salmonella* were observed on livers and in pâté. More specifically, when liver or pâté were subjected to 483 MPa, pathogen numbers decreased by approximately 1.7 to 4.9 log CFU/g, whereas when liver or pâté were subjected to 600 MPa, pathogen numbers decreased by approximately 2.2 to 6.0 log CFU/g. In addition, when chicken livers were subjected to 100 to 600 MPa (without holding), pathogen numbers decreased by approximately 0.1 to 2.1 log CFU/g.

Significance: These results established the effectiveness of HPP as an intervention to inactivate *Salmonella* on chicken livers and in chicken liver pâté. Thus, pressurization can appreciably reduce the risk of salmonellosis associated with consumption of undercooked chicken liver and pâté.

P1-90 Recovery of Regulated Non-O157 Serogroups of Shiga Toxin-producing *Escherichia coli* from Ground Veal and Ground Beef Collected from Retail Stores in the Mid-Atlantic Region of the United States

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Introduction: Testing of regulatory samples of raw ground beef and veal components (RGBC) suggests a higher recovery rate of Shiga toxin-producing *Escherichia coli* (STEC) from veal than from beef. However, far less data are available on the recovery rate of non-O157 STEC in beef in general, and for veal in particular, from samples collected at retail.

Purpose: Determine the prevalence of the six regulated non-O157 STEC in ground veal and ground beef purchased at retail establishments in the Mid-Atlantic region of the United States.

Methods: Samples of ground veal (555) and ground beef (540) were randomly collected from retail establishments in Virginia, Maryland, Pennsylvania, Delaware, and North Carolina over a two-year period. Samples were individually enriched (325 g per sample) and screened (BAX System Real Time PCR) according to the methodology for non-O157 STEC approved by the United States Department of Agriculture Food Safety Inspection Service. Isolates were recovered from positive samples via serogroup-specific immunomagnetic separation and selective media, and then confirmed via multiplex PCR for the presence of selected virulence genes (i.e., *eae*, stx_1, and/or stx_2, and *ehx*) for the non-O157 serogroups.

Results: Non-O157 STEC were recovered from ground veal and ground beef at a rate of 7.0% (39 of 555) and 0.9% (5 of 540), respectively. Of the five non-O157 isolates recovered from ground beef, four were confirmed as serogroup O26 and one as O103. The 39 isolates recovered from ground veal were confirmed as serogroups O26 (four isolates), O45 (one isolate), O103 (33 isolates), and O145 (one isolate).

Significance: Similar to data for regulatory sampling, the recovery rate for non-O157 STEC in retail ground veal was appreciably higher than in ground beef and, thus, systematic interventions should be implemented across the food chain continuum to reduce the risk of STEC cells associated with veal products.

P1-91 Risk of Aerotolerant Strains of *Campylobacter jejuni* under Various Conditions

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Introduction: Poultry meat products are major vectors for *Campylobacter jejuni* foodborne illness. Since *C. jejuni* is microaerophillic, the cell counts gradually decrease during distribution and display under aerobic conditions. However, if *C. jejuni* can resist aerobic conditions, they can become a serious problem for food safety.

Purpose: This study determined the aerotolerance of *C. jejuni* and the risk of aerotolerant *C. jejuni* isolated from poultry.

Methods: Fourteen *C. jejuni* strains isolated from poultry were subjected to aerobic conditions in a shaking incubator at 500 rpm and 37°C for 120 h. The cell counts of *C. jejuni* strains were enumerated on modified CCDA-Preston medium at 0, 24, 72, and 120 h, and the *C. jejuni* strains, with a reduction of less than 2 log CFU/mL for 120 h, were determined as aerotolerant *C. jejuni* strains. One non-aerotolerant (SMFM2015-Du8) and two aerotolerant *C. jejuni* strains (SMFM2015-Du7 and SMFM2014-Du16) were then incubated at 4 and 37°C under aerobic conditions to compare the growth between aerotolerant and non-aerotolerant strains. In addition, transcriptomes for virulence (*cadF, cdtB, ciaB*, and *clpP*) were compared between non-aerotolerant and aerotolerant strains.

Results: Among 14 *C. jejuni* strains, seven strains (50%) showed less than 2-log CFU/mL reduction at 37°C after 24 h, and five strains still showed less than 2-log CFU/mL reduction after 48 h. In particular, *C. jejuni* strains SMFM2015-Du7 and SMFM2014-Du16 survived after 120 h under aerobic conditions, after which they were determined to be aerotolerant. However, at 4°C under aerobic conditions, there was no significant difference in the reduction of cell counts and virulence factor expressions between aerotolerant strains and non-aerotolerant *C. jejuni* strains.

Significance: There are aerotolerant *C. jejuni* strains, but their risk is similar to non-aerotolerant *C. jejuni* strains at 4°C under aerobic condition, which are the storage conditions for poultry meat.

P1-92 National Survey of *Salmonella* Prevalence in Lymph Nodes of Sows and Market Hogs

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Introduction: According to the Centers for Disease Control and Prevention, *Salmonella* in pork is the third leading cause of foodborne illness-related hospitalizations. This, coupled with the knowledge that lymph nodes (LNs) can be reservoirs for *Salmonella*, highlighted a need to assess current prevalence rates of *Salmonella* in the LNs of sows and market hogs in the United States.

Purpose: This project was designed to benchmark the national prevalence rates of *Salmonella* in the lymph nodes of sows and market hogs.

Methods: Twenty-one commercial pork harvest and processing facilities participated in the study. Facilities were categorized as north (*n*=12) or south (*n*=9) geographical regions. As processing volumes allowed, 25 carcasses were selected at each establishment. Left and right superficial inguinal LNs (*n*=1,014 LNs) were removed and pooled, yielding one sample per animal (*n*=507 total LN samples). LN samples were subjected to *Salmonella* prevalence determination. Type of chilling method used at each facility was also recorded.

Results: Within each region, *Salmonella* prevalence rates between hog types differed ($P<0.05$). In the north, *Salmonella*-positive sow samples (37.0%) occurred more often than positive market hog samples (6.4%). In the south, a higher rate of *Salmonella* prevalence was seen in LN samples from market hogs (13%) than those from sows (4.8%). *Salmonella* prevalence was highest ($P<0.017$) for the conventional chill method when compared to other chill types for samples collected in the north (conventional=20.0%; blast chill=1.3%; other=2.7%). No differences in *Salmonella* prevalence were found between chill methods in the south (conventional=20.0%; blast chill=0.0%; other=12.0%).

Significance: These results have the potential to influence decisions related to pre- and post-harvest interventions for reducing *Salmonella* in pork, which in turn could reduce the number of salmonellosis cases attributed to pork products.

P1-93 Effects of Cooling Time on the Growth of *Clostridium perfringens* in Roast Beef Treated with a Concentrated Buffered Vinegar Solution

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✦ ***Undergraduate Student Award Competitor***

Introduction: RTE meats should be chilled from 48°C (120°F) to 12.7°C (55°F) in no more than 6 h according to a United States Department of Agriculture (USDA) Appendix B guideline to limit the growth of *Clostridium perfringens* to less than 1 log. However, due to process deviations, RTE meat processors may be unable to consistently meet this standard.

Purpose: The purpose of this study is to provide evidence for future industrial applications that beef treated with antimicrobial ingredients, such as a concentrated buffered vinegar solution (CBV), can be cooled up to 2 h longer than USDA guidelines permit, without exhibiting a 1-log increase of *C. perfringens*.

Methods: Cuts of top round beef treated with CBV provided by a local processor were ground and 10-g portions were sealed into polyethylene sample bags inoculated with approximately 10^3 spores/g of a *C. perfringens* cocktail (NCTC 8238, 8239, and 10240). The meat was cooked to 60.6°C within 8 h and then cooled from 48 to 12.7°C for 6, 7, and 8 h in triplicate batches. Duplicate samples were collected from each batch at 48 and 12.7°C, plated, anaerobically incubated for 24 h, and enumerated for growth comparison.

Results: The results of the experiments showed that roast beef samples inoculated with *C. perfringens* exhibited less than 1 log of growth when cooled for 6, 7, and 8 h and are statistically significant at $P<0.05$ ($n=6$).

Significance: The significance of this study is to provide proof that CBV-treated beef can safely undergo increased cooling times when necessary due to uncontrollable process deviations.

P1-94 *Salmonella* in Shell Eggs from Non-commercial Sources

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Introduction: Salmonellosis is the leading cause of foodborne illness in shell eggs and has been the focus of safety in commercial production. Consumer interest in shell eggs from non-commercial sources (e.g., small and backyard flocks, local farms, farmer's markets) continues nationwide; however, few studies have evaluated the presence of *Salmonella* from these suppliers.

Purpose: The purpose of this study was to evaluate the occurrence of *Salmonella* from non-commercially sourced shell eggs to further understand the potential for public concern.

Methods: Convenience random sampling of 1,388 shell eggs were obtained from non-commercial sources within the state of Alabama. Isolation for *Salmonella* was performed according to the U.S. Food and Drug Administration's Bacterial Analytical Manual, modified with the elimination of composite sampling. Presumptive positive samples were confirmed by colony PCR with *Salmonella* detection primers TS-11 (5'-GTCACGGAAGAAGAGAAATCCGTACG) and TS-5(5'-GGGAGTCCAGGTTGACGGAAAATTT), and further verified with primers InvA5 (5'-GTGAAATTATCGCCACGTTCGGGCAA) and InvA3 (5'-TCATCGCACCGTCAAAGGAACC). Amplicons were observed through 1.2% agarose gel electrophoresis.

Results: PCR results indicated the presence of three samples positive for *Salmonella* in the 1,388 evaluated samples. According to this study, the occurrence of *Salmonella* is higher in shell eggs from non-commercial sources, compared to the national average of those commercially produced (1 in 20,000).

Significance: The data suggest that the risk of *Salmonella* is higher in shell eggs from non-commercial sources compared to those produced commercially, implying the necessity for additional egg safety and handling guidelines. However, further nationwide or compiled state-by-state, evaluations of these non-commercial shell egg suppliers would provide additional insight into the risk to public health.

P1-95 Survival of *Listeria monocytogenes* and *Staphylococcus aureus* on Ready-to-Eat, Shelf-stable, Poultry-based Meat Bars during Vacuum-packaged Storage

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❖ ***Developing Scientist Competitor***

Introduction: Shelf-stable meat snacks are at risk for post-processing contamination with pathogens. Understanding the magnitude of these risks is important due to the increasing popularity of these products.

Purpose: This study evaluated the fate of *Listeria monocytogenes* and *Staphylococcus aureus* on commercially available poultry-based meat bars during vacuum-packaged storage.

Methods: Three brands of commercially-produced poultry meat bars (approximately 11.5 by 4.5 by 1.0 cm each) were removed from their original packaging and were surface-inoculated (6 to 7 log CFU/g) with a five-strain mixture of *L. monocytogenes* or *S. aureus.* Bars were then individually vacuum-packaged and stored at 25°C for up to 50 days. Samples (three per bar brand, two replications each) were periodically analyzed for surviving populations of *L. monocytogenes* (modified Oxford agar) and *S. aureus* (Baird-Parker agar). Samples were also analyzed for pH and water activity (aw). Surviving bacterial counts for each bar were fitted with the Baranyi and Roberts mathematical model (DMFit version 3.5, ComBase) to assess shoulder periods and death rates for each pathogen.

Results: Bar brands A, B, and C had average pH values of 5.50, 4.55, and 5.26, respectively, and aw values of 0.86, 0.89, and 0.84, respectively. Both pathogens survived the longest on bar A; shoulder periods and death rates were 22.2 days and -0.08 log CFU/g/day, respectively, for *S. aureus*, and 9.4 days and -0.12 log CFU/g/day, respectively, for *L. monocytogenes*. No shoulder period and the highest death rate was obtained for both pathogens on bar B, with -0.30 log CFU/g/day for *S. aureus* and -0.28 log CFU/g/day for *L. monocytogenes.* Both pathogens were recovered from all three bar brands after 50 days of storage. This indicates further research may be needed to assess the risk of poultry-based meat bars with differing a_w and pH attributes.

Significance: These data provide awareness of the fate of post-processing pathogen contamination on ready-to-eat shelf-stable meat bar snacks.

P1-96 *Salmonella* and *Escherichia coli* O157 in Beef Retail Channels in Colombia

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Introduction: *Salmonella* and *E. coli* O157 prevalence data associated to raw beef is very scarce in Colombia. Data collected by the industry and government is typically not published. Baseline information is necessary to establish food safety goals and for improvement of meat safety regulations.

Purpose: To conduct a *Salmonella* and *E. coli* O157 baseline associated to raw beef in three retain channels in Colombia.

Methods: Ground beef ($n=60$) and whole beef cuts ($n=37$) were collected from three different retail channels (supermarkets, distribution centers, and butcher shops), in Bogota, Colombia. For the whole beef cuts, samples were swabbed with sponges containing 10-ml buffered peptone water and enriched in 90-ml modified tryptic soy broth (mTSB). Ground beef samples were enriched by placing 10g of sample into 90-ml mTSB filer bags. Enrichments were incubated at 37°C for 18-24h. *Salmonella* was detected using the Molecular Detection System (MDS, 3M Company) and *E. coli* O157 was detected using the BAX® system (Hygiena). Frequency tables were created and prevalence data calculated based on total of positive samples obtained.

Results: *E. coli* O157 was not found at any of the samples tested. *Salmonella* prevalence was highest in whole cuts (13 samples=30.9%) than in ground beef (13 samples=21.7%). With respect to the retail channel, the highest *Salmonella* prevalence was found at butcher shops (56 and 35% in whole cuts and ground beef, respectively). Supermarkets had the lowest prevalence (0 and 15% in whole cuts and ground beef, respectively). Distribution centers had a prevalence of 36.4% in whole beef and 15% in ground beef. Supermarkets and distribution centers keep their meat under refrigeration, whereas butcher shops maintain products at room temperature and lower hygiene standards.

Significance: This baseline data will contribute to implement focused measures for the reduction of pathogens prevalence during beef commercialization, therefore improving public health.

P1-97 Assess Hepatitis A Virus Survival on Dried Berries during Month-long Storage

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Introduction: Foodborne RNA viruses such as hepatitis A virus (HAV) can degrade in fresh produce during storage at cold and room temperatures. However, the extent of HAV degradation in dried fruits is largely unknown.

Purpose: To model survival of HAV on dehydrated strawberry slices during storage at room temperature for 2, 7, 14, 28, 42, and 56 days after freeze-drying.

Methods: Sliced fresh strawberries (7 mm thick) were inoculated with 5 log PFU HAV/sample, air-dried 20 min, frozen at -80°C 1 h, and freeze-dried 24 h (FreeZone 12, Labconco, Kansas City, MO). The dehydrated slices were transferred into 50-mL polypropylene tubes and stored 2 days, 1, 2, 4, 6, and 8 weeks at 22°C. HAV titers in strawberries were determined by plaque-assaying eluates on six-well FRhK-4 cell dishes.

Results: Our previous study (2012 *AEM*) demonstrated temperature dependence of HAV degradation on fresh produce ($D_{22°C}$=6.3 days) equivalent to 0.158-log reduction per day. The current study at the same temperature found slower HAV degradation in dried berries (aw≈0.1), except for the first few days immediately following freeze-drying. The degradation rates in stored dried berries during 2, 7, 14, 28, 42, and 56-day storage averaged 0.2, 0.09, 0.08, 0.04, 0.04, and 0.03-log reduction per day, respectively (24 trials). The degradation rate was not constant during storage; a slower degradation was observed as storage continued, although temperature and aw remained stable. The regression between accumulated HAV degradation on dried berries (Y) and storage time in day (X) fits a power equation: $Y=0.2882X^{0.4503}$, $r^2=0.97$.

Significance: The findings show that HAV persists for a long time and survives better on dehydrated fruits than fresh ones; the results also permit assessment of the hazard of HAV-contaminated strawberries after freeze-drying process.

P1-98 Inter- and Intra-host Nucleotide Variations of Hepatitis A Virus in Culture and Clinical Samples Detected by Next-generation Sequencing

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Introduction: High mutation rates are common in RNA viruses due largely to the lack of proofreading ability of most RNA polymerases. Thus, nucleotide sequence variations can occur among infected individuals (inter-host), or possibly within a single-infected individual (intra-host), posing a challenge for accurate detection and identification of viral (RNA) pathogens and accurate attribution of the sources of contamination and infection.

Purpose: The purpose of this study was (i) to detect and verify the inter- and intra-host single nucleotide polymorphisms (SNPs) of hepatitis A virus (HAV) in culture and clinical samples, and (ii) to establish a model of SNP detection from viruses using next-generation sequencing (NGS).

Methods: FRhK4 cells persistently infected with HAV HM175/clone1 (F4-c1) were periodically collected from 62 to 1,200 days post-infection (dpi). Viral RNA was isolated from cell pellets at each time point, or from a HAV-positive stool sample, quantified with real-time RT-PCR prior to RNA-based library generation. Libraries were sequenced on the MiSeq platform (Illumina), generating paired-end reads. CLC Genomic Workbench was used for data analysis of SNPs of F4-c1 from later time points compared to those of the earliest time point (62 dpi), or on SNPs of wild type HAV HM-175 between clinical samples. SNP calling was carried out on NGS data using the low frequency variation detection module, and confirmed by pyrosequencing.

Results: (i) Inter-host and intra-host variants were detected in cultured F4-c1 samples and clinical samples; (ii) NGS SNP identification was confirmed by pyrosequencing; (iii) Both read coverage and variation frequency were included in the development of our SNP identification model from NGS data.

Significance: Detection of viruses in foods is a major public health concern. Our study will help to provide the scientific basis for the U.S. Food and Drug Administration's regulatory mission of ensuring the safety and security of our nation's food supply.

P1-99 Inactivation of Tulane Virus on Blueberries with Gaseous Chlorine Dioxide

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Introduction: Berry fruits are prone to contamination with pathogenic human viruses due to irrigation with non-potable water sources, and in some cases non-hygienic manual picking. These viruses are very difficult to inactivate. Here we show that gaseous chlorine dioxide can inactivate a human norovirus surrogate on the surface of blueberries.

Purpose: To determine the effectiveness of gaseous chlorine dioxide against a human norovirus surrogate on produce.

Methods: Gaseous chlorine dioxide ($gClO_2$) was produced by acidifying sodium chlorite solution used to treat Tulane virus-coated blueberries in a 240-ml treatment chamber.

Poster

Results: Initial assessments indicated that blueberries treated with $gClO_2$ generated from ≤1 mg acidified sodium chlorite in a small chamber appeared unaffected, while 10 mg of acidified sodium chlorite altered the appearance and quality of the blueberries. Treatments of inoculated blueberries with $gClO_2$ generated from 0.1 mg sodium chlorite reduced the virus populations by >1 log after exposure for 30 to 330 min. For the 1-mg sodium chlorite treatments, the virus populations were reduced by >2.2 log after 15-min exposure and to non-detectable levels (>3.3-log reduction) after 180 min of exposure. Measured concentrations of $gClO_2$ peaked in the treatment chamber at 0.9 µg/L after 10 min for 0.1-mg treatments and 600 µg/L after 20 min for 1-mg treatments.

Significance: Overall results indicate that $gClO_2$ could be a feasible waterless intervention for blueberries and other produce. Once evaluated in larger scale for its efficacy and for product quality attributes, this dry, non-thermal intervention technology should be widely accepted by industry.

P1-100 Detection of *Cyclospora cayetanensis* in Prepared Food Dishes: Strengthening Laboratory Approaches for Future Outbreak Investigations

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Introduction: Cyclospora cayetanensis is a foodborne parasite associated with outbreaks worldwide. In past outbreak investigations, U.S. Food and Drug Administration (FDA) laboratories received leftovers of implicated foods, but there were no standards for the detection of *C. cayetanensis* in dishes containing multiple ingredients.

Purpose: The present study evaluated specific modifications of the validated FDA method to detect *C. cayetanensis* in prepared food dishes that were comparable to leftovers implicated in past cyclosporiasis outbreaks, i.e., in coleslaw and pico de gallo.

Methods: Prepared coleslaw and pico de gallo dishes were seeded with different numbers of *C. cayetanensis* oocysts and the standard FDA Bacteriological Analytical Manual (BAM) Chapter 19B protocol was followed; produce-washing procedure, DNA extraction, and molecular detection using a TaqMan assay specific for *C. cayetanensis* was performed. We modified the BAM chapter 19B wash procedure and evaluated techniques to further remove PCR inhibitors from the DNA extracts.

Results: In comparison to the BAM chapter 19B method, washing using a higher concentration of Alconox (1.0 instead of 0.1%) improved oocyst recovery in prepared coleslaw with dressing sauce. Detection rates were 80, 90, and 100% in samples seeded with five oocysts (*n*=10), 10 oocysts (*n*=10), and 200 oocysts (*n*=10), respectively. The original BAM method only allowed detection in samples at the 200 oocysts seeding level. When testing pico de gallo, aggressive washing, as used for leafy greens in BAM Chapter 19B, produced large wash pellets that were difficult to analyze; therefore; very gentle washing was essential to detect as low as five oocysts in 25-g samples (81.8%, *n*=11).

Significance: Development of detection standards for complex food matrixes implicated in *C. cayetanensis* outbreaks is necessary. Evaluating and optimizing laboratory methods for the detection of *C. cayetanensis* in such dishes will strengthen the use of laboratory applications for outbreak investigations.

P1-101 Effect of Bacterial Lipopolysaccharide and Peptidoglycan on the Resistance of Human Norovirus Surrogate, Tulane Virus, to Heat and Chlorine

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Introduction: Norovirus encounters bacteria in food systems, but data are lacking on the impact of bacteria on norovirus resistance to sanitation and processing treatments. Bacterial cell wall constituents reportedly increase the inactivation resistance of another enteric virus, poliovirus.

Purpose: The effect of lipopolysaccharide (LPS) and peptidoglycan (PEP) was evaluated on the thermal and chlorine resistance of human norovirus surrogate, Tulane virus (TV).

Methods: TV (10^4 PFU/ml) was incubated for 2 h at 37°C in suspensions of phosphate buffered saline alone or with 1 mg/ml of commercially-prepared LPS of *Escherichia coli* O111:B4 or PEP of *Bacillus subtilis*. TV was treated 2 min at 20, 55, 60, and 65°C and evaluated for infectivity by plaque assay in LLC-MK2 cells. TV was treated with 0, 2, 20, and 200 ppm chlorine for 5 min at 20°C. Chlorine was quenched by sodium thiosulfate (5%), and TV infectivity was assessed. TV detection limit was 10 PFU/ml. Controls included untreated TV and uninoculated PBS, LPS, and PEP. Three independent trials were conducted per treatment. Significant differences among treatments were determined by Student's *t* test.

Results: TV infectivity was reduced by 1.80, 1.95, and 0.88 log PFU/ml in PBS, LPS, and PEP, respectively, at 55°C compared to the control at 20°C. TV was not detected (>3.7-log PFU/ml reduction) after 60°C treatment when suspended in PBS and LPS, but was reduced by only 1.99 log PFU/ml in PEP (P<0.05). TV was not detected in any medium after 65°C treatment. TV was reduced by approximately 1 log PFU/ml after exposure to 2 and 20 ppm chlorine in PBS, LPS, and PEP. Chlorine treatment of 200 ppm rendered TV undetectable (>3-log PFU/ml reduction) in PBS and LPS; however, TV was still detected in PEP, reduced by 2.86 PFU/ml.

Significance: These data suggest more stringent treatments may be needed to inactivate virus in the presence of bacteria.

P1-102 A Cloth-based Hybridization Array System for Rapid Detection and Identification of the Food- and Waterborne Parasites *Giardia*, *Cryptosporidium*, and *Toxoplasma*

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Introduction: Infectious environmental stages (cysts and oocysts) of the food- and waterborne parasites *Giardia*, *Cryptosporidium*, and *Toxoplasma* are viable for weeks or months outside a host. Fewer than 10 (oo)cysts are sufficient to establish an infection in consumers who eat contaminated raw produce. Testing of fresh produce for the presence of these parasites requires the technical expertise of a specialized laboratory and is time-consuming.

Purpose: The purpose of this study is to develop a rapid PCR detection method that can reliably identify species and genotypes of *Giardia*, *Cryptosporidium*, and *Toxoplasma* on fresh produce without the need for DNA sequencing.

Methods: A multiplex PCR was developed to amplify selected genes of all three parasites in a single reaction mixture. An easy-to-use colorimetric readout was performed using a cloth-based hybridization array system (CHAS). Since not all *Giardia* and *Cryptosporidium* species and genotypes infect humans, specific PCR primers and CHAS probes were designed to distinguish *Giardia duodenalis* assemblages A and B from non-A and non-B, and *Cryptosporidium hominis* from *C. parvum* or *C. muris*. *Toxoplasma gondii* was not further typed because all three common genotypes are infectious to humans. The assay contains an internal amplification control to test for PCR inhibition.

Results: We subjected three *Giardia* strains, three *Cryptosporidium* strains, and one *Toxoplasma* strain, with different genotypic profiles, to the CHAS procedure. All parasites gave the expected pattern of CHAS reactivity for the different markers. No cross-reactivity between different genotypes was observed. The CHAS readouts were obtained within 1 h post-PCR.

Significance: This rapid detection method incorporates a colorimetric readout which makes analysis as simple as reading a pregnancy test. This novel technology provides reliable results without the need for a DNA sequencing facility, and results can be obtained within one day.

P1-103 Evaluation of Porcine Gastric Mucin as Control in Human Norovirus Bacteria Binding Experiments

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❖ ***Developing Scientist Competitor***

Introduction: Specific binding of human norovirus (HuNoV) to produce or phyllosphere microorganisms may play a role in persistence of the virus in leafy greens. This project aims to determine the role of cellular receptors, histo-blood group antigens (HBGAs), in binding of HuNoV surrogates, Tulane virus (TV) and murine norovirus (MNV), to Romaine and phyllosphere bacteria. In this study, we evaluate porcine gastric mucin (PGM) as a potential HBGA-positive control for use in HuNoV-phyllosphere bacteria binding experiments.

Purpose: Determine an appropriate positive HBGA binding control for use in HuNoV-bacterial interaction studies.

Methods: Partially purified PGM was cross-linked to magnetic beads (PGM-MB) in 0.1 M MES buffer. A sample of 100µl of PGM-MBs at 2.0×10^9 beads/ml were exposed to 100 µl of 2×10^6 PFU/ml of either TV (positive HBGA binding control) or MNV (negative HBGA binding) for viral-PGM-MB attachment. PGM-MBs with bound virus were isolated from unbound virus in solution using a magnetic separator. The level of infectious virus bound to the PGM-MB and remaining unbound in solution was determined by viral plaque assay.

Results: The level of TV bound to PGM-MB was determined to be 5.52×10^5 PFU/ml. The level of TV remaining unbound in solution was lower, at a titer of 4.22×10^5 PFU/ml. This correlates to 27.6% of the TV binding to the PGM-MB and 21.1% remaining in solution, with an approximate 51% loss of virus during the assay. The level of MNV detected bound to PGM-MBs was 1.94×10^4 PFU/ml and unbound in solution was 8.37×10^4 PFU/ml. These titers were lower than the starting titer of MNV used in the assay.

Significance: Results from this work indicate that the PGM-MB method may not be appropriate for use to assess HuNoV binding specifically to HBGA. Similar levels of virus were bound to PGM-MBs and found free in solution. This may indicate non-specific binding of both TV and MNV to the PGM-MB.

P1-104 Evaluation of a Hand Sanitizer for Evidence of Residual Activity against Human Norovirus

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Introduction: Hands of infected food workers are a major route of norovirus contamination to foods, particularly for RTE products. Effective hand hygiene, including hand sanitizers, can help interrupt human norovirus (HuNoV) transmission.

Purpose: To study the immediate and residual antiviral efficacy against HuNoV of a commercial hand sanitizer (Ultra GermFree24, Zoono USA, LLC, NJ) claiming residual antibacterial activity.

Methods: A 20% suspension of HuNoV GII.4 Sydney was used as inoculum. In Phase I, *in vitro* studies were done by suspension assay (ASTM E1052) and *in vivo* studies using the fingerpad method (ASTM E1838) on human volunteers (*n*=5). Exposure time was 60 s. The fingerpad method was modified in Phase II studies to evaluate residual antiviral activity. Briefly, sanitizer was applied to hands, allowed to dry, and then fingerpads were inoculated with HuNoV 5 min, 4, or 8 h post-application. After inoculum drying, the remaining virus was eluted. Virus concentrations in treatment and control eluates were determined as genome equivalent copies (GEC) as evaluated by RT-qPCR preceded by RNase treatment.

Results: In Phase I *in vitro* assays, the sanitizer was more effective than the benchmark control (60% ethanol), producing 2.5±0.02 log GEC versus 0.9±0.04 log GEC reduction, respectively (P<0.05). The product was also more efficacious compared to the benchmark in *in vivo* fingerpad assays (2.8±0.4 log GEC versus 1.7±0.5 log GEC reduction; P<0.05). For Phase II (residual activity of sanitizer post-application), a 1.3±0.4, 0.9±0.3, and 0.7±0.04-log GEC reduction was observed 5 min, 4, and 8 h after product application, respectively. These numbers represented an additional 0.25- to 1.0-log GEC reduction compared to the dry (no product pre-treatment) control.

Significance: The hand sanitizer product showed evidence of anti-noroviral efficacy on fingerpads and some residual activity up to 8 h post-application, a potentially unique feature for hand decontamination using a hand sanitizer.

P1-105 Dual Transcriptomic and Metabolomic Profiling of *Toxoplasma gondii* Infection Uncovers Parasite Manipulation of the Host Metabolome and the Discovery of a Novel Parasite Metabolic Capability

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Introduction: Toxoplasma gondii is one of the most common human parasites in the world. The Centers for Disease Control and Prevention classifies *Toxoplasma* as a leading cause of foodborne illness in the United States, accounting for 24% of deaths and 8% of hospitalizations caused by food contamination. A potential source for new therapeutics is the metabolic deficiencies of *Toxoplasma* because it is an obligate intracellular parasite.

Purpose: Our goal was to characterize the joint metabolome of *Toxoplasma* and its host cell during infection to better understand how the parasite changes its environment for optimal replication. A greater understanding of how infection changes the host metabolome will allow us to design host-directed metabolic therapies to starve the intracellular parasite.

Methods: Using high-pressure liquid chromatography and mass spectrometry, we have generated the joint host-parasite metabolome at nine time points over the 48-hour course of infection, compared to uninfected human fibroblast control cells. To address whether metabolic shifts are caused by the host or parasite, we sequenced the transcriptomes of *Toxoplasma* and the host over the same time period, which allows us to see which metabolic pathways are transcriptionally activated.

Results: Several areas of metabolism were more active during infection, including the tricarboxylic acid cycle, the pentose phosphate pathway, and nucleotide synthesis. These pathways were significantly more active during infection, as seen in the greater abundance of their metabolic intermediates and in the transcriptional activation of key regulatory steps.

Significance: This study has greatly expanded our understanding of how *Toxoplasma* optimizes the host cell's metabolism for infection. Using this foundation, we plan to repurpose existing host metabolism-targeted drugs for the treatment of *Toxoplasma* infection.

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P1-106 Evaluation of Pure Copper Surface for Inactivation of Human Norovirus Gii.4 Sydney by Porcine Gastric Mucin Binding Assay

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Introduction: Human noroviruses (HuNoVs) are the leading cause of foodborne illnesses. Copper is an effective antimicrobial agent and has been shown to inactivate many pathogens, including HuNoVs. Since it is difficult to cultivate HuNoVs, many inactivation studies use assays based on RT-qPCR to quantify the viruses. However, it is still unclear if assays based on RT-qPCR are able to accurately quantify infectious virus particles for copper treatments.

Purpose: This study evaluated the efficacy of pure copper surface for inactivation of the HuNoV GII.4 Sydney strain by porcine gastric mucin-conjugated magnetic beads (PGM-MB) binding assay followed by RT-qPCR (PGM-MB/PCR assay).

Methods: HuNoV GII.4 Sydney was inoculated and treated on copper coupons (100% copper) for 0, 2.5, 5, 10, 15, and 20 min. Stainless steel coupons (0% copper) were used for comparison, and the virus was inoculated on the coupons and treated for 0, 10, 20, and 40 min. The virus was eluted at the time points. Eluted virus was then treated by bovine pancreatic ribonuclease, bound by PGM-MB, and quantified by RT-qPCR for PGM-MB/PCR assay. Three replicates were included in each experiment.

Results: As assessed by PGM-MB/PCR, all replicates of copper treatments 10 min and over achieved more than 3-log reduction (maximum detectable reduction) of the HuNoV strain. However, no more than 1.0±0.1-log reduction (P<0.05) was observed from all stainless steel surface treatments. A 10-min treatment on copper surface achieved a significantly higher inactivation of the HuNoV strain than 20- and 40-min treatments on stainless steel surface (P<0.05).

Significance: These results suggest that copper surface can rapidly inactivate the HuNoV GII.4 Sydney strain.

P1-107 Efficacy of Sodium Hypochlorite and Peroxyacetic Acid in Reducing Levels of a Human Norovirus Surrogate in Chinese Cabbage and Green Onion

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Introduction: Fresh vegetables can potentially be contaminated directly through contact with foodborne norovirus from irrigation water, organic fertilizer, and washing. Therefore, disinfecting water with sanitizers is important to avoid cross-contamination of fresh produce with norovirus. Sodium hypochlorite (NaOCl) and peroxyacetic acid (PAA) are commonly used as oxidizing disinfectants.

Purpose: Because Chinese cabbage and green onion are used after being briefly washed with water, a disinfecting wash needs to be applied to inactivate norovirus. To address this, we examined the efficacy of two disinfectants (NaOCl and PAA) against murine norovirus-1 (MNV-1) in fresh Chinese cabbage and green onion.

Methods: Stems and leaves of Chinese cabbage, and white and green portions of green onions were prepared and were spot-inoculated with 200 µL of MNV-1 (approximately 6 log PFU/mL). The virus-inoculated food samples were soaked into the manufactured disinfectant (NaOCl at 100 to 500 ppm or PAA at 50 to 500 ppm) for 1 min, and then infectious viral particles were quantified with a plaque assay. The physical (Hunter color and hardness) and sensorial quality (7-point hedonic scale) of food samples were performed to confirm how each treatment affected their overall quality.

Results: The MNV-1 titers inoculated on produce gradually decreased with the stepwise increase in chlorine concentration (100 to 500 ppm; 0.15 to 1.43 log in Chinese cabbage, 0.07 to 1.48 log in green onion) or PAA concentration (50 to 500 ppm; 0.20 to 1.53 log in Chinese cabbage, 0.38 to 0.58 log in green onion). NaOCl or PAA treatment up to 500 ppm did not affect the Hunter color values of the two vegetables. While NaOCl treatment gradually decreased the hardness values of Chinese cabbage stems, PAA had no adverse effects on this parameter.

Significance: This study suggests that PAA treatment at 300 ppm for 1 min was suitable for inactivating MNV-1 in Chinese cabbage and green onion and does not adversely affect food quality.

P1-108 Detection of Protozoan Parasites Endemic to Surface Irrigation Water Using Three Water and Biofilm Sampling Methods

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❖ ***Developing Scientist Competitor***

Introduction: Globalization has increased yearly produce availability in the U.S. Produce outbreaks in the U.S. include berries, green onions, and herbs imported from Mexico. Irrigation water is a primary route of produce contamination and traditional sanitizers do not effectively remove pathogenic protozoa from produce.

Purpose: The objective of this study is to evaluate and identify better sampling methods to detect protozoan pathogens in surface waters and to explore biofilms as an alternative source for parasite testing.

Methods: *Giardia lamblia, Cryptosporidium* spp., and *Cyclospora cayetanensis* were detected using three sampling methods (toothbrush, swab, and hollow-fiber filter) to sample 3 rivers in Mexico. A total of 590 samples were collected monthly (January 2017-March 2018) from 6 locations along the rivers before and after cities. *Cryptosporidium* spp. and *Cyclospora* were detected using nPCR targeting the 18S rRNA gene. *Giardia* was detected using nPCR targeting the triosephosphate isomerase (TPI) gene.

Results: Positive *Cyclospora, Cryptosporidium* spp., and *Giardia* samples were collected in 17.67%, 12.65%, and 11.03% of samples, respectively. Water sampling (n=62) was significantly associated with collecting more positive samples (P = 0.0013) than biofilm sampling (n=528). Collection of *Cyclospora* (P = 0.0008), *Cryptosporidium* spp. (P = 0.0017), and *Giardia* (P < 0.0001) was linked to the sampling method with water sampling more likely to collect each parasite than biofilm sampling. There was no significant difference between brush or swab sampling methods (P = 0.5670) for parasite collection.

Significance: Dollar store toothbrushes are a cost-effective and more available tool for farmers looking to test their irrigation water for protozoan parasites. Understanding the microbial quality of their available irrigation waters is necessary for farmers to reduce the likelihood of contaminating their produce. Furthermore, knowledge of parasite species and subtypes native to an area can enhance the efficacy of public health investigations after an outbreak. Future subtyping of the confirmed *Cyclospora cayetanensis* isolates will provide information on subtypes native to Mexico.

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P1-109 Characteristics of Cau-STP-1 Bacteriophage against *Salmonella enterica* Serovar Typhimurium from Sewage in South Korea

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Introduction: Phages are suggested as promising alternatives to antibiotics to remedy bacterial diseases in humans, animals and food industry.

Purpose: This study investigated the characteristics of a lytic *Salmonella* Typhimurium bacteriophage capable of infection and inhibition of *Salmonella* Typhimurium strains.

Methods: In this study, a phage of *Salmonellla* Typhimurium was isolated from the public sewage facility in Anseong, South Korea. A total of seven characteristics were examined. The host range of the CAU-STP-1 was determined by spot tests using 34 different bacterial strains.

Results: The CAU-STP-1 phage was able to survive in a wide range of pH values between 3 and 12 and resistant at 90°C for 20 min. More than 50% of the phages were readily adsorbed into the host bacteria in 1 min. One-step growth kinetics showed that the latent period was less than 30 min, and the burst size was 197±5 PFU/cell for CAU-STP-1. Transmission electron microscopy revealed the bacteriophage belonged to the order *Siphoviridae*. The bacteriolytic activity of CAU-STP-1 at a multiplicity of infection 0.1, 1, and 10 indicated its efficiency for reducing bacterial growth.

Significance: The characteristics of the bacteriophage would be helpful in establishing a basis for adopting the application of the most effective bacteriophage treatment to control *Salmonella* Typhimurium.

P1-110 An Independent Evaluation of Novel Molecular Methods for the Detection of Hepatitis A Virus and Norovirus in Multi-component Foods and Dry Spices

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Introduction: It has been well-established that viruses are the leading cause of foodborne illness worldwide. Methodology for testing the food matrices most closely associated with outbreaks of hepatitis A virus and norovirus has been outlined in the recently released ISO 15216 standard. However, instances illustrating the persistence of these viruses in foods not encompassed by ISO standard are increasingly common, and customized viral extraction procedures may be necessary to improve recovery of the target viruses in these matrices.

Purpose: To evaluate the qualitative viral extraction methodologies outlined in ISO 15216-2 for use on food matrices not explicitly covered by the scope of the method, specifically dry spices (5 g) and multi-component foods (25 g), using the ceeramTools workflow.

Methods: A total of six matrices (three dry spices and three multi-component foods) were chosen based on their potential to challenge the ISO 15216-2 viral extraction and detection methods. Matrices chosen possessed dark coloration, high fat content, low pH, and/or low a_w. After inoculation with a mengovirus process control, viral particles were extracted using one or more modifications of the soft fruit/salad vegetable extraction protocol outlined in ISO 15216-2 to determine whether the >1.0% mengovirus recovery required for a valid assay could be achieved for that matrix.

Results: Mengovirus recoveries of >1.0% were able to be obtained for all test matrices analyzed through modification of the ISO 15216-2 and molecular detection assays.

Significance: The data from the study demonstrates that the ISO 15216-2 viral extraction methodology, specifically for soft fruits and salad vegetables, has the potential to be adapted to allow for extraction of hepatitis A virus and norovirus in challenging food matrices such as multi-component food matrices and dry spices using ceeramTools assays.

P1-111 Evaluation of Four Typing Strategies for *Cyclospora cayetanensis* Using Stool Samples from Past United States Outbreaks

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Introduction: Cyclospora cayetanensis is the causative agent of cyclosporiasis, a foodborne gastrointestinal illness associated with seasonal outbreaks in the United States (U.S.). Past outbreaks have been linked to fresh produce imported from countries where cyclosporiasis is endemic. However, outbreak investigations often fail to identify the contaminated food item, which makes it difficult to limit and prevent outbreaks. A robust molecular genotyping tool to aid in linking cases has yet to be developed.

Purpose: This study evaluated four typing strategies, based on 13 genetic markers, for their usefulness in discriminating cyclosporiasis outbreaks.

Methods: PCR amplification and Sanger sequencing were used to characterize the 13 genetic markers from 136 *Cyclospora*-positive stool samples collected during U.S outbreaks between 1997 and 2017. The four typing strategies were compared based on amplification rates, quality of the DNA sequences, and ability to discriminate isolates.

Results: A previously described microsatellite-based multilocus sequence typing method was not effective for typing cyclosporiasis cases due to the common occurrence of uninterpretable DNA sequences. Conversely, a highly variable region in the mitochondrial genome was successfully amplified and analyzed in 99% (134 of 136) of samples and separated the samples into 13 sequence types. Markers based on nuclear loci frequently exhibited evidence of intra-isolate heterozygosity (polymorphic sites). We developed a novel bioinformatic approach to analyze these heterozygous markers, which resolved 84% (78 of 88) of stool samples into clusters that agreed with available epidemiologic data. A typing method based on four nuclear protein-coding genes has so far been evaluated on 60 stool samples and shows similar typing results compared to the other methods but with a 23% (14 of 60) failure rate.

Significance: Three of the evaluated typing strategies displayed good amplification efficiency and enough sequence diversity to distinguish samples from separate epidemiologically implicated outbreaks. While further evaluation of these eight markers is required, they have potential as an efficient typing tool to improve cyclosporiasis outbreak investigations.

P1-112 Inactivation of Hepatitis A Virus on Strawberries and Blueberries by High-pressure Processing

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Introduction: Multiple outbreaks associated with foodborne viruses have occurred in recent years due to the consumption of contaminated berry products. High-pressure processing (HPP) has been recognized as a nonthermal processing technology for the food industry that is capable of inactivating viral pathogens while still retaining organoleptic quality.

Purpose: The objective of this study was to evaluate the application of HPP on inactivation of hepatitis A virus (HAV) on strawberries and blueberries.

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Methods: Fresh strawberries and blueberries (25 g) were spot-inoculated with HAV at approximately 4 log PFU/sample. Fresh and frozen berries were vacuum-sealed, packaged, and HPP treated at 200, 300, 400, 500, and 600 MPa for 3 min with an initial temperature at 4°C. After treatment, viruses were extracted and recovered from the samples and quantified by viral plaque assay.

Results: The initial inoculum level of fresh and frozen strawberries was 3.6, 3.5, 3.8, and 4.0 log PFU/sample, respectively. HPP treatment was effective in inactivating HAV in berries, and higher viral inactivation was observed as pressure levels increased. At 400 MPa, greater than 2-log reductions were achieved in strawberries. HAV was reduced to below detection level after 500 MPa treatment in both fresh and frozen strawberries. The inactivation was significantly lower in fresh and frozen blueberries, with 1.1- and 1.2-log reductions at 400 MPa, respectively.

Significance: Effective inactivation of HAV is achievable with HPP treatment for strawberries and blueberries. These results suggest HPP is a promising technology to improve microbial quality while retaining sensorial characteristics of berries.

P1-113 Verification of Thermo Scientific SureTect *Salmonella* Species PCR Assay on Dairy Matrices, Raw Ingredients, and Environmental Samples for an Accredited Laboratory

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Introduction: The Thermo Scientific SureTect Salmonella Species PCR Assay is a real-time PCR assay for the detection of salmonellae in food and environmental samples. The assay has been extensively tested and validated with over 20 different matrices from various categories including meat, dairy products, fish, fruit and vegetables, and production surfaces.

This study was performed in collaboration with a Finnish dairy company's accredited laboratory whose routine test method differs from the validated SureTect enrichment protocol; samples of ≤1kg are tested from a non-selective enrichment. The Thermo Scientific Food Protection Team verified the performance of the assay for the Finnish laboratory to show that the method can be applied as their routine test method.

Purpose: To verify performance of the assay with a range of dairy products, raw ingredients, and environmental samples.

Methods: A total of 45 different food and environmental samples in different sample sizes (25g to 1kg; 1–4 swabs) were artificially contaminated with three different *Salmonella* serotypes using two different spike levels. Samples were diluted 1/10 and enriched for 20 hours in Buffered Peptone Water (ISO). Post enrichment, samples were tested with the assay using the Applied Biosystems 7500Fast platform and confirmed using an RVS secondary enrichment and plating onto XLD medium.

Results: The assay was comparable to the confirmation method for the samples tested except for probiotic foods; modified enrichment with double-strength BPW greatly improved the detection resolved this. The assay reliably detected the presence of *Salmonella* in the selected matrices.

Significance: The study proved that the SureTect Salmonella method offers a reliable workflow for the detection of *Salmonella* in dairy products, raw ingredients, and environmental samples from different sample sizes, and can be applied as a routine test method for the laboratory.

P1-114 Rational Design of Bacteriophage-based Antimicrobial to Eliminate the Formation of Bacteriophage Insensitive Mutants

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Introduction: The use of bacteriophages as antimicrobials in foods can be affected by the appearance of bacteriophage insensitive mutants (BIMs), which decrease antimicrobial activity.

Purpose: The objective of this study was to characterize the formation of BIMs in order to enhance the activity of phage-based antimicrobials.

Methods: Phage T4 and its host *Escherichia coli* B were used as a model system. Six BIMs were isolated by incubating suspensions of the bacteria with 10-fold dilutions of phage T4. BIMs were characterized by whole genome sequencing, and permeability of the outer membrane (OM) to different antibiotic and non-antibiotic compounds was assessed by determining the MIC and uptake of plasmid DNA (via transformation) in comparison to the wild type.

Results: Compared to the wild type, all BIMs showed mutations in genes *WaaG* (a 18bp deletion) and *WaaO* (a 21bp insertion and a 11bp substitution), which encode glucosyltransferases that add glucose residues to the outer core of lipopolysaccharides (LPS), suggesting that the LPS of the BIMs may be truncated. All BIMs were highly sensitive to sub-therapeutic levels of sodium dodecyl sulfate (SDS, MIC<4 mg/ml) compared to wild type (MIC=200 mg/ml) and also had significantly lower MICs to kanamycin, ampicillin, and polymyxin B; they were also more permeable to plasmid uptake than the wild type (P<0.05), implying increased permeability of the OM. Sensitivity to SDS implied that a combination of SDS at sub-therapeutic concentrations and phage T4 might decrease BIM formation. No BIMs were recovered in broth culture after 18 h incubation with T4 and SDS (10 mg/ml) and an 8-log reduction in *E. coli* B bacterial concentration was observed, in contrast to incubation with just phage T4 or SDS, which led to BIM formation and no bacterial reductions.

Significance: Combination of T4 phage with a sub-therapeutic concentration of an anionic surfactant enhances the antimicrobial activity of phages and reduces the chances of BIM formation.

P1-115 Parasitic Inactivation in Processed Food

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Introduction: Foodborne parasitic disease outbreaks are on the rise. In 2017, there were 1,065 cases of cyclosporiasis (an illness caused by the protozoan parasite *Cyclospora cayetanensis*). There was no specific transmission vehicle identified in these cases. Although fresh produce is often implicated as the vehicle for transmission of *Cyclospora cayetanensis*, and both *Cyclospora* and other protozoans such as *Cryptosporidium* have been isolated on fresh produce, the processed food industry should not be considered immune to this threat.

Purpose: Consumer preferences towards minimally processed foods could push more fresh ingredient usage in the processed food category, increasing the likelihood of contamination with parasitic pathogens. Although current processing guidelines control for vegetative and spore forming pathogens, these have not been validated against parasitic organisms.

Methods: This study reviewed current thermal processing control guidance for vegetative pathogens, and accepted industry wide practices and compared this against literature for deactivation temperatures for the protozoan parasites *Cryptosporidium* and *Cyclospora*. Few studies have looked at thermal inactivation for either *Cryptosporidium* or *Cyclospora* and those that have do not have clear guidelines for inactivation in complicated food matrices found in this food category. The limited data available for parasitic inactivation was used to draft recommendations and guidance as to how the processed food industry can address this ever-growing threat.

Results: Per published literature, example guidance for a 6D inactivation for the foodborne pathogen *Listeria monocytogenes* is not adequate to insure deactivation of *Cyclospora* in water, let alone food matrices such as yogurts, smoothies, or frozen entrées. Current heat cooking models also may not be adequate for *Cryptosporidium* deactivation in complex matrices.

Significance: More research is needed to determine adequate deactivation for these parasites in different matrices, and processed food companies should ensure adequate treatment of fresh produce before addition to a processed food product.

P1-116 Effect of Vinegar on the Viability of *Cryptosporidium parvum* Oocysts

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Introduction: Cryptosporidiosis can be acquired by ingestion of contaminated food and water containing viable *Cryptosporidium* oocysts. The oocysts can be found in food, water, soil, and on surfaces that have been in contact with the fecal matter of infected humans and animals. Vinegar is a common sanitizer that is easily accessible and used worldwide to reduce bacterial loads in foods and surfaces.

Purpose: To evaluate the effect of three types of commercial vinegars on the viability on *Cryptosporidium parvum* oocysts.

Methods: Three types of vinegar (wine vinegar, rice vinegar, and cleaning vinegar with pH values ranging from 2.0 to 3.02) were tested to determine their effect on the viability of *Cryptosporidium* oocysts. Oocysts were collected from a local dairy farm, filtered, and incubated at 23°C for 5, 10, and 15 min with the three types of vinegar. The treatment was stopped using F12 medium. A second treatment was conducted, followed by 1.4% chlorine for 10 min at 23°C. The treatment was then stopped and the preparation examined by *in vitro* testing for *Cryptosporidium* using HCT-8 cells and for fecal coliforms using tryptic soy agar plates. Experiments were done in triplicate and repeated twice.

Results: Rice vinegar, cleaning, and wine vinegars killed 3 log CFUs/ml of fecal bacteria at all treatment times. If treatment included the second incubation with chlorine, the reduction varied from 6 to 9 CFU/ml. *Cryptosporidium* oocysts were not affected by either treatments as demonstrated by *in vitro* testing.

Significance: Cryptosporidium oocysts are highly resistant to vinegars and chlorine treatments, even at the extended incubation times of 15 and 10 min, respectively.

P1-117 Factors Affecting the Virucidal Efficacy of Cold Plasma against Hunov as Compared to Its Surrogate, Feline Calicivirus

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❖ ***Developing Scientist Competitor***

Introduction: Cold atmospheric gaseous plasma (CAP) is a powerful technology for decontamination of foodborne bacterial and viral pathogens. Recently we reported >5-log reduction in $TCID_{50}$ of feline calicivirus (FCV) on stainless steel discs and romaine lettuce leaves after 3 min wet exposure to air plasma generated by a novel 2D air-based dielectric barrier discharge cold microdischarge plasma array (2D-APMA). However, when human norovirus (HuNoV GII-4) was treated for 5 min under the same conditions, only ~2.6 log genome copy reduction was observed as measured by ethidium monoazide bromide (EMA)-coupled RT-qPCR (EMA-RT-qPCR). To investigate the reasons for this difference, two hypotheses were formulated: i) the presence of organic fecal impurities in the HuNoV sample competes with the viral particles to interact with CAP-reactive species, and ii) the EMA-RT-qPCR method underestimates viral inactivation.

Purpose: This work was done to test the above two hypotheses to explain the differences between virucidal efficacy of CAP against FCV and HuNoV GII-4.

Methods: Inactivation of FCV by CAP as determined by cell culture method was compared to inactivation of FCV and HuNoV-GII-4 as determined by EMA-RT-qPCR method. In addition, CAP inactivation of FCV suspended in distilled water (free of fecal impurities) was compared to that of FCV suspended in fecal extract, mimicking the conditions in which HuNoV was treated.

Results: The inactivation of FCV determined by cell culture method was higher than that determined by the EMA-RT-qPCR method for the same sample, indicating that the latter method (used for HuNoV titration) underestimates the amount of virus inactivated. We also found that fecal impurities present in HuNoV samples partially suppress the virucidal activity of CAP.

Significance: The results indicate that our 2D-APMA is a promising plasma setup for decontaminating food and food contact surfaces of HuNoVs.

P1-118 Assessment of Virulence Using a *Galleria mellonella* Model for *Listeria monocytogenes* Grown in Different Foods

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Introduction: Listeria monocytogenes associated with different foods, including cantaloupe, caramel-coated apples, and packaged salads, have been linked to listeriosis outbreaks. Our knowledge regarding the effect of food type on *L. monocytogenes* virulence is limited. Understanding the roles of different foods in modulating the virulence of *L. monocytogenes* can be useful in risk assessments and for developing control measures.

Purpose: We evaluated the impact of different foods on the virulence of *L. monocytogenes* using the greater wax moth *Galleria mellonella* model.

Methods: Fifteen strains of *L. monocytogenes* related to listeriosis outbreaks were evaluated using the non-human pathogen *Listeria innocua* as a control. Strains were inoculated in parallel in brain heart infusion (BHI) broth and on the surfaces of cantaloupe and apple fragments at 10^5 CFU/fragment. Following a five-day incubation at 10°C, *L. monocytogenes* was recovered from the fruits and BHI, washed twice, and used to inoculate *G. mellonella* at 10^6 and 10^5 CFU/larva. The larvae were incubated at 37°C and monitored for mortality (LT_{50} = time taken to kill 50% of the larvae) and phenotypic changes over seven days.

Results: Differences in larva mortality after infection with *L. monocytogenes* were dose- and growth-matrix dependent. *L. monocytogenes* grown on fruit revealed higher virulence than when grown in BHI. Statistical significance of these differences was strain-related. *L. monocytogenes* infection doses of 10^6 CFU/larva resulted in an LT_{50} of ≤30, 54, and 72 h on cantaloupe, apples, and BHI, respectively. These results represent a 2.5- to 4-fold increase in mortality compared with an LT_{50} ≥120 h in larvae infected with the same doses of *L. innocua* grown in corresponding matrices. Similar trends were also recorded at the infection dose of 10^5 CFU/larvae.

Significance: In the *Galleria mellonella* insect larvae model, cantaloupe-grown and apple-grown *L. monocytogenes* resulted in higher virulence than *L. monocytogenes* grown in BHI, demonstrating the role of the food matrix in virulence.

Poster

P1-119 Independent Performance Evaluation of a Real-time PCR for the Detection of *Salmonella* in Poultry Primary Production Samples

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Introduction: Despite several interventions by the poultry industry, *Salmonella* prevalence in poultry products and the disease burden due to *Salmonella* remains a persistent issue. In line with certain European Union countries, it has been recently hypothesized that (in the United States) increasing the *Salmonella* test frequency and interventions at the primary production sites prior to poultry processing may further reduce the *Salmonella* load on processed poultry meat products. Although the efficacy of this practice is being debated, the need for sensitive and rapid methods for *Salmonella* detection is clearly understood. A number of commercial DNA detection-based systems are available; however, for the most part, they remain burdened with cumbersome sample preparation and complex instrumentation and interpretation tools.

Purpose: The purpose of this study was to independently evaluate the performance of GENE-UP based *Salmonella* (SLM) assay for the detection of *Salmonella* spp. in poultry primary production samples (PPS).

Methods: In total, 180 PPS were tested: unpaired (*n*=85) were tested against a tetrathionate and modified semisolid Rappaport-Vassiliadis (TT/MSRV) method, while separate unpaired (*n*=95) were tested against the buffered peptone water (BPW) method in accordance with the United States Department of Agriculture Poultry Improvement Plan standards. For SLM, the PPS were enriched in BPW (1:10), followed by incubation at 42°C for 18 to 22 h. All presumptive results were confirmed with their respective culture methods, i.e., TT/MSRV or BPW methods. The 2×2 contingency table analysis was performed to compute the sensitivity and specificity with a 95% confidence interval.

Results: SLM sensitivity was 100% (True positive (TP)=30; False negative (FN)= 0) and 97.62% (TP=41, FN=1), and the specificity was 96% (True negative (TN)=53, False positive (FP)=2) and 94.34% (TN=50, FP=3) against the TT/MSRV and BPW methods, respectively. The overall SLM accuracy was ~96%.

Significance: SLM provides, i) highly sensitive and comparable results to the culture methods and ii) a significant time advantage (~48 h) over the culture methods, and thereby presents a viable alternative for *Salmonella* detection in PPS.

P1-120 Performance Feasibility of Ceeramtools Hepatis A and Norovirus GI/Gii Kits

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Introduction: Foodborne illnesses related to virus outbreaks are steadily increasing, along with the need for rapid methods of detection. ceeramTools virus detection kits provide a standardized method that is compliant with ISO 15216-1 and ISO 15216-2 and meets this food safety need.

Purpose: The purpose of this study was to evaluate the performance of the hepatitis A and norovirus GI and GII duplex kits in berries.

Methods: This feasibility consisted of a five replicate study at two levels for all three virus targets. The matrix tested was strawberries. All samples and levels were analyzed by both the ISO method and the candidate method. The strawberries were spiked at the BIPEA Proficiency Testing organization and shipped to the Silliker Food Safety Center for analysis. The viral levels assigned from BIPEA for each target were 1,968 and 984 (log [µg/g]) for norovirus GI; 2,788 and 1,394 (log [µg/g]) for GII; and 1,969 and 935 (log [µg/g]) for hepatitis A. The standard berry protocol for both methods was followed for testing.

Results: For hepatitis A, nine of the replicate samples were positive by both the candidate and ISO method. For norovirus GI, nine of the replicates samples were positive by the candidate method, with 10 samples positive for the reference method. The retained extract for the single negative sample was tested again in duplicate and was positive for both retest PCR runs. For norovirus GII, all test replicates were positive by both methods.

Significance: These data suggest that the candidate method and ISO method are equivalent for the recovery of viruses in strawberries. This feasibility supports the use of ceeramTools for the detection of hepatitis A and norovirus GI and GII in berries.

P1-121 Photodynamic Inactivation of Hepatitis A Virus on a Contact Surface Mediated by Grapeseed Extract and Light

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❖ ***Developing Scientist Competitor***

Introduction: Hepatitis A virus (HAV) is an epidemiologically significant foodborne virus that continues to spread worldwide. Novel inactivation strategies are being researched for HAV control. Photodynamic inactivation of microorganisms (PDI) that occurs in the presence of oxygen (air), photosensitizers, and light to produce reactive oxygen species are being researched to control foodborne pathogens. Grapeseed extract (GSE), a natural antimicrobial rich in polyphenols, has potential to be used in microbial PDI.

Purpose: The purpose of this study was to determine the effectiveness of GSE-mediated PDI to decrease HAV titers.

Methods: HAV (100 µl; 6-log PFU/ml) was aseptically dried for 10 min on sterile formica coupons in sterile petri dishes and treated with equal volumes of GSE (10 mg/ml) or phosphate buffered saline (pH 7.2; control). This was followed by either ultraviolet (UV; 254 nM) light treatment, or no treatment for 0, 1, 3, 5, 15, and 20 min in a biosafety hood. At each time point, HAV was eluted using cell culture media containing 8% newborn calf serum and serially diluted 10-fold. Viruses were enumerated by infecting confluent host fetal rhesus kidney-4 cells in six-well plates. Each treatment in duplicate was replicated thrice, and data were statistically analyzed.

Results: GSE-mediated PDI decreased HAV titers by 1.18±0.68, 1.6±0.51, 2.03±0.42, and 2.34±0.54 log PFU after 1, 3, 5, and 15 min, respectively, and to non-detectable levels after 20 min. UV treatment alone decreased HAV titers by 1.69±0.58, 1.13±0.47, 1.47±0.83, 1.05±0.34 and 1.72±0.77 log PFU after 1, 3, 5, 15, and 20 min, respectively. Lower HAV titer reduction was obtained with 10 mg/ml GSE alone, ranging from 0.11±0.48 to 1.38±0.81 log PFU reduction after 1 to 20 min.

Significance: GSE-mediated PDI showed slighter higher HAV titer reduction after 20 min than UV or GSE treatment alone. Thus, GSE-mediated PDI shows promise as a surface decontamination technology to reduce HAV transmission.

Poster

P1-122 Internalization of Murine Norovirus in *Pseudomonas aeruginosa* Biofilm

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Introduction: Biofilms are defined as cohesion of bacterial entrapments within a highly hydrated matrix of extracellular polymer substances. There are well-known to protect bacteria, including pathogenic varieties, against antimicrobial substances and adverse conditions. Released in the environment, enteric viruses such as norovirus may be captured in bacterial biofilms.

Purpose: The objective of this work was to study the internalization of murine norovirus (MNV) in *Pseudomonas aeruginosa* biofilm grown in a dynamic bioreactor system.

Methods: P. aeruginosa (ATCC 15442) biofilms were produced on polypropylene and stainless steel coupons with high shear and continuous flow using a CDC bioreactor according to the ASTM E2562-12 method. A concentration of 8 log genome-copies/ml of MNV was used to contaminate biofilms for 24 h with the same debit. The MNV was added to the biofilm at two moments in its formation: i) after six days of biofilm formation, or ii) after the batch step during the first 24 h of the biofilm formation. Bacterial and viral counts, as well as protein and sugar contents, were determined in duplicate for each assay.

Results: After a contact of 24 h with the six-day biofilm, 7.5 log (genome-copies/cm^2) of MNV were detected. In contrast, 5 log (genome-copies/cm^2) of MNV were determined after one week of biofilm formation when MNV was introduced at an early stage of the biofilm formation. These results suggest that developing biofilm may progressively embed MNV particles and release them over time, while already existing biofilm may internalize them by absorption into the matrix. No significant difference in the bacterial and the viral counts was observed for both tested surfaces.

Significance: According to our results, bacterial biofilms may contribute to internalization, protection, and release of enteric viruses in the environment.

P1-123 A Comparison of the Prevalence of Protozoan Parasites in Potential Alternative Sources of Agricultural Water

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❖ ***Developing Scientist Competitor***

Introduction: According to the 2010 United States (U.S.) Geological Survey, agriculture accounts for 80 to 90% of the U.S. consumptive water use, and 38% of total fresh water withdrawals are used for irrigation alone. Climate change presents numerous challenges to U.S. agriculture, especially in terms of fresh water availability. It may become necessary to utilize non-traditional or alternative sources of agricultural water to meet the needs of the nation. To determine the feasibility of utilizing non-traditional water sources, it is essential to evaluate associated food safety risks.

Protozoa are associated with drinking and recreational water and have been isolated from lakes, rivers, and reservoirs, further substantiating the need to include protozoa in food safety considerations and evaluation of non-traditional water sources.

Purpose: To evaluate the prevalence of protozoan parasites in potential alternative sources of agricultural water.

Methods: Water samples (*n*=11) were collected (July 2017 to October 2017) from tidal brackish water, vegetable processing, and wastewater treatment facilities, and 10 to 20 L were filtered using an Envirochek HV Capsule at a filtration rate of 2 L/min. Filters were eluted according to the EPA 1623 modified method. The DNeasy Power Water Extraction Kit was used and qPCR was performed using the QuantiNova Probe Assay Kit and confirmed by gel analysis.

Results: For *Cryptosporidium parvum,* 54% (*n*=6) of water samples tested positive. Each water type, 75% (*n*=3) of wastewater, 50% (*n*=2) of tidal brackish, and 33.33% (*n*=1) of vegetable processing, tested positive. For *Cyclospora cayetanensis,* 72% (*n*=8) of the water samples tested positive. For each water type, 100% (*n*=4) of wastewater, 100% (*n*=4) of tidal brackish, and 0% of vegetable processing water tested positive. There were no statistically significant differences amongst water types for presence of protozoa (*P*>0.05).

Significance: Understanding the prevalence of protozoan pathogens in alternative agricultural waters will enable the establishment of on-farm solutions and subsequent safe use of these waters for irrigation to reduce impending agricultural water challenges resulting from climate change.

P1-124 The Use of Pulsed Light to Inactivate *Cryptosporidium parvum* oocysts on Mesclun Lettuce

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❖ ***Developing Scientist Competitor***

Introduction: The Centers for Disease Control and Prevention recently reported a 45% increase in the number of foodborne infections caused by *Cryptosporidium* spp. from 2013 to 2016. There is growing evidence to suggest protozoa are becoming more common contaminants of fresh produce and herbs, emphasizing the need for novel interventions to prevent and manage contamination.

Purpose: This study examined the effects of pulsed light on the viability of *Cryptosporidium parvum* oocysts on the surface of mesclun lettuce.

Methods: Oocysts (1.56×10^6/per 0.125 mL phosphate buffered saline [PBS]) were applied to glass slides (control) or mesclun (0.5 g) in duplicate (*n*=4). Samples were air-dried for 60 min and treated with pulsed light for 0, 10, 45, and 90 s. Samples were processed with a modified U.S. Food and Drug Administration Bacteriological Analytical Manual methodology and final resuspension in 1 mL PBS, of which 0.8 mL was added to confluent HCT-8 cells in a six-well plate. After 60 min, inoculum was removed and replaced with fresh cell media. Following incubation at 37°C for 48 h, DNA was extracted and *Cryptosporidium* detected by qPCR. Data were analyzed using one-way analysis of variance. Microscopy was used in conjunction with integrated cell culture-qPCR detection.

Poster

Results: Overall, statistically significant differences existed between the infectivity of treated oocysts on mesclun for all treatment times compared to the 0-s mesclun control (P<0.0001). A 2.3-log reduction in infectivity was detected between oocysts treated on mesclun after 0 and 90 s of treatment compared to a 2.74-log reduction between 0 and 45 s. Overall, for the oocysts treated on glass slides, there was a ~1.72-log reduction between 0 and 90 s and a ~2.08-log reduction between 0 and 45 s. The infectivity of oocysts treated on the mesclun was significantly higher than the infectivity of oocysts treated on glass slides (P<0.001).

Significance: The data suggest that pulsed light treatment effects *C. parvum* oocyst viability without major changes in the physical appearance of mesclun lettuce.

P1-125 Application of High-pressure Processing for Inactivation of Norovirus and Quality Stability in Fresh Sea Squirt (*Halocynthia roretzi*)

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Introduction: Sea squirt (*Halocynthia roretzi*) is a tunicate and edible ascidian consumed primarily in Korea and Japan. Sea squirt is considered a potential cause of human norovirus (NoV) in Korea.

Purpose: This study investigated the effect of high-pressure processing (HPP) at 100 to 500 MPa for 5 min on the inactivation of murine norovirus-1 (MNV-1, initial inoculum of 6 to 7 log PFU/ml) as a human NoV surrogate in fresh sea squirt. The effects of HPP on Hunter colors and pH were also examined as the main indices of quality.

Methods: A 500-µL aliquot of MNV-1 as a human NoV surrogate (6 to 7 log PFU/mL) was spot inoculated onto the meat surface of sea squirt. For the HPP treatments, the samples were pressurized for 5 min (holding time) to the respective pressures of 100, 200, 300, 400, and 500 MPa. Viral titers were calculated as the number of PFU per mL. Hunter colors of "L" (lightness), "a" (redness +, greenness –), and "b" (yellowness +, blueness –) and pH were determined in HPP-treated samples.

Results: No reductions in MNV-1 titers were observed in sea squirt treated at 100 to 400 MPa. However, MNV-1 in sea squirt was completely inactivated by 500 MPa of HPP. Furthermore, the Hunter colors ("L", "a", and "b") and pH values (6.10 to 6.19) were not significantly (P>0.05) different between non-HPP treated sea squirts and all HPP-treated sea squirts.

Significance: This study suggests that 500 MPa of HPP may be an optimal treatment for tunicate meat without altering the color, pH, or food quality.

P1-126 Synergistic Effects of Chlorine and Thiamine Dilauryl Sulfate Combination on the Reduction of Norovirus Titers in Raw Shucked Oyster (*Crassostrea gigas*)

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Introduction: Human norovirus (NoV) can persist in oysters for weeks and cannot be effectively removed during commercial depuration. Fresh oysters are most commonly consumed in the United States, Korea, and Japan.

Purpose: This study investigated the synergistic effects of combined chlorine and thiamine dilauryl sulfate (TDS) on the reduction of MNV-1 as a NoV surrogate in fresh shucked oyster and evaluate the overall quality (pH, texture, and sensory attributes) of the treated oysters.

Methods: A 200-µL aliquot of MNV-1 as a human NoV surrogate (6 to 7 log PFU/mL) was spot inoculated onto the meat surface and gills of oyster. For the combined treatments, the chlorine (200, 500, 700, or 1,000 ppm) was first conducted as a primary disinfectant, and the TDS (1,000, 2,000, or 3,000 ppm) followed immediately as a secondary disinfectant. Viral titers were calculated as the number of PFU per mL. Texture, pH, and sensory evaluations were performed.

Results: The combined treatment of chlorine and TDS resulted in a 0.52- to 1.97-log-reduction of MNV-1. The synergistic reduction in MNV was not dependent on the concentrations of chlorine and TDS, and it ranged between 0.08 and 1.03 log PFU/mL. The largest synergistic reduction observed was for the combined 700 ppm chlorine and 1,000 ppm TDS. The pH and instrument-measured texture parameters of the oysters were not significantly changed by the combined 0 to 1,000 ppm chlorine and 3,000 ppm TDS. The overall sensory acceptability was significantly reduced in oysters treated with 1,000 ppm chlorine and 3,000 ppm TDS than in those treated with 0 to 700 ppm chlorine and 3,000 ppm TDS.

Significance: The combined 700 ppm chlorine and 3,000 ppm TDS could be used to reduce NoV in fresh shucked oysters without changes in the mechanical texture, pH, or sensory qualities of the oysters.

P1-127 Decontamination of Cattle Carcasses by a Commercial Steam Vacuuming Treatment Implemented after Slaughtering in a Cattle Abattoir

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Introduction: Complete prevention of carcass contamination during slaughter is nearly impossible to accomplis. Thus, there is increasing interest in effective decontamination treatments for carcasses. With regard to steam vacuuming, only a few studies have examined the antimicrobial effects under commercial conditions and for naturally contaminated cattle carcasses.

Purpose: The aim of the present study was to evaluate the effect of a newly implemented steam vacuuming system (Vapo-Vac System, Industrade, Strasbourg Cedex) on the microbial contamination of cattle carcasses during routine operations.

Methods: A total of 105 cattle carcasses (calves, feeder cattle, cows) were sampled by excision (5 cm^2) at four different areas (perineal area, rump, brisket, shoulder), both (i) after trimming (just before steam vacuuming) and (ii) after steam vacuuming. After homogenization (0.85% saline solution), each sample was examined (ISO 4833-1:2013) for total viable counts (TVC). Differences in TVC before and after steam vacuuming were analyzed by analysis of variance and Tukey's HSD test.

Results: Before steam vacuuming, mean TVC were comparable at the perineal area and brisket (3.0 to 3.1 log CFU cm^{-2}) or the hind leg and shoulder (2.6 to 2.7 log CFU cm^{-2}). Steam vacuuming reduced mean TVC by 0.9, 0.7, 0.6, and 0.4 log CFU cm^{-2} at the perineal area, hind leg, shoulder, and brisket, respectively. TVC from respective areas thereby differed significantly before and after steam vacuuming (P<0.05). With regard to the distribution of TVC, steam vacuuming increased the proportion of results <3.0 log CFU cm^{-2} from 75 (63 to 88% at carcass areas) to 87% (71 to 97% at carcass areas).

Significance: Steam vacuuming after slaughtering can be a useful tool for the reduction of contaminations in designated carcass areas, but the effect must not be overestimated. Decontamination treatments cannot replace slaughter hygiene practices and must always be seen as part of an integral food safety system.

P1-128 Establishment of Culture Method for the Detection of *Clostridium difficile* in Meat Samples

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❖ ***Developing Scientist Competitor***

Introduction: Clostridium difficile have been recognized as the main cause of antibiotic-associated diarrhea in humans. Emerging epidemiological studies have shown the evidence of endemic *C. difficile* infection (CDI), for which foods, especially meat products, can be a vehicle. To investigate the prevalence of *C. difficile* in various foods, each study has employed several culture media due to the lack of standard culture methods for this bacterium in foods.

Purpose: The purpose of this study is to evaluate enrichment and selective media for detecting *C. difficile* and, ultimately, to establish the most effective culture detection method in meat samples.

Methods: A total of six enrichment media (cycloserine-cefoxitin fructose broth [CCFB], cycloserine-cefoxitin mannitol broth [CCMB], *Clostridium difficile* moxalactam norfloxacin broth [CDMNB], CCFB with sodium taurocholate and lysozyme [TAL], CCMB-TAL, and CDMNB-TAL) and four selective media (cycloserine-cefoxitin fructose agar [CCFA], CDMN agar, cycloserine cefoxitin egg yolk agar [CCEY], and chromID *C. difficile* agar) were compared for their enriching and recovering performance, respectively. To test the sensitivity and specificity, a total of 160 artificially inoculated meat samples were tested with the newly established detection method.

Results: CDMNB-TAL was the best medium and showed the highest capability of lessening enrichment time to reach the maximum growth level (24 h to 8 log CFU, P<0.05). For selective media, ChromID *C. difficile agar* generated the most colonies among four media (P<0.05). The detection limit of this method was 2.9×10^3 CFU/mL.

Significance: The results showed 99.0% sensitivity and 89.5% selectivity, indicating the novel method could be applied to investigate the prevalence of *C. difficile* in meat samples.

P1-129 Co-Regulation of Fumonisin Risk in the Texas High Plains

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Introduction: Co-regulation as a form of risk management relies upon a government-private regulatory partnership that uses government-backed codes of practice or action plans. In Texas, implementation of co-regulation to manage aflatoxin risk has been practiced since 2011 and the strategy to manage fumonisin risk was first utilized in the Texas High Plains beginning in 2017 to manage disposition of maize containing greater than 60 ppm fumonisin from the 2016 harvest and subsequently applied during the 2017 harvest.

Purpose: The present study evaluates implementation and effectiveness of the co-regulation program to improve fumonisin risk management in Texas.

Methods: A total of seven laboratories in the Texas High Plains participated in the program during the 2017 harvest, servicing 23 grain elevators. The implementation of the co-regulation program included an evaluation of the firms' food safety plans, onsite qualification of the firms' analysts, monitoring the firms' performance during harvest, and verification of 592 samples from participating firms' satellite laboratory results by the OTSC ISO 17025 accredited laboratory in College Station, Texas.

Results: Samples collected during the 2017 harvest reveal that 17% of the harvest maize was below 5 ppm, the lowest regulatory limit for animal feed. Maize containing greater than 60 ppm must be subject to a blend plan and 33% of the Texas High Plains maize contained fumonisin contamination levels exceeding 60 ppm. The 592 fumonisin verification samples analyzed by OTSC were correlated (r=0.86) with co-regulation laboratories. An operating curve using a 4th order polynomial model yielded a coefficient of determination (R^2) of 0.999 indicating that it explains 99.9% of the variability in analytical variation between commercial laboratories and OTSC. The cumulative probability of type I and type II error was 15%.

Significance: The implementation of co-regulation upon a quality system based code of practice successfully managed aflatoxin risk in Texas using co-regulation as a governance option.

P1-130 Assessment of Contaminants in Cottonseed and Rice Following Hurricane Harvey

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Introduction: In the aftermath of Hurricane Harvey, rice and cottonseed samples were analyzed for contaminants from flooded areas in Texas by the Office of the Texas State Chemist (OTSC).

Purpose: Associations representing producers, grain handlers, and processors worked with OTSC to facilitate recovery from potential damage associated with Hurricane Harvey. Damaged grain is defined as feed under the Texas Commercial Feed Control Act, which is administered by the Texas Feed and Fertilizer Control Service (FFCS) housed within OTSC.

Methods: The Agricultural Analytical Service of OTSC assisted farmers in assessing damage by providing testing service through their ISO 17025-accredited laboratory for contaminants of concern listed in the U.S. Food and Drug Administration's (FDA) guidance for industry titled *Evaluating the Safety for Flood-affected Food Crops for Human Consumption*. This response was part of an activation of the All Hazards Rapid Response Team involving the FDA, Department of State Health Services, and OTSC following Hurricane Harvey. FFCS field investigators, who are also credentialed FDA officers, collected sterile samples and contaminant samples using official sampling and chain of custody procedures.

Results: One hundred thirty three (133) rice samples were tested for contaminants listed in an FDA guidance for industry titled *Evaluating the Safety for Flood-affected Food Crops for Human Consumption* including mycotoxins (aflatoxin, fumonisin, vomitoxin (DON), ochratoxin, and patulin), heavy metals (cadmium, mercury, lead, and arsenic), microbiological hazards of significant public health impact including human pathogens (e.g., *Salmonella*, *E. Coli* O157:H7, other Shiga toxin-producing *E. coli*, *Clostridium perfingens*), and pesticides. Eighteen samples were positive for one or more contaminant. Ninety eight percent of the post Harvey rice tested by OTSC was successfully harvested and marketed.

Significance: OTSC provided legal certainty to the farm community and preserved market quality and integrity during the recovery process. These activities also included coordinating communication between farmers and the FDA regarding contaminated product disposition and assisting the Risk Management Agency of the United States Department of Agriculture in making crop insurance decisions during the early days of the recovery process.

P1-131 *Listeria monocytogenes Sigb* Allelic Type and Pulsotype Diversity in Scat and Agricultural Water Samples Collected on a New York Produce Farm

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❖ ***Developing Scientist Competitor***

Introduction: Preventing *Listeria monocytogenes* contamination of fresh produce is a challenge currently facing the produce industry. As such, a comprehensive understanding of the ecology and diversity of *L. monocytogenes* in produce production environments is key to developing effective methods for reducing preharvest produce safety risks.

Purpose: To characterize *L. monocytogenes* diversity in wildlife scat and agricultural water collected over one growing season from a New York produce farm.

Methods: Agricultural water samples were collected from a pond (*n*=80) and stream (*n*=51). Fecal samples (*n*=77) were opportunistically collected from areas <10 m from the water sources. All samples were tested for *L. monocytogenes*. One isolate per *Listeria*-positive sample was subtyped via PCR amplification and sequencing of the *sigB* gene. The prevalence of *L. monocytogenes* in each sample location was calculated and compared using chi-square and Fisher's exact tests. The *sigB* allelic type (AT) diversity for each sample type (i.e., pond water, stream water, feces) was calculated using Simpson's Index of Diversity (D) with 95% confidence intervals (CI).

Results: The *L. monocytogenes* prevalence in the stream samples (65%) was significantly higher than the prevalence in the pond samples (39%, *P*=0.007) and fecal samples (29%, *P*<0.001). Among a total of eight ATs identified, four were repeatedly isolated from all sample types, and the other four ATs were isolated three or fewer times. Additionally, two ATs were isolated from only one sample type. Overall, the *L. monocytogenes sigB* AT diversity in the stream (D=0.454; 95% CI=0.248 to 0.659), pond (D= 0.656; 95% CI=0.493 to 0.818), and feces (D=0.632; 95% CI=0.461 to 0.804) were similar.

Significance: The pattern of subtype isolation suggests the survival or continuous introduction of a diverse *L. monocytogenes* population into the farm environment. Therefore, typing multiple isolates per sample may facilitate traceback following detection of a *Listeria*-positive sample during routine product testing.

P1-132 Evaluation of Microbial Safety and Quality of Louisiana Strawberries after Flooding

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Introduction: Rainfall and flooding both affect the Louisiana strawberry industry. Floodwater serves as an ideal medium for microbiological contaminants, which may lead to contamination of soil, agricultural water, and fresh produce with foodborne pathogens.

Purpose: This study aimed to evaluate the microbial safety and quality of strawberries after flooding.

Methods: Three strains of generic *Escherichia coli* were used to establish a baseline population of approximately 10^6 CFU/L (high contamination) and 10^2 CFU/L (low contamination) in floodwater. Five raised beds were filled with water to simulate a flooding event. Simulated floodwater was mixed with cow manure and spiked with generic *E. coli*. Treatments included High Flooding High Contamination (HFHC), High Flooding Low Contamination (HFLC), Low Flooding High Contamination (LFHC), and Low Flooding Low Contamination (LFLC). One bed served as the control (C). Strawberry plants were flooded for 4 h. Strawberry fruit samples were collected and stored in the refrigerator for 0, 48, 96, and 144 h. Soil samples were collected onsite for up to a week. Population of foodborne pathogens and indicators were evaluated during the shelf life of the strawberries. Qualities of the strawberries were also evaluated.

Results: The presence of *E. coli* O157:H7 and *Listeria monocytogenes* in strawberries, soil, and plants was not detected. In strawberry samples, generic *E. coli* was only detected in HFHC samples right after flooding (0.41±0.57 log CFU/g). In soil samples, generic *E. coli* was significantly higher at 0 h after HFHC flooding (1.61±0.31 log CFU/g) and at 48 h (1.54±0.51 log CFU/g). Significant levels of coliform were present in strawberries and soil at 0, 48, 96, and 144 hours in all treatment beds. Generic *E. coli* or coliform were not present on plant foliage.

Significance: This study will provide local growers science-based information to determine the safety of food crops exposed to floodwater.

P1-133 Survival of *Escherichia coli* in Manure-amended Soils and Transfer to Tomato, Radish, and Spinach on a Maryland Certified Organic Farm

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Introduction: Raw, inadequately treated manure is used to fertilize and condition organic fruit and vegetable farm soils. Manure pathogens may contaminate fresh produce. National Organic Program (NOP) standards stipulate wait time intervals of 90 to 120 days for produce harvested from raw-manured soils. Current U.S. Food and Drug Admnistration Food Safety Modernization Act regulations await scientific data regarding appropriate wait time intervals.

Purpose: To evaluate manure pathogen and *Escherichia coli* survival in soil and transfer to fresh produce harvested 90 to 120 days post-application of raw manure to NOP-certified field soil.

Methods: Replicated field plots per treatment (*n*=4) on NOP-certified silty loam soils, amended with dairy manure (DM) or horse manure (HM), poultry litter (PL), or unamended (UnA), were spray inoculated (1.0 L/2 to 4m^2) with a three-strain cocktail of rifampicin-resistant *E. coli* at 6 log CFU/mL. Colony count and most probable number (MPN) methods were used to determine persistence and transfer of *E. coli* between 0 and 90 to 120 days post-inoculation (dpi) for 528 total samples. *Salmonella*, *Listeria monocytogenes*, and pathogenic *E. coli* were also determined for all manures and on harvested produce.

Results: For 90-dpi radish soil, rifampicin-resistant *E. coli* for all treatments were barely detectable (0.56 to 16 MPN/g) with PL treatment being the most populous; all radish bulbs were positive for rifampicin-resistant *E. coli*. For 120-dpi radish, rifampicin-resistant *E. coli* was barely detected in four PL plots and one UnA plot; bulbs were negative. For 90-dpi spinach, rifampicin-resistant *E. coli* was undetectable in plots UnA and DM, and barely detected in HM and PL plots (0.6 to 6.6 MPN/g); all plots were negative at 120 dpi. For 90-dpi tomato, rifampicin-resistant *E. coli* was present on all tomatoes in most plots, with clear evidence of intensive insect activity (fall army worms) and *Phytophthora infestans* blight.

Significance: Results show slightly greater and prolonged persistence of *E. coli* in PL-amended plots compared to other manures. These results specific to NOP-certified soils will contribute to risk-based assessments for development of application-to-harvest wait time intervals for fresh produce safety.

Poster

P1-134 Phylogenetic Characterization of *Listeria monocytogenes* Isolates Collected from Surface Waters Used for Irrigation in the Lower Mainland of British Columbia, Canada

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Introduction: The presence of *Listeria monocytogenes* in surface waters used for irrigation may pose a risk for fresh produce contamination. A knowledge of persistent versus sporadic occurrence of this pathogen is important to the development of risk-reducing strategies.

Purpose: Our objective was to characterize the phylogenetic relationships and genetic variability of *L. monocytogenes* isolates previously collected over an 18-month period in the Lower Mainland of British Columbia, with the goal of identifying trends in persistence.

Methods: Up to five isolates of *L. monocytogenes* were selected from 24 surface water samples (*n*=80) collected during a previous study in our laboratory. The whole genomes of these isolates were sequenced using the Illumina HiSeq platform in Genome Québec. The raw reads were quality trimmed and mapped to the reference genome, and the genetic relationship among isolates was inferred after phylogenetic analysis of single-nucleotide polymorphisms (SNPs).

Results: The SNPs data indicated the presence of 30 unique (i.e., non-clonal) isolates, which clustered evenly into two well-supported clades, representing lineages I (*n*=14) and II (*n*=16). Sixteen of these occurrences appear to be sporadic, while putative persistence was observed twice within each lineage. Within lineage I, a clade of phylogenetically related isolates from five sampling dates at two independent sampling sites was observed, while a second clade of genetically related isolates was observed at two independent sites on two different sampling dates. This suggests persistent contamination in the area, possibly from a mobile source, (e.g., feral animal). Within lineage II, the recurrence of highly related isolates at a single site on different sampling dates was observed twice, suggesting a persistent source of the pathogen upstream from these sampling sites.

Significance: These data show that environmental reservoirs of *L. monocytogenes* are capable of causing persistent contamination in surface waters used for irrigation.

P1-135 Food Safety Assessment of Fresh Produce Served at School Feeding Programs in South Africa

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❖ ***Developing Scientist Competitor***

Introduction: The National School Nutrition Programme (NSNP) was introduced in South Africa as one of Nelson Mandela's initiatives to improve the food security status of children in the educational system. Currently, more than 9 million school-going children benefit from the program.

Purpose: This study aimed to determine the level of safety of fresh produce served at schools and to determine the factors in the school environment that could lead to the contamination of fresh produce.

Methods: Fresh produce (*n*=84) and environmental samples (*n*=100) were collected from three schools during two site visits. Indicator organisms including coliforms, *Escherichia coli*, *Enterobacteriaceae*, and *Staphylococcus* species were enumerated. Detection of *E. coli*, *Staphylococcus aureus*, and *Salmonella* species was conducted, and presumptive isolates were identified using matrix assisted laser desorption ionization-time of flight mass spectrometry.

Results: Irrigation water stored in open refuge bins at school 3 was not of an acceptable standard (>1,000 CFU *Escherichia coli*/100 ml) according to the South African Water Quality Guidelines for irrigation water. Rain water used at school 1 and municipal water at school 2 was of acceptable quality according to the national guidelines. Water used for washing fresh produce at schools was also considered potable according to the national standard. Not all fresh produce grown at the school and delivered by the Department of Education were within the Department of Health microbiological guidelines for raw vegetables and fruit. Coliform counts exceeded the specified limit (2.3 log CFU/g) and *E. coli* was detected from some samples, also exceeding the specified limit (0 CFU/g). *Staphylococcus* species, including *S. aureus*, were found in irrigation water, fresh produce, and contact surface samples. No *Salmonella* was detected in any of the samples collected.

Significance: This study highlights the need for better food safety strategies at schools focusing on food safety training for food handlers and access to appropriate food preparation facilities to ensure proper hygiene that will enhance food safety at schools where meals are provided.

P1-136 Fate of 30 *Salmonella* Strains on Two Lettuce and Tomato Cultivars

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Introduction: *Salmonella* is the most common cause of bacterial foodborne disease outbreaks linked to fresh produce. Ten of more than 2,500 known *Salmonella* serovars have caused 90% of reported outbreaks, and leafy vegetables are the most often implicated food vehicle. The influence of serovar, strain, plant species, or cultivar on the fate of *Salmonella* on growing food plants is poorly understood.

Purpose: The purpose of this work was to assess the fate of *Salmonella* from a range of serovars on lettuce and tomato plants from two different cultivars.

Methods: Three-week-old plants from two lettuce and tomato cultivars were separately inoculated with 30 *Salmonella* strains from 23 serovars. *Salmonella* populations were measured on xylose lysine deoxycholate agar immediately after inoculation and after five days of incubation in a growth chamber at 21°C. Three replicate trials were carried out with each *Salmonella* strain.

Results: Only one (3.3%) of 30 *Salmonella* strains (from serovar Anatum) could not be recovered from lettuce or tomato plants five days after inoculation. Populations of three (10.0%) *Salmonella* strains increased on lettuce, but not on tomato plants. Six (20.0%) strains on one of two lettuce cultivars, four (13.3%) strains on one of two tomato cultivars, and populations of 18 (60.0%) strains from 18 different serovars increased on all plants. Populations of the latter were significantly (*P*>0.05) higher on lettuce than on tomato plants, and population increases were significantly (*P*>0.05) larger on one of the two lettuce cultivars examined (2.47±0.52 CFU/g versus 3.16±0.74 CFU/g).

Significance: The results of this study suggest that *Salmonella* from a range of serovars can colonize food plants but that individual strains differ in their ability to colonize specific plant species or cultivars thereof.

Poster

P1-137 Impact of Withdrawal Periods between Cattle Grazing and Harvest on Food Safety Risk of Native Pecans

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Introduction: Cattle grazing on native pecan orchards is a common practice in Oklahoma, Arkansas, and Texas; however, animal grazing may represent a potential contamination source of foodborne pathogens to native pecans. The application of waiting periods between grazing and harvest has been recommended to minimize such contamination.

Purpose: The objective of this study was to determine the contamination rate of *Salmonella* spp. and Shiga toxin-producing *Escherichia coli* (STEC), in native pecan production orchards as influenced by withdrawal periods between cattle grazing and pecan harvest.

Methods: Soil (10 g), cattle feces (10 g), and in-shell pecans (25 g) were sampled from five cattle grazing orchards in areas that were either electrically-fenced two or four months prior to harvest or without fencing. Also, five non-grazing orchards were sampled at harvest for comparison. Detection and isolation of STEC and *Salmonella* from pecans and environmental samples were performed by enrichment, selective isolation, and multiplex PCR. Statistical analyses were performed using contingency tables with Pearson's chi-squared test.

Results: STEC contamination rate in cattle-grazing orchards was higher (38%) at areas without fencing than these with cattle removed two (29%) and four (27%) months prior to harvest. *Salmonella* contamination rates were 30, 22, and 30% at areas without fencing, with two and four months of cattle removal, respectively. However, the contamination rates were not statistically significant different (P<0.05) among withdrawal periods for either pathogen. The contamination rate in non-grazing orchards was significantly lower for STEC (13%) and *Salmonella* (7%) compared to cattle-grazing orchards (P<0.05).

Significance: The application of two- and four-month withdrawal periods may not be effective in reducing foodborne pathogen contamination rates in native pecan production environment. The presence of STEC and *Salmonella* in non-grazed native pecan orchards suggests that grazing cattle may not the only source of foodborne pathogen contamination in native pecan production areas.

P1-138 Preliminary Data on the Prevalence and Concentration of Shiga Toxin-producing *Escherichia coli* in Bovine Manure in Florida

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Introduction: The interval between application of biological soil amendments of animal origin (BSAAO) and the harvest of fresh produce has not yet been set in the produce safety rule. Studies are needed to determine the prevalence and concentration of pathogens in BSAAO to inform regulatory efforts.

Purpose: The objective of this research was to determine the prevalence and concentration of Shiga toxin-producing *Escherichia coli* (STEC) in bovine manure.

Methods: A modified most probable number (MPN) (4x6) assay was utilized to screen samples (n=413) from nineteen farms (three/four piles per farm, seven replicates per pile) for *stx1* and *stx2*. Samples were enriched in mEHEC media, isolated on CHROMagar™ STEC, and screened for *stx1* and *stx2* via quantitative PCR (qPCR). Dynabeads™ anti-*E. coli* O157 were utilized to screen for *E. coli* O157:H7 presence/absence. CT-SMAC and Rainbow Agar O157 were utilized for the isolation from enrichment and qPCR was used for confirmation of *E. coli* O157.

Results: Of the nineteen farm visits, 59 piles, and 413 samples evaluated, 6 (32%), 11 (19%) and 15 (4%) were *stx1* and/or *stx2* positive, respectively. The average MPN/g for positive samples was 558 (0.45 to 6,800) with a median of 9.6 MPN/g. Of the nineteen farm visits, 59 piles, and 413 samples evaluated, 17 (90%), 33 (56%), and 81 (20%) were *E. coli* O157:H7 positive, respectively. Positive *E. coli* O157:H7 samples were further evaluated for *stx1* and *stx2*, and with the inclusion of *E. coli* O157:H7 positive isolates, 13 (68%) farm visits, 20 (34%) piles, and 33 (8%) samples were *stx1* and/or *stx2* positive.

Significance: This study provides data for risk assessments to determine the public health impact of the time to harvest interval between BSAAO application and harvest of fresh produce.

P1-139 Effect of Dry and Wet Heat Treatments on *Clostridium difficile* Endospores during Composting

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Introduction: Endospore-forming *Clostridium difficile* has been isolated from the manure of livestock animals. A few studies reported its existence in manure-based composts, as well.

Purpose: To assess the thermal resistance of *C. difficile* endospores upon exposure to dry and wet heat by simulating the early stages of composting.

Methods: The finished dairy compost was adjusted to 20, 30, and 40% moisture contents (MC) and inoculated with the endospores of three non-toxigenic *C. difficile* strains at 5 to 6 log CFU/g. Inoculated compost was exposed to 55 and 65°C for three days after the come-up times by both wet and dry heat treatments. The surviving endospores in duplicate compost samples were enumerated on brain heart infusion agar supplemented with yeast extract, L-cysteine, and sodium taurocholate, at 24-h intervals. The experiment was performed in triplicate.

Results: The come-up time of dry heat treatment was programmed to be two days, whereas come-up time for wet heat treatment was dependent on target temperature and MCs of compost. For both heat treatments, *C. difficile* endospore reductions during the come-up times ranged from 0.01 to 0.87 log CFU/g compost. For wet heat, further log reductions of endospores after reaching 55°C were 0.74, 0.79, and 0.80 as compared to the log reductions of 0.62, 1.01, and 3.04 after reaching 65°C in compost with 20, 30, and 40% MCs, respectively. For dry heat treatment at 55°C, no further reductions of endospores were observed but reductions of 0.08, 0.22, and 0.53 log were detected in compost with 20, 30, and 40% MCs, respectively, during dry heat treatment at 65°C.

Significance: The thermophilic phase of composting reduces the level of *C. difficile* endospores to some extent, depending on the type of heat treatment and MC of the compost. Importantly, some more resistant endospores could survive and pose risk for pre-harvest environmental contaminations.

Poster

P1-140 Soil Bio-remediation Practices to Reduce *Salmonella* Contamination in Melon Production Systems

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❖ ***Developing Scientist Competitor***

Introduction: Soil contamination with human pathogens represents a major problem for fruit and vegetable farmers due to lack of valid remediation practices to inactivate human pathogens.

Purpose: Develop control strategies to inactivate *Salmonella* spp (SAL) from soils used in melon production.

Methods: A four-treatment approach using a complete randomized design with 4 replicates per treatment (T1- control bare soil, T2- muscadine-pomace compost (MPC), T3- mushroom compost (MC) and T4- a combination of MPC and MC) combined with solarization and cultivation of a mustard cover crop (MCC) was implemented to determine inactivation of avirulent SAL rifampicin resistant (50mg/ml) (ASALrif; 2 L at Log 5 CFU/ml) from organic and conventional fields with identical soil type. Bacteria was recovered at 0, 4, 10, 24 and 31 days-post-inoculation (DPI), after solarization (75-DPI) and disking of a MCC (134 and 143-DPI). Soil sampling (150g) per subplot was used to determine the population of ASALrif, *Coliforms* and *Enterococci* by plating on TSA amended with rifampicin at 50mg/mL and ECC.

Results: Inactivation of ASALrif after 24-DPI was different between conventional (Log 4.7 CFU/150g soil) and organic (Log 2.9 CFU/150g soil) cropping systems ((P<0.05). In organic systems, T4 presented the largest inactivation of ASALrif (Log 3 CFU/150g-soil, (P<0.05)). After solarization, ASALrif inactivation from T1 was different between organic (Log 1.7 CFU/150g soil) and conventional (Log 3.8 CFU/150g soil) systems (P < 0.05). T3 presented the largest inactivation of ASALrif (Log 3.3 CFU/150g soil) when compared to T2 and T4 (Log 2.1 and 2.4 CFU/150g soil respectively, (P < 0.05) irrespective of cropping system. The MCC alone significantly reduced the populations of ASALrif by Log 1.98 CFU/150g soil (P < 0.05) after 42 days of crop establishment irrespective of cropping system. *Enterococci* populations were mainly impacted by solarization and T1, T3 and T4 (average Log reduction of 2.1 CFU/150g soil sample, (P < 0.05).

Significance: A combination of these sustainable bio-remediation practices generated a 3-log reduction of ASALrif after 3 months of inoculation.

P1-141 Effect of a Dairy-origin Probiotic Bacterium, *Propionibacterium freudenreichii* spp. *freudenreichii* Nrrl 3523, against Multidrug-resistant *Salmonella* Heidelberg in Turkeys

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Introduction: Multidrug-resistant *Salmonella* (MDRS) Heidelberg is an emerging threat to the pre-harvest microbiological safety of poultry, including turkeys. The pathogen is highly invasive in humans and possesses high antibiotic resistance potential. Cecal colonization of MDRS Heidelberg and subsequent fecal shedding of the pathogen could contaminate farm environments, fresh incoming flocks, and carcasses during processing. Therefore, preharvest interventions targeting the control of MDRS Heidelberg in turkeys are required.

Purpose: The objective of this study was to determine the efficacy of a generally recognized as safe probiotic bacterium of dairy origin, *Propionibacterium freudenreichii* subsp. *freudenreichii* NRRL 3523 against MDRS Heidelberg in 7- and 12-week-old turkeys.

Methods: Day-old turkey poults were purchased from a commercial hatchery and allocated to three treatment groups: negative control (NC; turkeys without *P. freudenreichii* supplementation or MDRS Heidelberg challenge), MDRS Heidelberg control (SC; turkeys challenged with MDRS Heidelberg without *P. freudenreichii* supplementation) and test group (PFS; turkeys supplemented with *P. freudenreichii* and challenged with MDRS Heidelberg). Turkeys (seven-week study = eight turkeys per group, two experiments; 12-week study = 10 turkeys per group, two experiments) were raised according to the current industry recommendations until 7 or 12 weeks of age with continuous *P. freudenreichii* supplementation in water (1,010 CFU/ml). At the 6th or 11th week, turkeys were challenged with MDRS Heidelberg at 106 CFU/bird by crop gavage. After two and seven days of MDRS Heidelberg challenge, cecal colonization of the pathogen and its dissemination to the liver and spleen were determined.

Results: Supplementation of *P. freudenreichii* reduced MDRS Heidelberg cecal colonization in the PFS group by 1 to 1.3 and 1.7 to 2.2 log CFU/g at seven and 12 weeks, respectively (P<0.05), compared to the SC group. In addition, *P. freudenreichii* supplementation significantly reduced MDRS Heidelberg dissemination to the liver and spleen.

Significance: *P. freudenreichii* subsp. *freudenreichii* could be used as an antibiotic alternative against MDRS Heidelberg in turkeys, thereby improving the preharvest safety of turkeys and turkey products (MAES Project# MIN-16-102).

P1-142 Preliminary Survey of Microbial, Chemical, and Physical Parameters of Chicken Litter in Florida

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Introduction: Application of chicken litter as a biological soil amendment of animal origin (BSAAO) in fresh produce production holds a risk of contamination from human pathogens. Legislation regarding the interval between applications of BSAAO and harvesting needs additional data. Research on factors contributing to the prevalence and concentration of pathogens in chicken litter may aid in providing guidance to producers.

Purpose: The purpose of this research was to survey the microbial (including the prevalence and concentration of *Salmonell*a), chemical, and physical parameters of chicken litter in Florida.

Methods: Raw chicken litter samples (n=36) from eighteen farms were surveyed for *Salmonella* using a modified (4×3) most probable number (MPN) method. Samples were selectively enriched in Rappaport-Vassiliadis R10 broth, and presumptive colonies were isolated on modified lysine iron agar and triple sugar iron agar. Presumptive colonies were further confirmed by serotyping and PCR for the *inv*A gene. Litter samples were serially diluted, and aliquots from each dilution were spread plated onto plate count agar. Plates were incubated at 35°C for 24 h, and colonies were counted to determine aerobic plate counts (APC). Chemical and physical parameters were measured by the University of Florida's Institute of Food and Agricultural Sciences Analytical Services Laboratories.

Results: Salmonella was recovered from 17 of 18 (94%) farms and 19 of 36 (53%) samples collected. Among positive samples, the average *Salmonella* (MPN/g) was 35.1 (0.04 to 460). The average APC from all samples was 8.7 log CFU/g. The average pH values of samples was 7.9 (7.3 to 8.6). The average content (g/kg) of nitrogen, ammonium, phosphorus (P_2O_5), and potassium (K_2O) in the samples were 22.4, 1.2, 19.1, and 25.6, respectively. Average moisture content and organic matter in the samples were 20 and 58%, respectively.

Significance: This data will be helpful in risk assessments to determine the time interval between BSAAO application and the harvest of fresh produce.

Poster

P1-143 Manure Pathogen Survey of *Salmonella* and Shiga Toxin-producing *Escherichia coli* in Untreated Poultry and Cattle Manure of the Mid-Atlantic Region

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❖ ***Developing Scientist Competitor***

Introduction: Biological soil amendments of animal origin are a valuable nutrient source, but the potential presence of microbial pathogens is a food safety risk.

Purpose: The purpose of this study is to quantify *Salmonella* in poultry litter and Shiga toxin-producing *Escherichia coli* (STEC) in cattle manure in the Mid-Atlantic United States.

Methods: Seven manure samples (30 g each) were collected upon each visit to 20 poultry and 12 cattle farms, on two separate sampling dates to each farm over a period of 18 months. Samples were mixed with tryptic soy broth and pathogens detected by most probable number (MPN) in 48-well blocks at 37°C for 24 h (poultry) or 42°C for 20 h (cattle). For *Salmonella*, enrichment proceeded in buffered peptone water, and then transfer to tetrathionate (TT) and Rappaport Vassiliadis (RV) tubes/MPN blocks. For STEC, cattle samples were transferred to modified enterohemorrhagic *Escherichia coli* tubes and MPN blocks. Incubations were typically overnight. Samples were plated on xylose-lysine-tergitol 4 agar and CHROMagar STEC from selective enrichment tubes for confirmation. MPN was quantified with a detection limit of -1.05 log MPN/g (0.089 MPN/g). Colonies from positive samples were confirmed by PCR. Statistical analyses were determined using a chi-square test.

Results: *Salmonella* was detected in 43% (120 of 280) of samples, non-O157 STEC in 18% (21 of 119), with no *E. coli* O157 confirmed in any of the cattle samples. Among the positive samples, log MPN/g ranged from -1.05 to 5.45 (average 2.53) for *Salmonella* and a range of -0.29 to 2.83 (average 1.24) for STEC non-O157. In *Salmonella* enrichment, RV and TT yielded similar detection for samples as positive for *Salmonella*; however, log MPN/g values were statistically greater ($P<0.0001$) for *Salmonella* in samples detected by TT.

Significance: *Salmonella* and STEC are present in non-composted manure in varying levels and could transfer to raw produce when applied to crops as biological soil amendments. Differences in *Salmonella* enrichment media should be further elucidated.

P1-144 Impact of Irrigation Run-off Water on the Survival of *Salmonella* and *Escherichia coli* in Soil and on Lettuce Plants

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Introduction: Ground water is the main source of irrigation water for growers in the Salinas Valley in California. For conservation reasons, there is interest in reuse of irrigation run-off or "tail water" for applications such as pre-irrigation and germination.

Purpose: To characterize irrigation and tail water inoculum carriers on the survival of *Salmonella*, generic *Escherichia coli*, or *E. coli* O157:H7 in soil and on lettuce plants.

Methods: Irrigation and corresponding tail water and soil samples were collected from one leafy green farm in the Salinas Valley. Cocktails of rifampicin-resistant *Salmonella*, generic *E. coli*, and *E. coli* O157:H7 were prepared separately and suspended in either tail, irrigation, or Milli-Q water. Inoculated water (150 ml) was mixed thoroughly with 500 g of soil to a target concentration of 4 log CFU/g dry weight. Individual leaves of two-month-old iceberg lettuce were inoculated with 10 2-µl drops of inoculum cocktail, for a target concentration of 4 log CFU/leaf. Soil and plants were incubated in a growth chamber at fluctuating relative humidity and temperatures set to mimic those in the Salinas Valley. Inoculated organisms were enumerated on tryptic soy agar supplemented with rifampicin.

Results: Concentration of organic carbon was significantly higher in tail water than irrigation water samples. The declines of *Salmonella*, *E. coli* O157:H7, and generic *E. coli* were similar in soil or on lettuce plants after either tail- or irrigation-water inoculation. In soil, *Salmonella*, *E. coli* O157:H7, and generic *E. coli* concentrations decreased slowly over 28 days, by 0.9, 1, and 0.8 log CFU/g dry weight, respectively. On the lettuce leaf surface, all three organisms declined rapidly, by 3 log CFU/leaf, reaching the limit of detection (1 CFU/leaf) by day seven.

Significance: Tail water did not significantly impact the survival of *Salmonella*, generic *E. coli*, and *E. coli* O157:H7 in soil or on lettuce leaf surfaces when compared with irrigation water.

P1-145 Sampling on Maryland's Eastern Shore Farms: Keeping an Eye Out for Pathogens and Providing a Service to Produce Growers

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Introduction: In 2014, a *Salmonella* outbreak on cucumbers that reached 275 people in 29 states and Washington, D.C. was traced back to the Delmarva Peninsula on the East Coast of the United States. However, studies conducting thorough on-farm environmental sampling over time are lacking in this region.

Purpose: The purpose of this study is to monitor *Salmonella* and *Listeria* spp. on the Eastern Shore of Maryland while providing a service to any grower wishing to verify that their cleaning and sanitation practices are effectively reducing these organisms.

Methods: A total of 213 environmental samples were taken from nine farms in five counties during the summer months between 2015 and 2017. Blossoms, soil, irrigation water, and nearby surface water were aseptically collected. Buckets, harvest bins, packing lines, packing tables, vehicle tires, and manure spreaders were swabbed. All collected samples were sent to Maryland Animal Health Laboratories where they were tested for the presence of *Listeria* and *Salmonella* species using FDA BAM methods.

Results: Positive samples were recovered from only two of the five counties tested (9% and 2% of Wicomico and Caroline County samples, respectively). Five of the seven positive *Salmonella* samples were from a nearby creek or soil; the remaining two positive samples were isolated from a packing line. *Salmonella* was recovered once from creek water in Caroline County in 2016 (no samples were collected in Caroline County in 2017). *Listeria* was recovered once each in 2015 and 2016 from Wicomico County from a cooler and from creek water, respectively, and once in 2016 in Caroline County, again from creek water.

Significance: This study demonstrated that *Salmonella* and *Listeria* species are in surface waters of Wicomico and Caroline Counties. Positive samples from packing lines, coolers, and soil informed corrective actions in cleaning and sanitation practices.

P1-146 *Escherichia coli* O157:H7, Non-O157 Shiga Toxin-producing *E. coli*, and Generic *E. coli* Survival in Manure-amended Sandy and Clay Soils

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❖ ***Developing Scientist Competitor***

Introduction: The Food Safety Modernization Act Produce Safety Rule established standards for the use of biological soil amendments of animal origin (BSAAO). Standards for the application of untreated BSAAO and harvest of covered produce have not been set. Further research is needed to generate data on pathogen dynamics in BSAAO.

Purpose: This study investigated the growth and survival of different *Salmonella*, *Escherichia coli* O157:H7, non-O157 Shiga toxin-producing *E. coli*, and generic *E. coli* strains in poultry litter and bovine amended soils.

Methods: Greenhouse experiments were arranged in a randomized complete block design totaling 90 pots (3 replicates×(12 strains(daily_H_2O)+3 strains(weekly_H_2O)×2 soil types). Each culture was mixed with each soil type and BSAAO. Target inoculum was 4-5 log CFU/g. All strains were exposed to daily water application, while three of the strains were exposed to weekly water application to maintain soil moisture. Soil was sampled at 0d, 0.167d, 1d, 2d, 4d, 7d, 14d, 21d, 28d, 56d, and 84d post-inoculation. Population levels were enumerated on selective agar. An MPN method was used to detect lower levels (-0.6 log MPN/g). Significance was analyzed using Tukey's HSD test *($P<0.05$)*.

Results: Pathogen populations were significantly higher in soils amended with BSAAO. Individual strains, even within group, exhibited different survival dynamics. *E. coli* strain populations were significantly higher in amended sandy soils, while *Salmonella* strain populations persisted at higher populations in amended clay soils. No significant difference was observed between strain survival in either amended soil when exposed to daily or weekly irrigation to maintain soil moisture. All *Salmonella* strains survived 84d post-inoculation, while most *E. coli* strains were below the limit of detection 84d post-inoculation.

Significance: Soil type influenced the survival of pathogen strains in amended soils, compared to moisture. Data will be included in risk assessments to develop standards for the safe application of BSAAO.

P1-147 *Listeria monocytogenes* Transfer Potential during Field-pack Handling of Cantaloupe

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Introduction: One cantaloupe-associated *Listeria monocytogenes* outbreak in 2011 devastated the industry. Since then the industry has been proactively supporting research efforts aimed to limit contamination events.

Purpose: The goal was to quantify *L. monocytogenes* transfer from contaminated gloves to cantaloupes, and vice-versa, evaluating different contact times (5, 10, and 20s) and pressures (none, mild, and vigorous).

Methods: Experiments were performed using cantaloupe with three glove materials (nitrile, cotton, and rubber) typically worn by harvest crews. Two different transfer scenarios were evaluated: inoculated cantaloupe to glove and inoculated glove to cantaloupe. To simulate contamination in a dry environment, a dry inoculum protocol (inoculum mixed with sand) was used at a final concentration of 10^6 CFU/g. *L. monocytogenes* populations were enumerated on nonselective and selective agar supplemented with 50 ug/mL nalidixic acid. Log transfer coefficients (TCs) were calculated and significance determined by least squares test ($P\leq0.05$).

Results: The transfer of *L. monocytogenes* from a contaminated cantaloupe to surface or contaminated surface to cantaloupe was low using a dry inoculum. *L. monocytogenes* TCs from inoculated glove material to cantaloupe were highest on rubber gloves (0.16 ± 0.12 to 0.43 ± 0.13), regardless of the contact times and pressures. While, *L. monocytogenes* TCs from inoculated glove material to cantaloupe were lowest on nitrile gloves (0.10 ± 0.09 to 0.22 ± 0.15), regardless of the contact times and pressures. *L. monocytogenes* TCs from cotton gloves to cantaloupes were extremely variable by contact times (less transfer when 20s contact time, compared to 5s contact time) and pressures (less transfer when vigorous pressure, compared to no pressure).

Significance: The likelihood of *L. monocytogenes* cross-contamination was influenced by different glove materials. Since TCs in each scenario were highest in rubber gloves, eliminating the use of rubber gloves may reduce the risk of cross-contamination events during cantaloupe handling.

P1-148 How Evaporating Water Can Promote Internalization of Bacteria through the Leaf Stomate

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❖ ***Developing Scientist Competitor***

Introduction: Evaporation of a water film on a leaf surface is a process that frequently happens as leafy greens move from field to fork. As surface water evaporates, capillary forces generated by the surface tension of water can push microorganisms present in water toward the leaf surface, facilitating their adhesion to the substrate surface and infiltration into the leaf openings and crevices. Despite this risk, there is no study concerning the sole effect of evaporation on the infiltration of bacteria into fresh produce.

Purpose: In this work, we model the transport of bacteria within an evaporating sessile droplet on a leaf surface.

Methods: The model includes fluid flow within the droplet and gas phase, gas-water interface tracking during evaporation, transport of vapor in the gas, transport of sugar in water, passive and active transport of bacteria within water, and heat transfer. The model results for bacteria distribution and infiltration are validated by conducting drop evaporation experiments on patterned polydimethylsiloxane (PDMS; as a surrogate leaf surface) and real leaf surfaces.

Results: Our results indicate that evaporation can cause internal flows within the droplet that can move and accumulate bacteria close to the leaf surface and within the stomatal opening. Wetability of the leaf surface plays an important role in the bacteria infiltration, and there is more infiltration on hydrophillic leaves. The model is further used to study the effects of the most important factors (e.g., leaf topology and roughness, stomatal opening size, availability of sugar at the leaf surface, bacteria chemotaxis and motility ability, evaporation rate, and liquid surface tension) contributing to the infiltration of bacteria into the leaf openings.

Significance: Deep understanding about the major contamination mechanisms obtained from this work should lead to improvement of the level of safety of fresh produce processing. Considering the fact that the consumption of fresh produce has been on the rise in recent years, such an understanding can have a major impact on public health.

Poster

P1-149 Sanitizing Role of Berry Pomace Extracts in Controlling Enteric Pathogens on Fresh Produce

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❖ ***Developing Scientist Competitor***

Introduction: Produce-borne infections, particularly through organic produce in the United States, are rising faster than ever, causing thousands of hospitalizations and deaths annually. It is a huge economic burden and a major reason for recalls of produce and fresh-cut fruit products. Therefore, postharvest processing with more effective and consumer-friendly sanitizers is urgently required. As organic farmers cannot use any synthetic chemicals or antibiotics, newly developed sanitizers should be natural antimicrobials that can be used for both conventional and organic produce.

Purpose: Determination of the efficacy of berry pomace extract (BPE), which is rich in phenolic compounds, as a sanitizer for washing produce products in order to control and eradicate enteric pathogens.

Methods: Leafy vegetables, specifically spinach and celery, were inoculated with either *Salmonella enterica* Typhimurium (ATCC 14028) or multidrug-resistant *Salmonella* strain (a laboratory isolated strain), then dipped into water supplemented with BPE (3.0 mg/mL) for various time periods (15, 30, 45, 60, 120, 180, and 1,440 min) at two different temperatures (24 and 4°C).The reduction in the bacterial CFU was determined. Expression of bacterial virulence genes (*hilA/C/D, invA/C/E/F*) in the presence of BPE was also examined. For statistical analysis, Student's *t* test was used.

Results: For spinach dipped in BPE, there was significant (P<0.05) reduction of both *Salmonella* Typhimurium and multidrug-resistant *Salmonella* from a range of 0.2 to 1.2 log CFU/mL at 24°C. At low temperature (4°C), the reduction was observed at a range from 0.5 to 5 log CFU/mL. For celery, the reduction of both *Salmonella* Typhimurium and multidrug-resistant *Salmonella* was also significant (P<0.05) at both temperatures (24 and 4°C). There was no significant (P<0.05) alteration of virulence gene expression in the bacteria isolated from spinach, but for celery *hilA/D/A* and *invC/E/F* of *Salmonella* Typhimurium showed significant (P<0.05) alteration in expression at 4°C.

Significance: Our findings indicated that BPE can be a potential natural alternative to replace synthetic chemical sanitizers in order to eliminate or reduce the major enteric bacterial pathogen *Salmonella* on produce products and to limit salmonellosis in humans.

P1-150 Withdrawn

P1-151 Survival of *Salmonella* in Tomato Stem Scars as Affected by Sanitizer Wash and Antimicrobial Coating

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Introduction: Tomatoes have been frequently implicated in outbreaks of foodborne illness. The stem scar region of the tomato has been indicated as the potential source for enteric pathogen contamination. Current chlorine-based sanitizer washes have limited efficacy. There is a need for a safe and effective alternate intervention strategy.

Purpose: The purpose of the present study was to evaluate the antimicrobial efficacy of an integrated treatment method utilizing organic acid wash (AW) and chitosan-allyl isothiocyanate (CT-AIT) coating against *Salmonella* in the stem scar region of tomatoes.

Methods: A bacterial cocktail containing three serotypes of *Salmonella enterica* was used for this study based on their association with produce-related outbreaks. Fifty microliters of the mixed culture suspension containing about 10^8 CFU/ml were carefully spotted on the stem scar sites of cherry tomatoes at ambient air temperature. The recovered initial population (control) was 7.8 ± 0.33 log CFU/g. Tomatoes were then immersed in an organic acid solution containing 0.5% (volume/volume) acetic (AA) and formic (FA) acid for 1 min at ambient temperature (22°C), followed by 1 min dipping in a coating solution containing 6 ml AIT/g CT. The treatment effect on microbial loads and fruit quality during storage was also determined.

Results: AW for 1 min reduced Salmonella by 2.7±0.34 log CFU/g from an initial load of 7.8 log CFU/g, while the AW treatment reduced the pathogens on stem scars to undetectable levels (< 0.7 log CFU/g), achieving greater than 5-log CFU/g reduction of the pathogen. During storage at 10°C, *Salmonella* population in the control remained unchanged while the population in treated (AW plus CT-AIT) stem scars remained at undetectable levels on day 1 and no regrowth was observed. The treatment significantly (P < 0.05) reduced total aerobes to approximately 1.3 log CFU/g and completely inactivated mold and yeast on day 1 with no growth reoccurrence through day 21 at 10°C.

Significance: Results obtained indicate that the integrated treatment of organic acid wash (AW) and antimicrobial (CT-AIT) coating can be a safe and effective intervention strategy for cherry tomatoes.

P1-152 Apple Peel Morphology and Attachment of *Listeria innocua* through Aqueous Environment as Shown by Using Scanning Electron Microscopy

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❖ ***Developing Scientist Competitor***

Introduction: Industrial apple packing process begins with submerging the harvested or stored apples into the water dump tank and carrying them by water flumes through various washing steps before drying and packing. Constant introduction of organic matter and water recirculation introduces the risk of apple being cross-contaminated with the pathogenic bacteria such as *Listeria monocytogenes.*

Purpose: This study investigated the relationship between apple peel microstructure and attachment of bacteria in aqueous environment.

Methods: Whole fresh unwaxed apples of three varieties (viz., Gala, Golden Delicious, and Granny Smith) were inoculated with *L. innocua*, a surrogate strain of *L. monocytogenes*, by submerging the apples into the inoculum solution at ~10^6 CFU/mL level. Apples were carefully turned around in the inoculum for 10 min simulating industrial dump tank and flumes conditions. Attachment of *L. innocua*, to different apple peel sections, was demonstrated using scanning electron microscopy (SEM).

Results: Textural characteristics varied considerably among apple varieties. Generally, apple peel was covered with amorphous layer of wax, disrupted by microcracks, lenticels, and occasionally overlaid with various types of epicuticular wax platelets. *L. innocua* attached primarily to apple peel in stem bowl and calyx sections, embedding in the microcracks, lenticels and on the surface of the trichomes. Bacteria were difficult to be located on the smooth surfaces peel in the equatorial section of the apples.

Significance: Results provide valuable information on *Listeria* attachment on apples which can be used in developing new intervention methods of microbial decontamination.

P1-153 Evaluation of a Batch Wash Ozone Sanitation System for Reduction of Microorganisms on Fresh Produce

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Introduction: Numerous foodborne illnesses are related to the contamination of produce. Produce is often consumed raw by the consumer. Sanitizing agents such as ozone can be implemented at retail for the reduction of microorganisms.

Purpose: The efficacy of a batch wash ozone sanitation system (BWOSS) was investigated for reduction of microorganisms on fresh produce and prevention of cross-contamination.

Methods: Head lettuce was inoculated with 1 mL bacterial cocktail (6 log CFU/mL). Eight heads were submerged in the BWOSS and covered with ice. Experiments were performed with and without ozone injection. Microbial analysis from heads (exterior and inner leaves) and BWOSS water were performed at times (T) of 0, 10, 20, 30, and 40 min. Surface swabs were collected from the drained BWOSS at T=40. Aerobic plate count (APC) and total coliform/*Escherichia coli* were enumerated using the respective petrifilm. *E. coli* C-3000, *Salmonella* Typhimurium LT2, and *Listeria innocua* were enumerated by spread plate method on selective media.

Results: Data indicate significant (P<0.05) reduction of bacteria on head lettuce when treated with ozone compared to water-only wash. Mean concentration of inoculated bacteria at T=0 was 6.13 log CFU/g. At 0.65 to >0.77 ppm ozone, *Salmonella* Typhimurium and *L. innocua* were not detected at T=30 and T=40, respectively. *E. coli* was reduced by 99.98% at T=40. Native APC reduction by BWOSS was also evaluated. Starting concentration of APC was 6.38 log CFU/g. Ozone exposure significantly reduced APC compared to water-only wash, with 99.99% and 89.83% reduction at T=40, respectively. Ozone eliminated microbes in water and on surfaces compared with water. Evaluation of the BWOSS using other leafy greens, as well as reduction of murine norovirus — an enteric virus surrogate — is ongoing.

Significance: Ozone is an effective sanitizer for reduction of microorganisms on leafy greens. Implementation of a BWOSS during retail food service may result in fewer foodborne illnesses.

P1-154 Overview of Leafy Greens-related Incidents with a California Link: 1996 to 2016

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Introduction: An increase in the number of outbreaks associated with produce has been noticeable in the literature, with leafy greens as the most common produce category. As one of the largest leafy green-producing regions in the United States, many related foodborne illness incidents have been traced to California.

Purpose: Conduct a historical overview of leafy greens incidents linked to California through complaints, routine surveillance sampling, disease outbreaks, and investigations covering 1996 through 2016. Develop a risk assessment tool based on overview outcomes to modernize emergency response efforts to foodborne outbreaks related to leafy greens by the California Department of Public Health Food and Drug Branch (CDPH-FDB).

Methods: A systematic review was performed of all California leafy greens-related incidents based on data available covering 1996 through 2016. A database including environmental, epidemiologic, and laboratory information of each incident was developed and descriptive analysis performed to identify trends.

Results: In the 21-year period analyzed, 135 incidents were identified, the majority of which were surveillance incidents. Approximately 1,600 cases of illness were reported in the United States, including ~300 California cases resulting in 55 hospitalizations. Most illnesses occurred in the fall season. The most prevalent hazard type was microbiological, in particular bacterial, and more specifically, pathogenic *Escherichia coli*. Romaine and iceberg lettuce were the most implicated vehicles.

Significance: In California, the overview provided CDPH-FDB with a platform to (1) enhance its Food Safety Program, Emergency Response Unit, and California Food Emergency Response Team; (2) assist in more efficient investigation, response, control, and ultimately prevention of California-linked foodborne illness incidents; and (3) identify knowledge gaps and develop effective definitions, procedures, training, guidelines, and policies that will serve to prevent future outbreaks. Nationally, outcomes provide insight into the findings of the largest leafy greens-producing states. Results may be used to prioritize limited food safety resources and aid in future leafy greens-related foodborne incident investigations.

P1-155 Colonization and Internalization of *Salmonella enterica* in Cantaloupe Plants

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Introduction: Cantaloupes have emerged as significant vehicles of widespread foodborne illness outbreaks caused by bacterial pathogens, including *Salmonella*.

Purpose: To examine the pre-harvest risks of cantaloupe when exposed to *Salmonella* on blossoms and in soil.

Methods: Cantaloupe plants (*Cucumis melo* 'reticulatus') from two cultivars 'Athena' (Eastern) and 'Primo' (Western) were grown from commercial seed. Plants were maintained in the NCSU BSL-3 phytotron greenhouse. *Salmonella* (a cocktail of serovars Javiana, Newport, Panama, Poona and Typhimurium) contamination was introduced via blossoms or soil at 4.4 log CFU/blossom or 8.4 log CFU/root zone. Cantaloupes were analyzed for *Salmonella* by enrichment in accordance with modified FDA-BAM methods. Five randomly chosen colonies from each *Salmonella*-positive sample were serotyped using the Agilent 2100 bioanalyzer following multiplex PCR. Data were analyzed for prevalence of contamination and serovar predominance in fruit, stems and soil.

Results: Of the total cantaloupe fruit harvested from *Salmonella*-inoculated blossoms (n=14), 86% (12/14) were contaminated and had *Salmonella* internalized into the fruit; of the presumptive-*Salmonella* positive colonies screened, greater than 47% (23/48) and greater than 66% (76/115) were identified as *S.* Panama on the surface and inside the fruit, respectively. When soil was inoculated, only 5% (2/40) of the plants were shown to translocate *Salmonella* to the lower stem (ca. 1-1.5 cm) after 7 days post inoculation (dpi), and of those positive, *S.* Javiana and *S.* Poona were most prevalent. We observed *Salmonella* persistence in the soil up to 20 dpi. S. Javiana (11/118), Newport (37/118), Panama (28/118), Poona (30/118) and Typhimurium (12/118) were identified at 10-30% (n=118) after 9 dpi and serovar Newport was identified at 60% (42/70) after 20 dpi (n=70).

Significance: These data demonstrate that contaminated soil and blossoms can lead to *Salmonella* internalization into the plant or fruit. Blossom infection resulted in a higher percentage of *Salmonella* internalization at a much lower level of inoculum (4.4 CFU/blossom versus 8.4 CFU/root zone).

Poster

P1-156 Internalization of *Salmonella* Newport in Transplanted Tomato Plants through the Roots

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❖ ***Developing Scientist Competitor***

Introduction: The risk of pathogen internalization in produce commodities is of concern due to the difficulties in eliminating pathogens during post-harvest handling. Transplanting is a common agricultural practice that adds stress to plants, and may allow the internalization of microorganisms.

Purpose: Therefore, the objective of this study was to determine the potential for *Salmonella* to internalize in tomato plants through the roots following transplanting.

Methods: Tomato plants were transplanted in triplicate (n=9) and exposed to one of four treatments; soil inoculated with a nalidixic acid resistant strain of *Salmonella* Newport (4 log CFU/g; T1), irrigation water inoculated with *Salmonella* Newport (4 log CFU/mL) administered once following transplanting (T2), irrigation water inoculated with *Salmonella* Newport (4 log CFU/mL) administered for 7d following transplanting (T3), and a control (T4). Plants were sampled 1, 2, 3, 7, and 90 d post-transplant. Plants were surface sanitized, aseptically sectioned into roots, stems, leaves, flowers, and fruit, and enumerated for *Salmonella* to determine internalized populations using XLT-4 plates treated with nalidixic acid. If the limit of detection (<4 CFU/plant section) was reached during plating, samples were enriched for *Salmonella* by MPN.

Results: *Salmonella* was recovered from 29/45 (64%), 0/45, and 13/45 (29%) of the root sections transplanted in T1, T2, and T3 respectively. Root sections transplanted into T1 had significantly higher *Salmonella* recovery, compared to other treatments (P-value<0.05). *Salmonella* was recovered less frequently in stem sections transplanted in T1, T2, and T3. No *Salmonella* was recovered from flowers or fruits for all treatments, while *Salmonella* was recovered from only two leaf samples exposed to T3.

Significance: Data show that while *Salmonella* Newport may be internalized by tomato plant roots following transplanting, it is very unlikely that *Salmonella* moves into the stem, leaves, flowers, or fruits. Internalization events seem to be associated with other environmental stressors, thus further research is needed to determine likely internalization scenarios to develop effective control measures.

P1-157 Prevalence and Levels of Shiga Toxin-producing *Escherichia coli* and *Salmonella* in Untreated Cattle and Poultry Manure in the West Coast of United States

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Introduction: Application of untreated biological soil amendments of animal origin (e.g., raw manure) is a potential source of fresh produce contamination. In the Produce Safety Rule, the U.S. Food and Drug Administration (FDA) has deferred its decision on appropriate time interval(s) between manure application and crop harvesting pending research and risk assessment outcome.

Purpose: This study aimed to determine the prevalence and levels of Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* spp. in untreated cattle and poultry manure in California and Arizona.

Methods: Manure samples were collected over a one-year period from farms or facilities. Seven samples were collected from each location using a modified stratified composite sampling scheme from different depths within each pile. Each sample was tested for pathogens using a 30-g screening for *Salmonella* (BPW, RV, TT media) and STEC (TSB and mEHEC media), and simultaneous transfer of 5-ml serially diluted samples to a 48-well reservoir plate for MPN analysis. Statistical analyses were performed using Fisher's exact test and Donner's chi-square test for clustered binomial data.

Results: Of 48 cattle manure piles/sources (*n*=336 samples) and 31 poultry manure piles/sources (*n*=216 samples), the prevalence (with 95% confidence interval) in piles and in samples were: 20.8% (10 to 35%) and 8.9% (6.1 to 13%) for STEC O157 in cattle manure; 31.2% (19 to 46%) and 8.3% (5.6 to 11.8%) for STEC non-O157 in cattle manure; and 51.6% (33 to 70%) and 28.2% (22 to 34%) for *Salmonella* spp. in poultry manure. Among positive samples, the level ($\log_{10}$ MPN/g) ranged from -1.05 to 2.36 for STEC O157; -1.05 to 0.66 for STEC non-O157; and -1.05 to 4.89 for *Salmonella* spp. When clustering effect is considered, prevalence in samples is not significantly (*P*>0.05) associated with facility type, state, and sample type.

Significance: This study provides critical data for quantitative risk assessment relevant to provisions of the FDA's Food Safety Modernization Act Produce Safety Rule.

P1-158 Assessing the Inactivation of *Listeria monocytogenes* on Raspberries by Chlorine and Peroxyacetic Acid Spray Treatments

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Introduction: *Listeria monocytogenes* contamination compromises the safety of fruits and vegetables. Sanitizer spray is one strategy used by the raspberry industry to minimize food safety risk. However, the treatment's efficacy remains unclear.

Purpose: This project evaluated the inactivation of *L. monocytogenes* on raspberries using chlorine and peroxyacetic acid (PAA) spray treatments. Sanitizer decay on raspberries was also studied during frozen storage.

Methods: A lab-scale spray bar device was fabricated to simulate industrial settings. Fresh raspberries (25 g) were spot-inoculated with *L. monocytogenes* cocktail (ca. 10^5 CFU/g) and sprayed with 50 ppm chlorine or 80 ppm PAA. Post-spray hold times ranging from 5 to 60 s, as well as frozen storage at -20°C for 1 and 24 h, were evaluated. Surviving *L. monocytogenes* were extracted and enumerated by plating on trypticase soy agar with yeast extract and a modified Oxford agar overlay.

For the sanitizer decay study, un-inoculated raspberries were sprayed with chlorine or PAA and stored at -20°C for up to two hours. At different time points, samples were removed from the freezer and sanitizer on the raspberry surfaces was extracted with 30 ml deionized water. The sanitizer concentration was measured with a free chlorine or PAA test kit at a detection limit of 0.1 ppm. The concentration on the berries was calculated by: [sanitizer on berries]=[sanitizer in water]/(30/sanitizer weight on berries). All experiments were repeated three times in triplicate.

Results: Chlorine spray (50 ppm) on raspberries resulted in a 0.61±0.16-log CFU/g reduction in *L. monocytogenes*. Post-spray holding time and frozen storage did not significantly increase *L. monocytogenes* reduction. PAA spray (80 ppm) demonstrated a similar reduction of 0.62±0.45 log CFU/g; however, post-spray frozen storage for 1 or 24 h provided further reduction of 3.63±0.56 and 2.33±0.77 log CFU/g, respectively. The sanitizer decay study revealed that active chlorine on raspberry surfaces was below the limit of detection within 30 min. In contrast, the PAA concentration on raspberries decreased by 50% after 120 min.

Significance: PAA spray treatment reduces *L. monocytogenes* populations on raspberries and could contribute to postharvest sanitation.

P1-159 Identity, Prevalence, and Chlorine Demand of Major Organic Compounds in Fresh Produce Wash Water Concerning Food Safety

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Introduction: The washing of fresh-cut produce generates large industrial volumes of spent water loaded heavily with organic compounds liberated from plant tissues. The associated large chemical oxygen demand (COD) and chlorine demand (CLD) negatively impact the efficacy of sanitization during washing and complicate safe reuse of the water. Identification of the major chemicals contributing to COD/CLD provides valuable information for improving food quality and safety.

Purpose: To identify by systematic chemical analyses the chief sources of COD and CLD during fresh produce washing.

Methods: Water samples, prepared by washing diced cabbage, were measured for total COD and CLD. The samples were then fractionated through centrifugation, ultrafiltration, and solid phase extraction. Sugars, organic acids, and phenolics were profiled by high-performance liquid chromatography, and peptides were extracted directly from the wash water. Regression equations were established to predict the time-dependent chlorine demand of each identified compound at their respective concentrations.

Results: Contributions to COD ranked from greatest to least as: sugars (81.6%), proteins/peptides (5.3%), organic acids (3.6%), and phenolics (0.5%). Contributions to CLD ranked roughly from greatest to least as proteins/peptides, phenols, organic acids, and sugars, but this ranking varied over time. Proteins/peptides accounted for >50% of total CLD during the cabbage-washing process. Phenols reacted rapidly with chlorine, contributing to 21% of the total CLD at 5 min, but this percentage diminished over time. Organic acids (citric, malic, oxalic, and ascorbic) and sugars (fructose and glucose) reacted continuously, making up for 22 and 16%, respectively, of the total CLD at 2 h. Collectively, the identified compounds accounted for 94% of the total CLD in 2 h.

Significance:
This is the first systematic report on sources contributing to COD and CLD in produce wash water. It underscores distinctions that will influence development and selection of improved wash water treatment strategies to achieve improved safety of fresh-cut produce.

P1-160 Presence of Bacterial Pathogens in Fresh Produce from Local Retail Markets in Maryland Region

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Introduction: Foodborne illnesses associated with the consumption of fresh produce have increased in last decades. Increased consumption of fresh produce, changes in production and distribution systems, and active surveillance by health agencies have been cited as factors contributing to increase in illnesses.

Purpose: The objective of this study was to evaluate the prevalence of bacterial pathogens in fresh produce obtained from local retailers in Maryland.

Methods: Organic and conventionally grown fresh produce (*n*=125) were purchased locally from independent and national retailers. A 25-g sample was transferred to 100ml buffered peptone water, pummeled for 2 min, and appropriately diluted samples were plated on specific agar for total microbial populations and coliforms. Presence of bacterial pathogens (*Salmonella, Escherichia coli* O157:H7, and *Listeria monocytogenes*) was determined by primary and secondary enrichment (RV and TT broth for *Salmonella*, mEHEC broth for *E. coli* O157:H7, and Fraser broth for *L. monocytogenes*) followed by plating on selective media (XLD, CT-SMAC, and *L. mono* agars). The presumptive positive colonies were confirmed by RT-qPCR.

Results: Total populations and coliforms ranged from 4.26-8.29 and 2.95-7.21 log CFU/g, respectively, in fresh produce. There was no significant effect of retailer on total or coliform populations on produce. However, the population varied significantly ($P < 0.05$) with the type of produce. Coliforms in Italian parsley were significantly higher than those in bok choy, kale, cilantro, and cabbage. Microbial quality of organic produce was not different ($P > 0.05$) from conventional fresh produce. Three samples (2.4%) were confirmed positive with bacterial pathogens. Organic green chard was contaminated with *E. coli* O157:H7 and *Salmonella,* whereas *L. monocytogenes* was found in red cabbage. There was no correlation between the presence of pathogens and bacterial populations in fresh produce.

Significance: The results shed light on the microbial safety and distribution patterns of pathogenic bacteria in the locally purchased fresh produce and need for additional safeguards for fresh produce consumption to minimize foodborne illnesses.

P1-161 Assessment of Preparation Methods to Create a Postharvest Wash Water Model for Food Safety Validation

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Introduction: The use of sanitizers in agricultural wash water has shown to be a good water quality-control measure. However, choosing an appropriate sanitizer can be challenging due to differences between produce commodities, processing conditions, and interference with organic load. Current research has been conducted using a variety of methods to model the organic load of vegetable processing water, but different produce can introduce additional elements, affecting the physiochemical properties of the wash water and making it challenging to make comparisons of effectivity among sanitizers from published work.

Purpose: This work aims to identify a suitable model for processing water that is representative of small-scale farming operations that can be used for future validation work.

Methods: The physiochemical properties of processing water from 10 farms in the Pioneer Valley of Massachusetts were analyzed, and laboratory controls for artificial organic load replication were determined as 100 and 50 mg/L chemical oxygen demand (COD) and 100 turbidity (NTU). These were then used to prepare produce processing wash water using two different models (paddle mixing versus homogenized leafy matter), and the wash water was analyzed for pH, oxygen reduction potential, NTU, and COD. The experiment was repeated at 400, 700, 1,000, 2,000, 2,100, and 2,200 mg/L. Data was analyzed using analysis of variance and Duncan's Multiple Range Test (*P*=0.05).

Results: Results reported that there were no statistical differences in physicochemical characteristics between model organic load methods at determined levels of 100 and 50 mg/L and 100 NTU. However, a significant difference between models was seen at COD levels of 2,000. In addition, COD showed to be a stronger indicator of organic load in comparison to turbidity in heavy organic matter conditions.

Significance: Our data suggest that the use of different methods to model organic load can impact the physiochemical properties of the agricultural wash water, making it difficult to compare sanitizer validation work.

Poster

P1-162 Impact of Wastewater and Roof-harvest Water Irrigation on Microbial Quality of Spinach

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Introduction: Secondary-treated wastewater (STWW) and roof-harvest rainwater (RHW) have been considered as alternative irrigation waters to overcome water scarcity. The impact of these waters on microbial safety of fresh produce requires further investigation.

Purpose: The objective of this study was to investigate the effect of STWW and RHW irrigation on microbiological quality of spinach in the field.

Methods: Two field trials of spinach in the summer and winter season were conducted in this study. Six-week old spinach grown in the field was spray irrigated by STWW, RHW, or ground water (GW, control) once a week for two weeks. Four replicate spinach and soil samples from each group (n=160) were collected weekly before irrigation and on 0, 1, 2, and 4 days-post-irrigation (dpi). Water, spinach, and soil samples were analyzed for the indicator bacterial populations and the presence of bacterial pathogens (*Salmonella, Escherichia coli* O157:H7 and *L. monocytogenes*).

Results: For both trials, there was no significant increase in total coliforms and *E. coli* populations on STWW or RHW-irrigated spinach as compared to control irrigated-spinach. Fecal coliforms were significantly ($P < 0.05$) increased from 1.8 log to 3.2 log CFU/g on summer-grown control spinach (average temp. 70°F) at 0 dpi due to the higher bacterial populations in GW. Bacterial die-off rate was higher on winter-grown spinach (average temp. 50°F), and *E. coli* in all irrigation waters used during winter (0.6-1.8 log CFU/100 ml) were below the Statistical Threshold Values (2.6 log CFU/100 ml) for irrigation water according to fresh produce safety rule. Bacterial pathogens were not detected in water or spinach samples.

Significance: STWW and RHW containing low indicator bacterial populations may be used as irrigation waters without affecting the microbiological safety of spinach. Growing season influences the irrigation water quality and bacterial persistence on spinach. Microbiological quality of alternative waters must be determined prior to their use for irrigation.

P1-163 Quantification of *Listeria monocytogenes* Transfer during Slicing of Fresh Produce Based on Inherent Product Characteristics

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❖ ***Developing Scientist Competitor***

Introduction: Many studies have investigated the extent of microbial cross-contamination during slicing of fresh-cut produce,;however, few have examined how product characteristics influence pathogen transfer.

Purpose: This study aimed to quantify the impact of intrinsic produce characteristics (water content, pH, texture, soluble solids content, and surface hydrophobicity) on *Listeria monocytogenes* transfer during mechanical slicing.

Methods: Locally purchased whole onions, radishes, tomatoes, potatoes, carrots, zucchini, cantaloupe, apples, and cucumbers were dip-inoculated with a three-strain avirulent cocktail of *L. monocytogenes* containing ca. 7 log CFU/cm^2 and air-dried for 1 h. Thereafter, one intact sample was sliced to contaminate a Nemco vertical slicer, followed by 15 uninoculated samples of the same product type. Three slices per sample were homogenized by stomaching; they were then then appropriately diluted and surface-plated on modified Oxford agar. For each of the nine different products assessed, a two-parameter exponential decay model was fitted to *Listeria* populations obtained during slicing. Moreover, the intrinsic produce data, including water content, pH, texture, soluble solids content and surface hydrophobicity, were determined and fitted to a linear model to assess their effects on *Listeria* transfer. All experiments were conducted in triplicate.

Results: A multiple comparison of all product transfer data yielded significantly different ($P<0.05$) *Listeria* decay rates, ranging from -0.008±0.002 to -0.275±0.059 for cucumbers and apples, respectively. After fitting a linear model to describe the effects of these inherent produce characteristics on the *Listeria* decay rate, only surface hydrophobicity (radish 32.1 to pears 112^0) and texture (cantaloupe 3.2 to apples 17.3 N) had a significant effect ($P<0.05$), with parameter estimates of 0.002±0.001 and -0.018±0.004, respectively.

Significance: Based on product characteristics, some types of fresh produce such as apples are more prone than cucumber to spreading *Listeria* during slicing. These findings should help define the best order for product slicing and aide in the development of improved predictive models for risk assessment.

P1-164 The Efficacy of Peroxyacetic Acid-based Sanitizer for Disinfection of Seeds Artificially Inoculated with *Salmonella* as Affected by Treatment Time, Concentration, and Seed Type

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Introduction: Certain organic sanitizers including those based on peroxyacetic acid (PAA) have been approved for disinfection of produce but their efficacy in the treatment of seeds for sprouting remains to be evaluated.

Purpose: Evaluate the impact of Tsunami 100 (consisting of 15.2%PAA) on the germination rate and *Salmonella* reduction in artificially inoculated seeds, as affected by treatment time, concentration, and seed type (alfalfa, clover, broccoli, onion, radish, mung beans).

Methods: Ten g of uninoculated seeds were treated with different concentrations of the sanitizer (80, 200, 400 and 1000 ppm PAA) for different periods of time (0.25, 1, 3, 5 and 24h). After rinsing with water twice, 100 seeds were allowed to germinate at room temperature. The germination rate was determined in 3 or 8 days (for onion seeds). Additionally, 10 g of seeds inoculated with 4-6 log cfu/g of a cocktail of five *Salmonella* serotypes (Muenchen, Saintpaul, Cubana, Tennessee, and Mbandanka) were treated with the sanitizer (80 and 400 ppm PAA) for 0.25, 3 or 5h. After treatments, seeds were rinsed twice and analyzed for *Salmonella* by plate counts.

Results: PAA was rapidly consumed by alfalfa and clover seeds (from 1000 to <0.02 ppm within 3h) but less so by other types of seed. The germination rate was >90% for all seeds treated with lower concentrations of PAA (80–400ppm) and shorter periods of time (0.25–5h), but was much lower (1 to 17%) for all but the onion seeds after treatment with 1000 ppm PAA for 24h. Treatment with 80 ppm PAA for 15 min resulted in a greater lot kill for clover and onion seeds, achieving a reduction of 1.0 and 0.7 log units, respectively (on TSA with XLD overlay). Extending the treatment time from 0.25 to 3h did not result in an increase in *Salmonella* reduction. Treatment with 400 ppm PAA for 5h only reduced *Salmonella* on these seeds by 1.2 and 0.9 log units.

Significance: The PAA-based sanitizer did not appear to be effective in reducing *Salmonella* on seeds for sprouting.

Poster

P1-165 Validation of Three-step Wash Process with Commercial Antimicrobials for Control of *Salmonella* and *Listeria monocytogenes* on West Virginia Locally Grown Tomatoes, Cucumbers, and Squashes

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❖ ***Developing Scientist Competitor***

Introduction: The prevalence of *Salmonella* and *Listeria* on locally grown produce sold at West Virginia (WV) and Kentucky farmers market was 16 and 3.78%, respectively. The three-step washing process is suggested by the WV Small Farm Center for removing pathogens from produce surfaces.

Purpose: This study aims to evaluate the three-step wash process with various commercial antimicrobials to reduce pathogens on WV locally grown fresh produce.

Methods: Fresh WV locally grown tomatoes, cucumbers, and squashes were surface-inoculated with *Salmonella* serovars Typhimurium and Tennessee and three-strain mixture of *L. monocytogenes* by dipping method. Produce was washed through three-steps (10 s each), including water rinse, antimicrobial dip, and final water dip, followed by draining for 2 min on paper towels. Tested antimicrobials were: i) lactic and citric acid blend (LCA; 2.5%); ii) sodium hypochlorite (SH; 100 ppm); and iii) a H_2O_2-peroxyacetic acid mixer (SaniDate-5.0, 0.25 and 0.50%). Surviving bacteria were recovered in buffered peptone water and spread plated onto XLT-4 (*Salmonella*) and modified Oxford agar (*L. monocytogenes*). Data (two replicates, three to four samples per replicate) were analyzed using the one-way analysis of variance function of SAS software (P=0.05).

Results: Counts of *Salmonella* recovered on unwashed tomatoes, cucumbers, and squashes were 3.70, 3.80, and 2.67 log CFU/ml, respectively. Antimicrobials reduced (P<0.05) *Salmonella* on tomatoes, cucumbers, and squashes by 0.90 to 1.67, 1.82 to 2.44, and 1.05 to 2.51 log CFU/g, respectively, with the best (P<0.05) reductions showed by 0.5% H_2O_2-peroxyacetic acid mixer. Counts of *L. monocytogenes* recovered on unwashed tomatoes, cucumbers, and squashes were 4.39, 4.31, and 3.72 log CFU/ml, respectively. Antimicrobials reduced (P<0.05) *L. monocytogenes* on tomatoes, cucumbers, and squashes by 0.93 to 1.72, 1.22 to 1.74, and 0.52 to 1.22 log CFU/ml, respectively. The lowest survival on tomatoes and cucumbers were achieved by 0.5% H_2O_2-peroxyacetic acid mixer.

Significance: These results are useful for developing antimicrobial three-step washing protocols to control foodborne pathogens during the postharvest processing of locally grown produce.

P1-166 Environmental Microbial Evaluation in a Papaya (*Carica papaya* L.) Packing Facility Located in Mexico

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✦ ***Undergraduate Student Award Competitor***

Introduction: In recent years, the number of foodborne outbreaks associated with consumption of fresh produce has increased. Five *Salmonella* outbreaks, one in 2011 and four in 2017, were linked to papayas produced and packed in Mexico. Packing facility surfaces and water can act as reservoirs for *Salmonella*. Environmental monitoring provides useful information about potential sources of microbial contamination and sanitation practices.

Purpose: To enumerate indicator microorganisms and detect *Salmonella* in food contact and non-food contact surface samples and water samples collected in a papaya packing facility.

Methods: Food contact and non-food contact surface samples (n=59) and water samples (n=18) were collected on two different days from four zones (following U.S. Food and Drug Administration [FDA] guidelines) in a papaya packing facility. *Enterobacteriaceae*, total coliforms (TC), *Escherichia coli*, and yeast and molds (YM) were enumerated from 400-cm^2 sponge surface samples using Petrifilm plates; TC and *E. coli* were enumerated from 100-mL water samples using the FDA membrane filtration method. *Salmonella* presence was simultaneously investigated for all samples using a loop-mediated isothermal amplification (LAMP) bioluminescence-based method and the FDA Bacteriological Analytical Manual method.

Results: Mean counts (log CFU±SD/sample) for *Enterobacteriaceae*, TC, *E. coli*, and YM on surface samples collected from zone 1 were 2.0(±2.2), 0.7(±1.5), 0.4(±1.3), and 1.6(±1.6), respectively; from zone 2 were 1.1(±1.8), 0.2(±0.8), 0.2(±0.7), and 1.8(±1.2), respectively; from zone 3 were 2.8(±2.4), 2.2(±2.2), 0.4(±0.9), and 2.7(±1.7); and from zone 4 were 2.7(±2.4), 1.5(±2.0), <0.2, and 2.2(±2.0), respectively. All water samples tested negative for *E. coli*. *Salmonella* was not detected in any sample, either by the LAMP-bioluminescence or the FDA-BAM method.

Significance: Good manufacturing practices and sanitation procedures should be reinforced at the packing facility. Data collected could be used to correct problems in specific areas and to prevent product contamination. Further investigation is required to complete a risk assessment.

P1-167 Gamma Irradiation Reduces the Survival and Regrowth of Inoculated Antibiotic-resistant Bacteria and Antibiotic Resistance Genes on Romaine Lettuce

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❖ ***Developing Scientist Competitor***

Introduction: Gamma irradiation effectively reduces foodborne pathogens and spoilage microorganisms on fresh produce. However, limited research is available regarding the effect of gamma irradiation on survival and regrowth of antibiotic-resistant bacteria (ARB) or persistence of antibiotic resistance genes (ARGs) on fresh produce.

Purpose: The objective of this study was to determine the effect of gamma irradiation on the survival and regrowth of inoculated ARB and ARGs on romaine lettuce at two different time points over two weeks of 4°C storage.

Methods: To provide a background inoculum representing potential carryover of ARB from the field, lettuce leaves (n=3, 100 g) were dip-inoculated in compost slurry derived from manure from dairy cows previously dosed with antibiotics and inoculated with multi-drug resistant *E. coli* O157:H7 and *Pseudomonas aeruginosa*. Lettuce was washed with XY-12 (50 ppm free chlorine), packaged in modified atmosphere, treated with 0 or 1.0 kGy gamma irradiation, and stored for 14 days at 4°C. ARB were enumerated on days 1 and 14 by serial dilution and plating onto antibiotic-supplemented eosine methylene blue or *Pseudomonas* isolation agar. Relative abundance of *tet*A was quantified via real-time PCR for each treatment.

Results: Irradiation resulted in ca. 3-log reduction of *E. coli* O157:H7 (P=0.04) and *P. aeruginosa* (P<0.0001). No significant regrowth or declines in ARB populations were observed in irradiated or control samples between 1 and 14 days of storage (P>0.05). Washing lettuce samples led to significant reductions in *tet*A/16S rDNA compared to unwashed samples. However, no statistical difference in *tet*A/16S rDNA copies was noted between irradiated and control lettuce over time.

Poster

Significance: Results suggest that gamma irradiation is an effective treatment to reduce ARB populations on romaine lettuce, but not necessarily for reducing ARGs. Analysis of 16S rRNA sequencing data is underway to determine the effect of irradiation on the overall bacterial community composition of lettuce.

P1-168 Plant Water Stress Limits the Growth of *Salmonella* on Lettuce

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Introduction: Salmonella *enterica* can colonize lettuce plant surfaces utilizing nutrients from surfaced-leached exudates. Our previous work reported a negative correlation between presence of specialized (secondary) metabolites and *Salmonella* on tomato surfaces. When plants are under water stress they synthesize more specialized compounds.

Purpose: Evaluate the effect of water stress on the growth of *Salmonella* on lettuce leaf surfaces.

Methods: Four-week-old lettuce plants (red loose leaf lettuce cultivar 'Mascara') were subjected to water stress for 6 days, or watered regularly (control). Colonies of *Salmonella* Newport and Typhimurium adapted for rifampicin resistance were grown overnight on tryptic soy agar (TSA) at 35°C , then suspended in 0.1% Peptone Water (PW) to a concentration of 10^7 CFU/ml. Aliquots (100 µl) of the cell suspension were inoculated onto the abaxial side of the third or the fourth true leaf of each plant. The inoculated leaf on each plant was clipped 24 hours post-inoculation (hpi), and put in a Whirlpak bag with 30 ml of 0.1% PW. Serial dilutions from the rinsate were plated onto TSA with rifampicin for bacterial enumeration.

Results: Population levels of *Salmonella* retrieved from water-stressed and control plants differed. When leaves were inoculated with ~6.0 logCFU/ml *Salmonella*, the number of *Salmonella* Newport recovered from water-stressed plants after 24 hpi was 1.6±0.2 logCFU/ml of leaf rinsate (n=29), compared to control plants at 2.4±0.2 logCFU/ml of leaf rinsate (P < 0.05) (n=29). *Salmonella* Typhimurium recovered from water-stressed plants was estimated at 1.3±0.3 logCFU/ml of leaf rinsate (n=9), while the level on control plants was 2.2±0.3 logCFU/ml of leaf rinsate (P < 0.05) (n=9).

Significance: These data suggest that lettuce plants responding physiologically to water stress may provide a less favorable environment for *Salmonella* colonization. Understanding how human pathogen-plant interactions are affected by extreme fluctuations in climate is important as climate variability increases.

P1-169 Microbial Quality of Aquaculture Water for Produce Irrigation

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Introduction: Aquaponics, which uses recycled water from a fish tank to irrigate produce, has grown rapidly in recent decades. Because the water contains waste, there are concerns of produce contamination by foodborne pathogens (e.g., *Escherichia coli, Salmonella,* etc.).

Purpose: The purposes of this study were to evaluate the microbial quality of irrigation water from a tilapia production tank and soil used for produce growth, and to determine whether *Listeria monocytogenes* and *Salmonella* were present in the tomatoes and cucumbers grown with the water.

Methods: Populations of generic *E. coli* and coliforms, indicators of fecal contamination, were monitored every two weeks. Water effluents from the fish tank and the plant soils were collected, five replicates for water samples and 15 replicates for soil. *E. coli* and coliform populations were detected using 3M Petrifilm *E. coli*/coliform plates, and data analyzed using ANOVA. *L. monocytogenes* and *Salmonella* were monitored on produce by plating method and confirmed by PCR.

Results: The *E. coli* population in the tank effluent had a geometric mean (GM) of 49 CFU/100mL and a statistical threshold value (STV) of 62 CFU/100 mL in November 2016, decreased to an undetectable level during winter, and rose to a GM of 30 CFU/100mL and a STV of 127 CFU/100mL in May 2017. Coliform populations followed a similar trend, with the highest and lowest populations having a GM of 1820 and 3 CFU/100mL, respectively. Populations of *E. coli* and coliforms in soil were typically higher than water, the highest at 4256 CFU/g for coliforms and 559 CFU/g for *E. coli*. *L. monocytogenes* was detected in five cucumber and one tomato samples; *Salmonella* wasn't found in any samples.

Significance: The *E. coli* population in irrigation water is lower than the regulation limits of 126 CFU/100mL (GM) and 410 CFU/mL (STV) set by the U.S. FDA's Produce Safety Rule. *L. monocytogenes* may be present in produce.

P1-170 Nature Versus Nurture – Survival and Growth on Fresh Produce of Pathogens When Pre-Grown under Different Conditions

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❖ ***Developing Scientist Competitor***

Introduction: Studies evaluating pathogen growth and survival in produce are essential in order to create accurate and industry relevant risk assessments. However, limited comprehensive data is available that describe the effects of strain diversity and growth conditions on subsequent bacterial growth or survival on produce. We thus evaluated selected produce-relevant pathogens, pre-grown under different conditions, for growth and survival on produce.

Purpose: To provide industry-relevant data on the effect of pathogen genetic diversity and growth conditions on produce-relevant growth phenotypes. These data are essential for rational selection of strain cocktails and pre-growth conditions for challenge studies.

Methods: Pathogen strains including *Salmonella* (n=4), *Listeria monocytogenes* (n=4), and *Escherichia coli* (n=5), as well as surrogate, index, and indicator organisms (n=3), were pre-grown under seven different conditions (e.g., low pH, 21°C) and inoculated on tomatoes, cantaloupe, and lettuce. Inoculated produce was incubated for up to seven days and strains enumerated on days 0, 1, 3, 4, and 7. A crossed random-effects model was used to analyze the contributions of random choice in strain or condition.

Results: Pre-growth conditions showed a large effect on the variation of growth and die-off as compared to the variation attributed to different strains grown under a given condition. For example, *Salmonella* strains inoculated on tomatoes showed less strain variation within one condition (e.g., for pre-grown to stationary phase, day seven recovery of strains ranged from 6.98 to 7.74 log). For a single *Salmonella* strain pre-grown under different conditions recovery on day seven ranged from 5.1 to 8.15 log (for pre-growth at 21°C and NaCl, respectively).

Significance: In combination with previous data, our results indicate that pre-growth conditions effect pathogen stress resistance, as well as growth and die-off in challenge studies. This knowledge now needs to be translated into guidance information that can be used in the design of validation studies.

Poster

P1-171 Effect of Residual Chlorine and Organic Acids on the Survival and Attachment of *Listeria monocytogenes* and *Escherichia coli* O157:H7 on Spinach

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Introduction: Post-harvest washing and sanitation is important to reduce microbial food safety risks. However, subsequent steps such as handling, storage and packaging may pose a risk of cross contamination.

Purpose: This study investigated the effect of residual chlorine and organic acids on the survival and the attachment of *L. monocytogenes* and *E. coli* O157:H7 on spinach under refrigerated storage conditions.

Methods: Baby spinach leaves were sanitized with chlorine solution (100 ppm) or 0.5% organic acids (lactic acid or acetic acid), for 3 minutes. At 0 and 30 minutes after the treatment, a cocktail (3 strains) of *E. coli* O157:H7 and *L. monocytogenes* were spot inoculated on the surface (8 cm^2) of the leaves and spread using sterile glass rod. The inoculated leaves were stored at 4°C, and the survival and attachment of the tested organisms were examined for 48 h.

Results: A significant reduction (P<0.05) of *L. monocytogenes* was observed within 15 min on the chlorine treated spinach leaves. The reduction was 2.15 and 1.23 log CFU/cm^2 on the leaves contaminated at 0 min and 30 min after the treatment, respectively. After 48 h, the reduction was up to 3.16 log CFU/cm^2, which was significantly higher (P<0.05) than in untreated controls (0.43 log CFU/cm^2). The proportion of attached cells (S_R) of *L. monocytogenes* was significantly higher (P<0.05) in chlorine treated leaves (0.47 to 0.99) compared to the controls (0.16 to 0.73). A significant reduction of both *E. coli* O157: H7 and *Listeria monocytogenes* population was observed on lactic acid treated samples; however, the level remained same on the acetic acid treated samples. The effect of organic acids on the attachment of *L. monocytogenes* was variable with storage time.

Significance: The results indicate that residual sanitizers may affect the activity of contaminated microorganisms during post-sanitation period.

P1-172 Influence of Plant Defense Response on Survival and Interaction of *Escherichia coli* O104:H4 on *Arabidopsis* Plant and Lettuce

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) has caused illnesses and outbreaks associated with fresh vegetables. As an emerging non-O157 STEC, *E. coli* O104:H4 was linked to very large outbreaks, including hemolytic-uremic syndrome, in 2011. There is limited information on interaction mechanisms of *E. coli* O104:H4 with plants, including plant defense response regulating microbial fitness.

Purpose: This study aimed to evaluate survival and persistence of *E. coli* O104:H4 on plants and leafy vegetables, particularly the potential influence of plant defense response and pathogen capsular polysaccharide (CPS) on survival.

Methods: To investigate survival ability, four week-old *Arabidopsis thaliana* and romaine lettuce were dip-inoculated with *E. coli* O104:H4 (RG1 and C3493) and *E. coli* O157:H7 strains (Sakai and 7386). On days 1 and 5 post-inoculation, plants were harvested and populations of each *E. coli* strain were determined. CPS production by the *E. coli* strains were quantified using hot phenol-water extraction. Plant pathogenesis-related gene (*PR1*) expression was examined by reverse transcription quantitative PCR.

Results: E. coli O104:H4 strains showed better survival on *Arabidopsis* and lettuce compared with *E. coli* O157:H7 strains. On day 5, populations of *E. coli* O104:H4 RG1 (4.1 log CFU/g) and C3493 (3.6 log CFU/g) on *Arabidopsis* were significantly (P <0.05) greater compared to *E. coli* O157:H7 7386 (2.3 log CFU/g) and Sakai (2.3 log CFU/g). Plants inoculated with *E. coli* O104:H4 strains showed weak expression of the *PR1* gene, a marker of plant defense response, compared with *E. coli* O157:H7 strains. In addition, *E. coli* O104:H4 strains produced significantly (P<0.05) greater amounts of CPS.

Significance: This study provides better understanding of the survival behavior of *E. coli* O104:H4 on plants and leafy vegetables in pre-harvest environments, which is helpful for designing intervention strategies that provide safe vegetable crops. The overall finding also demonstrates the importance of studying emerging non-STEC strains.

P1-173 Investigation of Microbial Contamination Source during Production of Dried Red Pepper

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Introduction : In recent years, spices have been involved in a number of cases of foodborne illness. To enhance the safety of red pepper powder, a common spice in Korea, it is necessary to identify and prevent the microbial contamination source in the production process of dried red pepper which is raw material of red pepper powder.

Purposes : The purpose of this study was to investigate the main source of contamination of dried red pepper by assessing microbial loads on red peppers, washing water, washing machines, harvesting containers, and worker gloves that had come in contact with the dried red pepper.

Methods : To estimate microbial loads, indicator bacteria (total bacteria, coliform bacteria, and *Escherichia coli*) and pathogenic bacteria (*E. coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*) were investigated. After each sample individually was cultured in enrichment broth, each culture was streaked on each selective agars including including CHROMagar O157 for *E. coli* O157:H7, Xylose Lactose Tergitol™ 4 (XLT-4) agar for *Salmonella* spp., and CHROMagar Listeria for *L. monocytogenes*, respectively. The identity of suspected colonies on each selective agar was confirmed with VITEK.

Results : The results showed that the number of indicator bacteria increased significantly after washing red peppers compared with that before washing (P < 0.05). Moreover, *E. coli* and *Listeria* spp. were recovered from the red peppers after washing and from the ground water used in the washing process. The number of indicator bacteria on red peppers dried in the green house was lower than that on red peppers dried in a dry oven (P < 0.05). However, *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* were not detected.

Significance : These results suggested that a disinfection technique may be needed during the washing step in order to prevent potential contamination. In addition, hygienic practices during the drying step using the dry oven, such as establishment of an optimal temperature, should be developed to enhance the safety of dried red pepper.

Poster

P1-174 Antimicrobial Effects of Chlorine Dioxide on Pathogenic *Escherichia coli* and *Salmonella* spp. Colonized on Alfalfa Seeds

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Introduction: In recent years, alfalfa have been involved in a number of cases of foodborne illness. It has been reported that the cause was seeds contaminated with pathogens. To prevent the outbreak associated with alfalfa sprout, it is necessary to reduce these pathogens in alfalfa seeds using the sanitizer, such as chlorine dioxide (ClO_2).

Purpose: The purpose of this study was to establish conditions for maximizing the efficiency of ClO_2 to control pathogenic *E. coli* and *Salmonella* spp. contaminated with alfalfa seeds.

Methods: Alfalfa seeds were inoculated with pathogenic *E. coli* (four strains isolated from irrigation water and sprout in sprout farm), *Salmonella* spp. (ATCC 4931, ATCC 19586, and NCCP 13697) at a level of 6.0 log CFU/g, respectively. Alfalfa seeds were treated with ClO_2 at different concentrations (50, 100, 150, and 200 ppm) for 15, 30, 45, and 60 min after 5 hour soaking seeds. After exposure to 200 ppm of ClO_2 for 60 min after 5 h soaking seeds, the growth of pathogens was investigated during 3days of germination from seed to sprout. For investigation of antimicrobial effect on pathogenic *E. coli* and *Salmonella* spp., 1- mL of each treated sample was serially diluted with 9 mL of 0.1% peptone water, and 0.2 mL aliquots of each dilution were spread-plated onto TSA-R agar, followed by incubation under 37°C for 24 h.

Results: The reduction level of pathogens on seeds exposed to ClO_2 after 5 h soaking was higher than those exposed to ClO_2 without soaking ($P < 0.05$). As the concentration of ClO_2 and the treatment time were increased, the killing effect of the pathogens increased. Pathogenic *E. coli* and *Salmonella* spp. in alfalfa seeds which were not treated with ClO_2 proliferated up to 9 log CFU/g during 3 days of germination from seed to sprout. However no growth of these pathogens were observed in seeds treated with 200 ppm of ClO_2 for 60 min after 5 h soaking.

Significance: The results suggest that the treatment of 200 ppm of ClO_2 for 60 min after 5 h soaking seeds is more effective to control pathogens contaminated with alfalfa seeds.

P1-175 Papaya-associated Outbreaks of *Salmonella* Illnesses in 2017 – Traceback and Laboratory Results

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Introduction: In 2017, four papaya-associated outbreaks of *Salmonella* (multiple serotypes) were reported with a total of 244 ill persons. Traceback and laboratory results were critical to confirming papaya as the vehicle and determining the source origins of products.

Purpose: The purpose was to determine the source of papayas that ill persons consumed, supporting the epidemiologic evidence that papayas were the vehicle implicated in four outbreaks of salmonellosis. Papaya sampling from imported products was conducted by FDA to monitor for *Salmonella* contamination.

Methods: State and FDA laboratories used the FDA BAM method to isolate *Salmonella* from papaya samples collected at various points of distribution. Serotyping, pulsed field gel electrophoresis (PFGE), and phylogenetic analyses of whole genome sequencing (WGS) data were conducted to characterize the isolates. Local, state, and federal officials collected records (invoices, bills of lading, and import entries) that were reviewed and compiled to determine the sources of papayas.

Results: Four different suppliers of papayas were linked by record review and *Salmonella* positive laboratory results to four separate outbreaks of *Salmonella* associated with papayas. Multiple states tested papaya samples from their states, and Maryland and Virginia investigators recovered outbreak strains of *Salmonella*. One hundred eighty-three papaya samples were collected by FDA in 2017, many in response to the outbreak investigations. A total of 11 FDA papaya samples were positive for *Salmonella*, yielding 62 isolates. Eleven serotypes and 17 different PFGE patterns were identified. WGS was conducted on all 64 FDA isolates obtained. Nine serotypes of *Salmonella* identified in papaya samples were related to clinical outbreak isolates of *Salmonella* by PFGE and WGS.

Significance: Four specific supplying farms were identified, and their names were released to the general public, retailers, and foreign authorities. This information was used to protect public health through individual awareness, recalls, and import alerts.

P1-176 Development of Hot Water Process for Inactivating *Salmonella enterica* on Inoculated Mung Bean Seeds for Enhancing Microbial Safety of Mung Bean Sprouts

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Introduction: The sprouting conditions of mung bean seeds provide optimal conditions of temperature and humidity for any potential pathogenic contaminant on the seeds to grow. The lack of a kill step post-sprouting is a major safety concern. The use of a kill step on the seeds prior to sprouting would enhance the safety of fresh sprouts.

Purpose: The objective of this work was to develop a hot water process for 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 80°C for 90 s with or without mixing was investigated. The effect of this hot water treatment on seed sprouting and recovery of *Salmonella* cells from sprouted seeds were also investigated.

Results: Hot water treatments at 80°C for 90 s, in combination with mixing, was capable of eliminating this pathogen on seed surfaces (in excess of 5 log CFU/g). Sprouts prepared from these seeds were negative for this human pathogen. The complete inactivation of this pathogen on the seeds could be attributed to increased uniformity in heat transfer and exposure to hot water medium. Scanning electron microscopy imaging indicated that *S. enterica* cells were completely removed from the surface of the inoculated mung bean seeds. All treated seeds were capable of germinating as well as the non-treated controls.

Significance: These results suggested that hot water treatments are capable of penetrating and inactivating cells that are attached to inaccessible sites and/or within biofilms on the seed surface. Therefore, this hot water treatment would be a viable process for enhancing the safety of fresh sprouts without compromising yield and quality.

P1-177 Effectiveness of Aqueous Chlorine Dioxide Treatment in Reducing Microbial Food Safety Risk during Sprouting of Alfalfa Seeds

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❖ ***Developing Scientist Competitor***

Introduction: Sprouts are considered high-risk foods because the sprouting conditions provide a favorable environment for the growth of microorganisms. Aqueous chlorine dioxide treatment during the sprouting process can be used as a potential intervention.

Purpose: This study evaluated the effectiveness of aqueous chlorine dioxide (ClO_2) treatment on the survival of generic *Escherichia coli* during the sprouting of alfalfa seeds.

Methods: Alfalfa seeds were first inoculated with a three-strain cocktail of generic *E. coli* (ATCC 23716, 25922, and 11775) and dried overnight inside a biological safety cabinet. The dried seeds (~5 log CFU/g) were then pre-soaked in distilled water for 8 h and then germinated for 5 days. On each day of germination, the contaminated seeds/sprouts were rinsed with aqueous ClO_2 (3 ppm) or water (1:10 weight/volume) for 10 min. Seed/sprout samples before and after daily sanitizing treatments were collected for microbiological analysis. On the 5th day, untreated inoculated seeds were segregated into roots, stems, and leaves for microbial analysis. Enumeration of generic *E. coli* was done by using three Petrifilm *E. coli* count plates.

Results: On soaking seeds in distilled water for 8 h, the levels of generic *E. coli* significantly increased from 5.01±0.10 to 7.07±0.26 log CFU/g. During five days of sprouting, the levels increased to 8 log CFU/g in both ClO_2 treated and untreated seeds. However, on a given day, analyzing samples after treatment with aqueous ClO_2 the generic *E. coli* levels were significantly (P<0.05) reduced. A maximum reduction of 0.95 log CFU/g was observed on the first day of sprouting with aqueous ClO_2, while the deionized water rinse did not achieve any reduction. After five days of sprouting, the roots had the highest count (7.29 log CFU/g), followed by the leaves (6.92 log CFU/g) and stalks (5.60 log CFU/g).

Significance: Aqueous chlorine dioxide showed promise to reduce the levels of generic *E. coli* during the sprouting of alfalfa seeds.

P1-178 Growth Potential of *Listeria monocytogenes* in Artificially Contaminated Cut Apple

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Introduction: Listeria monocytogenes is a foodborne pathogen that causes listeriosis in humans. Apples have been identified as the vehicle in some recent *L. monocytogenes* outbreaks, raising questions regarding the growth potential of *L. monocytogenes* in apples.

Purpose: Understanding biochemical parameters of apple varieties on *L. monocytogenes* growth may have important implications in risk assessment and disease mitigation.

Methods: Five apple varieties (Braeburn, Gala, Red Delicious [RD], Golden Delicious [GD], and Granny Smith [GS]) were used in this study. The growth potentials of *L. monocytogenes* on artificially inoculated cut apple chunks were tested after incubation at 10°C. A rifampicin-resistant serotype 1/2b strain (LS748), was used as an inoculum at 6 to 9×10^4 CFU/5 g chunk. Samples were collected on alternate days for 12 days, plated onto brain heart infusion agar containing selective antibiotics, and incubated at 37°C for 24 h before colonies were counted. The three-parameter Gompertz bacterial growth model was modeled on time (t=days) for each apple type. Biochemical parameters such as pH, titratable acidity, total polyphenol, and total antioxidant contents were also measured.

Results: L. monocytogenes grew approximately 2 log (P<0.0001) in the chunks of all apple varieties, except GS where concentrations remained fairly stable over time with no measurable change (P>0.99). The growth rates of inoculated *L. monocytogenes* (log CFU/day) were comparable for Gala (0.44), GD (0.4), and RD (0.53), although the growth rate for Braeburn was slightly higher (0.84). The pH values of different apple varieties varied between 3.2 and 4.1. The polyphenol, antioxidant, and acidity for GS were significantly higher than those of other apple varieties (P<0.05), suggesting that these factors, or a combination thereof, may have an inhibitory effect on *L. monocytogenes* growth in GS apples.

Significance: These results show that *L. monocytogenes* has variable growth potentials in different apple varieties and that biochemical properties may have a role in controlling the growth of *L. monocytogenes*.

P1-179 Microbiological Quality and Prevalence of Pathogens in Strawberries (*Fragaria x ananassa*) in the United States

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Introduction: As the consumption of strawberries increases and the year-round availability of this fresh produce from abroad increases, microbiological quality must be surveilled to identify potential hazards following the approach of the FDA Food Safety Modernization Act.

Purpose: To evaluate the levels of indicator organisms and prevalence of pathogens in strawberries from domestic production and imported from Mexico.

Methods: Fresh strawberries (50 samples) were collected from local markets in the Baton Rouge, Louisiana area. Samples (25 g) were analyzed for aerobic plate count (APC), total coliforms, and *Escherichia coli* using 3M™ Petrifilms™ at 37°C for 48 h. Ten g were enriched in Tetrathionate Broth Base with Iodine solution for 24 h at 35°C, and plated in XLD agar at 35°C for 24 h. An additional 10 g were enriched in Listeria Enrichment Broth at 30°C for 48 h, and plated in Oxford agar at 35°C for 24 h. Colonies resembling *Listeria monocytogenes* were isolated on blood agar at 20°C for 24 h. Presumptive positives where identified with a Microgen® Listeria latex test.

Results: APC on imported strawberries ranged from 3.6 to 7.01 log CFU/g and <1 to 3.06 log CFU/g for coliforms. APC in domestic strawberries ranged from 3.82 to 6.11 log CFU/g. Coliforms ranges from <1 to 3.32 log CFU/g. *E. coli* was below detection limits (<1 log CFU/g). No significant differences in microbial indicators between domestic and imported strawberries was found (p>0.05). Prevalence of pathogens was 0 and 10% for *Salmonella* and *Listeria monocytogenes*, respectively.

Poster

Significance: Prevalence of pathogens in fresh strawberries raises concerns regarding the potential safety of fresh produce.

P1-180 Field Validation of Minimum Application Intervals for Raw Animal Manure Used as a Soil Amendment at a Certified Organic Research Farm in California

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Introduction: Raw animal manure used as a soil amendment in fresh organic produce production is valuable for soil health, but also increases the public health risk from foodborne pathogens. The United States Department of Agriculture's National Organic Program (NOP) standards for raw manure are based on time-interval criteria between application of manure and crop harvest, but have not been validated in the context of microbial food safety.

Purpose: To conduct an experimental field trial to evaluate *Escherichia coli* survival in manure-amended soil and transfer to tomato fruits.

Methods: Organic heirloom tomatoes were transplanted to field plots (4 m^2) amended with untreated dairy solids (DS), horse manure (HM), poultry litter (PL), and unamended controls (UA) at a University of California - Davis certified organic research farm in May 2017. Tomatoes were grown with plastic mulch and buried drip irrigation. Four replications per amendment type were spray inoculated with a three-strain cocktail of rifampicin-resistant (*rif*) *E. coli* at 7 to 8 log CFU/ml. Soil samples (n=200) were collected serially from 0 to 180 days post inoculation (dpi). Composite tomato samples (n=60) were harvested once ripe. *rif E. coli* was enumerated from samples by direct plating and most probable number (MPN).

Results: Soils at 90 dpi had log reductions in *rif E. coli* populations of 4.3 (DS), 5.1 (HM), 5.3 (PL) and 6.3 (UA); at 120 dpi, log reductions were 5.3 (DS), 5.8 (HM), 6.2 (PL) and 5.5 (UA). On the final sample day (180 dpi), the concentration of *rif E. coli* (MPN/g) had decreased to 3.7 (DS), 18.0 (HM), 0.7 (PL), and 0.6 (UA). The inoculum was not detected from any tomato fruits. Experiment day and *rif E. coli* concentration were significantly associated (P<0.001), but not by manure type.

Significance: The outcomes of this project will benefit organic farmers and consumers by generating data to inform policy related to wait periods for the safe use of raw animal manure.

P1-181 Efficacy of Two Hand-hygiene Interventions at Reducing Hand Contamination among Produce Farmworkers in Northern Mexico

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Introduction: Studies on the hands of farmworkers during jalapeño harvest showed that hand washing reduced soil, while alcohol-based hand sanitizers (ABHS) reduced indicator bacteria. However, it is unknown whether hand hygiene intervention results are specific to produce type.

Purpose: To compare the efficacy of two hand hygiene interventions at reducing contamination on melon farmworkers' hands in Mexico and to determine if intervention efficacy differs between melon and jalapeño farmworkers.

Methods: A total of 129 melon and 159 jalapeño farmworkers from four farms in Mexico were randomly assigned to three groups: hand washing, two-step ABHS (Sanitwice), or no hand hygiene (control). After one 30-min harvest cycle, hand hygiene interventions were performed and hand rinse samples collected. Rinse samples were tested for soil (absorbance, A_{600nm}) and bacterial indicators (coliforms, generic *Escherichia coli*, *Enterococcus*, and AllBAC and BFD *Bacteroidales* markers). Groups were compared using linear regression (α=0.05) and adjusted for multiple comparisons (Tukey's test). Melon and jalapeño data were compared using two-way, fixed-effects models.

Results: Compared to the control group (geomean A_{600nm}=0.138), both handwashing (geomean A_{600nm}=0.014; P<0.0001) and Sanitwice (geomean A_{600nm}=0.043; P<0.0001) interventions yielded significantly lower absorbance levels on melon farmworkers' hands, with the hand washing group having the lowest (P<0.0001). The concentrations of bacterial indicators on melon farmworkers' hands did not differ across intervention group (P=0.1238 to 0.4168). The efficacy of Sanitwice and hand washing, compared to controls, differed between melon and jalapeño farmworkers; fixed-effects interactions between intervention group and produce type were significant for absorbance (P=0.0018), *E. coli* (P=0.0050), and coliforms (P=0.0005), but not for *Enterococcus* (P=0.2797).

Significance: Although both Sanitwice and hand washing reduced soil on melon farmworkers' hands after one 30-min harvest, neither intervention reduced indicator bacteria. The efficacy of Sanitwice and hand washing interventions differed for melon and jalapeño farmworkers, suggesting it may be necessary to develop produce-specific hand hygiene interventions in agricultural settings.

P1-182 *Salmonella* Infiltration into Whole Mangoes

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Introduction: Salmonella outbreaks have been linked to imported mangoes, which undergo a heat treatment prior to hydrocooling in order to control and prevent importation of the Mediterranean fruit fly.

Purpose: The purpose of this study was to quantify internalization of *Salmonella* into different mango varieties.

Methods: To mimic commercial fruit fly heat treatments, three mango varieties, Ataulfo, Kent, and Tommy Atkins, underwent a 75 to 100 min heat treatment (based on size) prior to submersion hydrocooling for 30, 40, or 50 min in 21°C water containing a 6.0 log CFU/ml *Salmonella* cocktail. Alternatively, mimicking commercial fruit fly treatments, mangoes rested for 30 min at room temperature following heat treatment and before hydrocooling. Infiltration was determined by sampling flesh from the stem end, middle equatorial section, and blossom end. Internalized *Salmonella* populations were determined by plating onto rifampicin-supplemented tryptic soy agar or by most probable number in triplicate with duplicate samples (n=6).

Results: Salmonella infiltration was significantly (P<0.05) impacted by variety, fruit region, hydrocool time, and addition of a rest period between heating and hydrocooling. Mangoes hydrocooled for 50 and 40 min resulted in greater infiltration (3.2 and 3.1 log CFU/segment) compared to 30 min (2.7 log CFU/segment). Tommy Atkins and Atualfo varieties (3.1 and 3.2 log CFU/segment, respectively) were more susceptible to infiltration than Kent (2.7 log CFU/segment). Greater concentrations of *Salmonella* were present in flesh sampled from the stem ends for all three varieties (4.0 to 4.7 log CFU/segment) than from the blossom or middle sections (1.9 to 2.8 log CFU/segment). The addition of a 30-min rest period resulted in a significant reduction of internalized *Salmonella* from 3.1 to 2.8 log CFU/sample (P<0.05) across all varieties and flesh regions.

Significance: Salmonella internalizes into whole mangoes, with some varieties and regions having greater susceptibility. The addition of a rest period significantly reduces *Salmonella* infiltration.

P1-183 Effect of Aqueous Ozone Treatment on the Survival of *Listeria monocytogenes* during Sprouting of Alfalfa Seeds

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Introduction: Listeria are bacteria which are ubiquitous to the environment and associated with significant food safety burdens. Optimum sprouting procedures, which require warm and humid conditions, are favorable for the growth of *Listeria monocytogenes*.

Purpose: This study evaluated the effect of ozonated water (2 ppm) on the survival of *L. monocytogenes* during the sprouting of alfalfa seeds.

Methods: Alfalfa seeds were inoculated with a three-strain cocktail of *L. monocytogenes* and germinated at 25°C and 78 to 87% relative humidity for five days. Inoculated seeds were rinsed with distilled water every 12 hours to maintain proper sprouting conditions. On each day during germination, contaminated seeds were rinsed with ozonated water (2 ppm) (10:1 volume/weight) for 5 min. Seed/sprout samples before and after daily sanitizing treatments, spent treatment solutions, and rinsing water were collected for microbial analysis. On the 5th day, untreated inoculated seeds were segregated into roots, stems, and leaves for microbial analysis. Enumeration of *L. monocytogenes* was done by spread plating on Oxford agar.

Results: Ozonated water did not have a significant (P<.05) effect on reducing *L. monocytogenes* counts during alfalfa seed sprouting. Throughout five days of sprouting, inoculated microorganisms increased from 3.88 to 7 log CFU/g. Reductions in *L. monocytogenes* from daily aqueous ozone (2 ppm) treatments ranged from -0.04 to 0.63 log CFU/g. The most contaminated portion was the roots (7.30 log CFU/g), followed by the stalks (7.01 CFU/g) and leaves (6.75 CFU/g). The level of *L. monocytogenes* in the spent rinsing water and sanitizing solutions increased throughout germination. Spent ozone treatment solutions contained significantly lower levels (4.6, 4.55, and 5.00 log CFU/ml) of *L. monocytogenes* compared to the control (5.11, 6.15, and 6.80 log CFU/ml) from day three to day five of germination, respectively.

Significance: Sequential aqueous ozone (2 ppm) treatment is insufficient at significantly reducing *L. monocytogenes* on alfalfa seeds and sprouts during sprouting.

P1-184 Survival of *Listeria* in Imazalil with Added Peracetic Acid and in Soda Ash Fresh Citrus Fungicide Solutions

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Introduction: Green and blue molds can result in significant postharvest losses of citrus fruit. To reduce such losses, fungicide solutions are applied to oranges or lemons in recirculating tanks or sprays. The impact of these fungicides on the survival of *Listeria monocytogenes* is not known.

Purpose: Evaluate the survival of *L. monocytogenes* in imazalil (IMZ) with and without peracetic acid (PAA) and in 3% soda ash.

Methods: Imazalil (~300 ppm) with 0 to 60 ppm PAA and soda ash (3%, pH >11) solutions were prepared with water obtained from citrus packinghouses. The preparations were inoculated (5.5 log CFU/ml) with a six-strain rifampin-resistant cocktail of *L. monocytogenes* and held at either 16 or 40°C for 0, 0.5, 1, 3, and 5 min. Cross-contamination potential was assessed by commingling uninoculated and *L. monocytogenes*-inoculated oranges or lemons in fungicide solutions for 1 min. Samples (n=6) were neutralized in Dey-Engley broth and plated onto tryptic soy agar with 50 µg/ml rifampin; remaining samples were enriched at 37°C for 48 h and then streaked onto selective agar.

Results: At 16°C, reductions of ≥5 log of *L. monocytogenes* were achieved in ≥5 or ≥3 min at 20 and 25 ppm, ≥3 or ≥2 min at 30 and 45 ppm, or ≥2 or ≥1 min at 60 ppm PAA in the absence or presence of IMZ, respectively. At 40°C, a ≥5-log reduction was observed within 1 min exposure of IMZ with 20 ppm PAA. No significant reductions of *L. monocytogenes* were observed in 3% soda ash solutions within 5 min. Cross contamination from inoculated to uninoculated fruit was prevented in 20 ppm PAA/IMZ, but not in soda ash solutions.

Significance: Cross-contamination of citrus fruit with *L. monocytogenes* was prevented in IMZ by addition of 20 ppm PAA; cross-contamination was not prevented in soda ash.

P1-185 Biofilm-forming Capability of *Salmonella enterica* on Papaya Epicarp (*Carica papaya* L.)

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Introduction: The consumption of papaya (*Carica papaya* L.) has been associated with outbreaks of *Salmonella enterica*. Evaluations carried out in other fruits revealed that chemical disinfection does not achieve the complete inactivation of pathogenic microorganisms. One of the factors that can influence the effectiveness of disinfectants is the formation of bacterial biofilms on the surface of the fresh product.

Purpose: The objective of this work was to capability *Salmonella enterica* to form biofilms on the epicarp of papayas var. Maradol.

Methods: Fifty microliters (~ 1 x 10^5 Log mL^{-1}) of a cocktail *S. enterica* (*S.* Enteritidis ATCC 13076, *S.* Typhimurium ATCC 23595, *S.* Typhimurium ATCC 14028, *S.* Thompson ATCC 8391, *S.* Montevideo ATCC 8387) resistant to rifampicin were inoculated on the epicarp of papaya (3 cm by 3 cm by 2 mm) (n=32). Papayas were stored at 25 and 30°C and 97% relative humidity for up to 72 h. Biofilm formation was monitored by taking measurements at 0, 12, 24 and 72 h of incubation. At each sampling time, the following assessments were performed: 1) Planktonic cells were detached by homogenized the portion of papaya in stomacher and they were counted in triptic soy agar; 2) polymer formation was determined by crystal violet assay in microtiter plates; 3) samples were reviewed with scanning electron microscopy.

Results: At 25°C, population of *Salmonella* increased 1.2 Log CFU g^{-1}; optical density (ΔDO_{595nm}) also increased 0.32 with respect to a negative control. After 48 h *Salmonella* population and polymers showed an increase of 2.2 Log CFU g^{-1} and 0.65 DO_{595nm}, respectively. There were no statistically significant differences at 72 h (P > 0.05). Similar results were observed at 30°C. Electron micrographs showed aggregates of bacterial cells embedded in polymeric material.

Significance: These results indicate that *Salmonella* is able to grow and form biofilms on papaya tissue after 24 h under conditions of this study; therefore, the efficacy of chemical disinfectants could be affected.

Poster

P1-186 Efficacy of Aqueous Chlorine Dioxide in Reducing *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on Sweet Potatoes

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Introduction: Current postharvest practices utilize chlorine-based sodium hypochlorite, despite its limited efficacy in reducing human pathogens. Aqueous chlorine dioxide, a stronger oxidizing agent than chlorine, is an effective sanitizing agent against a broad spectrum of microorganisms.

Purpose: This study evaluated the efficacy of aqueous chlorine dioxide (ClO_2) treatment in reducing the levels of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* in sweet potatoes.

Methods: Louisiana-grown sweet potatoes were spot inoculated (10^7 CFU/sweet potato) with multi-strain cocktails of *S. enterica*, *E. coli* O157:H7, and *L. monocytogenes* and dried for 90 min inside a biosafety cabinet. The inoculated samples were dipped in buckets containing 5 ppm aqueous ClO_2 or water (1:4 weight/volume) for 10, 20, and 30 min. The treated samples were collected into stomacher bags containing 100 mL of 0.1% peptone water and gently hand massaged for 2 min. The rinsate after massaging and the wash solutions were used to enumerate the pathogens. The data was analyzed by analysis of variance test using SAS software.

Results: Aqueous ClO_2 treatment significantly (P<0.05) outperformed water treatment after 20 min, reducing *Salmonella* by 2.14 log CFU/cm² compared to 0.87 log CFU/cm² for water treatment, though 30 min of aqueous ClO_2 treatment did not exhibit significant further reduction. Similar results were observed for *L. monocytogenes* with a reduction of 1.64 log CFU/cm² after a 20 min treatment with aqueous ClO_2, compared to 0.76 log CFU/cm² for water treatment. However, no significant difference on *E. coli* O157:H7 counts was observed between samples treated with aqueous ClO_2 and water after 30 min of treatment. None of the pathogens were recovered from ClO_2 wash solutions, while water treatment wash solutions had pathogen levels from 3.47 to 4.63 log CFU/mL.

Significance: The findings of this study indicate that aqueous ClO_2 is effective in reducing cross-contamination during postharvest washing of sweet potatoes.

P1-187 Survival of Human Pathogens at Room and Refrigerated Temperatures on Tomato and Kale

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❖ ***Developing Scientist Competitor***

Introduction: Contamination of fresh produce with human pathogens remains an important public health concern. Inadequate consumer storage practices increase the risk of bacterial growth and survival. There is limited research available on the survival of pathogens during specific domestic storage conditions on fresh kale and cherry tomatoes.

Purpose: This study was conducted to characterize the survival and growth of *Listeria monocytogenes* and *Salmonella* spp. on fresh tomatoes and kale at both room and refrigerated temperatures.

Methods: Cocktail inocula of *L. monocytogenes* and *Salmonella* spp. were used to spot inoculate kale and tomato, at 6 log CFU/mL. Kale leaves and whole grape tomato samples were placed at either room temperature (24°C) or refrigerated temperature (4°C) storage for up to 14 days. The samples were homogenized and serial dilutions were made for enumeration after 24 h of incubation at 37°C. The experiments were replicated six times. The concentrations were calculated and the collected data were analyzed using the statistical computation program, R.

Results: Room temperature supported better of pathogens on both produce variants (P < 0.001). *Listeria* demonstrated better persistence at refrigeration temperature than *Salmonella* spp. at each day of storage (1, 3, 7, and 14) post inoculation (P < 0.05; P < 0.01; P<0.001; P < 0.001, respectively). *Salmonella* spp. persisted better than *Listeria* at room temperature for every day of storage (P<0.001). Pathogen growth did not differ between tomato and kale, when stored at room temperature. At refrigeration temperature, both pathogens grew better on kale than on tomato on each day of storage (P < 0.01), with the exception of day seven. On this day, lower counts were observed and were similar to count on tomato (P = 0.297). Over the duration of storage, *Listeria* survived better in the refrigerator than at room temperature on tomato (P < 0.01), but temperature had no effect on *Listeria* persistence on kale (P = 0.902). *Salmonella* spp. demonstrated better survival at room temperature on both kale and tomato, with concentrations significantly increasing every day of storage at room temperature (P < 0.01). At refrigerated temperature, *Salmonella* spp. persisted but did not grow.

Significance: The data from these experiments highlight the importance of proper storage practices to minimize pathogen growth and survival. Pathogens are able to persist and even grow on foods at refrigeration temperatures. For food safety, storage of whole tomatoes in the refrigerator is better to prevent *Salmonella* growth.

P1-188 Influence of Outside Factors on the Concentration and Stability of Peracetic Acid-based Produce Sanitizers over Time

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❖ ***Developing Scientist Competitor***

Introduction: Produce sanitizers are a method to manage water quality and food safety risks on farm. However, established quality controls are limited on the management of these sanitizers. Peracetic acid (PAA) based sanitizers are a common sanitizer option yet there is little guidance on the proper handling of these sanitizers. Therefore, more work is needed to better understand PAA based systems so that best management practices can be established.

Purpose: This study investigated a commercially available PAA based sanitizer to assess the product quality over time and under different stressed conditions.

Methods: A commercially available sanitizer was aliquoted and stored at 5°C, 20°C, and 37°C. For 12 weeks, a sample was pulled and tested at 30ppm PAA in deionized water. Samples were titrated with ceric sulfate and sodium thiosulfate to determine concentrations of hydrogen peroxide (H_2O_2) and PAA in the sample. The same commercial sanitizer was also tested at four organic loads (0, 100, 500, 750 COD) over time by adding 30ppm PAA to the sample and titrating upon initial addition (T=0) and after three hours (T=3).

Results: The shelf life study showed that PAA concentrations displayed highly significant difference throughout the time stored. There was also a significant interaction between time stored and temperature (5, 20, 37°C). Significant differences among temperatures were seen at week 1 (55.5, 60.4, 61.6ppm respectively), week 8 (64.3, 59.8, 56.6ppm), week 11 (64.3, 54.5, 58.5 pm), and week 12 (54.1, 57.5, 51.3ppm). H_2O_2 showed a significant difference between the storage temperatures and time stored. With organic load present, H_2O_2 concentrations showed no change over time. PAA concentrations were maintained over three hours for all but the 750 COD.

Significance: Outside influences affect the concentration and stability of PAA systems leading to the need for established guidelines on proper handling to ensure food safety management on farm.

P1-189 Transfer of Indicator *Escherichia coli* to Spinach Grown in Soil Amended with Raw Animal Manure Associated with Heavy Winter Rains in California, 2016 to 2017

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❖ ***Developing Scientist Competitor***

Introduction: Biological soil amendments of animal origin (BSAAO) have been identified as a potential source of fresh produce contamination with zoonotic enteric pathogens. Vegetables grown close to the ground and fertilized with BSAAO are at increased risk for microbial contamination.

Purpose: To investigate the persistence of indicator *Escherichia coli* in soil amended with different manure types and subsequent transfer to fresh spinach.

Methods: Cattle manure (CM) and chicken litter (CL) were surface-applied separately onto four replicate soil plots (2 by 1 m) at a University of California - Davis vegetable crop field in October 2016. Plots were inoculated with a three-strain cocktail of generic *E. coli* ($10^{6\ to\ 7}$ MPN/g). Controls included un-amended (UA) and un-inoculated (UI) plots. Spinach was planted ~50 days post application (dpa). Soil (n=364) and spinach (n=96) leaf samples were collected from day 0 to 202 and from day 115 to 148, respectively, to enumerate *E. coli* (MPN/g).

Results: ***E. coli*** populations were higher in CL (6.03 to 2.51×10^7 MPN/g) than CM (5.01×10^{-1} to 9.12×10^5 MPN/g) and UA (2.24×10^{-2} to 9.55×10^2 MPN/g) plots from day 1 to 202; UI controls were negative. *E. coli* persisted in soil up to 202 dpa in all plots. Spinach leaves collected from different plots were *E. coli*-positive from day 115 to 148. From day 0 to 148, there were 53 days of rainfall with a total of 24.61 in, and from day 115 to 148, there were 14 days of rainfall with a total of 7.64 in. Rainfall was significantly higher than average throughout the study.

Significance: In contrast to previous trials with other vegetables (melons, tomatoes), this is the first time we observed transfer of *E. coli* to vegetables grown with untreated BSAAO. Our findings suggest that longer wait periods may be needed to protect leafy greens from microbial contamination via raw manure-amended soil, especially during rainy seasons.

P1-190 Control of *Salmonella* on Fresh Spinach by Application of a Sodium Bisulfate/Peroxyacetic Acid Solution

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❖ ***Developing Scientist Competitor***

Introduction: Fresh produce has become a commonly implicated product in foodborne illness outbreaks, including outbreaks of *Salmonella*. The use of antimicrobial washes is a common intervention to control pathogens in fresh-cut produce. While chlorine is a frequently used antimicrobial, it is only moderately efficacious on the product surface; thus, exploring other chemical interventions is warranted.

Purpose: The purpose of this study was to quantify the destruction of *Salmonella* spp. on fresh-cut spinach following a sodium bisulfate and peroxyacetic acid (SBS-PAA) wash and to compare reductions to other interventions.

Methods: Fresh-cut spinach was inoculated with a cocktail of *Salmonella* spp. at a target concentration of 5 log CFU/g, allowed to dry to facilitate attachment, and then submerged in one of three washes: SBS-PAA (80 ppm peroxyacetic acid and 0.5% sodium bisulfate), chlorine (150 ppm at pH 7.0), or water for 2 min, plus a non-washed control. Spinach was immediately sampled (d 0) or packaged in plastic retail display bags and stored at 7°C for 1, 3, 5, and 10 d until sampling. At each sampling point, *Salmonella* populations were enumerated using xylose lysine tergitol-4 (XLT-4) agar, or XLT-4 agar plus a tryptic soy agar (TSA) overlay to enumerate injured populations, and then incubated at 37°C for 18 h.

Results: When plated on XLT-4, SBS-PAA and chlorine washes achieved significant reductions compared to water wash and control (P<0.05). SBS-PAA and chlorine were similar (P>0.05) in efficacy. When plated on XLT-4 with TSA overlay, all treatments differed from the control (P<0.05), and SBS-PAA was the most effective, with a reduction of 1.77 log CFU/g (P<0.0001) and 0.46 log CFU/g (P=0.0270) in comparison to the control and chlorine washes, respectively.

Significance: A wash of SBS-PAA is an effective tool for controlling *Salmonella* on fresh-cut spinach and may serve as an alternative to traditional chlorine washes.

P1-191 Effects of Low Salt Concentration on the Microbial Safety of Spontaneously Fermented Cabbage

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Introduction: Fermented vegetables have gained popularity among consumers due to the potential health benefits of live cultures. The U.S. Food and Drug Administration has issued industry guidance recommending voluntary reduction of sodium in foods. Therefore, interest in low-salt fermented foods may be increasing. However, more information is needed to validate the safety of low-salt ferments.

Purpose: Objectives of this study were to evaluate the effects of salt concentrations (1.0, 1.5, 2.0, and 2.5% [weight/weight]) on the survivability of bacterial pathogens (Shiga toxin-producing *Escherichia coli* [STEC], *Staphylococcus aureus*, and *Listeria monocytogenes*) during spontaneous fermentation of cabbage.

Methods: A randomized complete block design was utilized with different sodium chloried (NaCl) concentrations as treatments and a 2.5% NaCl control, blocked by week (n=3). Cabbage was purchased from three locations, washed, shredded, salted, and packed in glass jars. Each jar was inoculated at 10^5 CFU/ml with a pathogen cocktail comprised of two strains each of STEC, *S. aureus,* and *L. monocytogenes*. Sauerkraut was fermented at room temperature (~23°C). Brine was plated throughout fermentation and pH was monitored. Samples were plated using the thin agar layer method on selective media until detection limit was reached. Data was analyzed using analysis of variance.

Results: Significant (P<0.05) increases in all pathogen populations were seen on day 1, followed by a sharp decrease in conjunction with acidification of all treatments. Salt concentration did not significantly (P>0.05) affect pathogen survival. By day 6, *L. monocytogenes* was found below the detection limit (10 CFU/ml) for all treatments. STEC was observed below the detection limit by day 12 for all treatments except the control. However, *S. aureus* was more resistant, surviving up to day 21 for all treatments. An initial increase of *S. aureus* (>6 log) was noted among all treatments, indicating a potential hazard of toxin production.

Poster

Significance: This data provides crucial information for producing safe low-salt sauerkraut for both home-fermenters and food processors.

P1-192 Survival of *Listeria monocytogenes* on Cantaloupe Field Pack Food Contact Surfaces

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Introduction: Survival information for *Listeria monocytogenes* on food contact surfaces during field packing operations is lacking.
Purpose: Five food contact surfaces (cotton, nitrile, rubber gloves, cotton rags, and stainless steel) were evaluated for pathogen survival.
Methods: Coupons were autoclaved (clean) or rubbed with a cantaloupe leaf for 20 s (fouled) and inoculated with a five-strain cocktail of *L. monocytogenes* (6 log CFU/ml or g). A wet inoculum was spot inoculated (100 µl) onto coupons and dried for 1h. A dry inoculum was prepared by mixing aqueous inoculum with sterile sand and drying for 24 h at 40°C, then mixing the coupon with 100 g of sand for two min. Coupons were held at 35°C (34% RH) for 8 h. Samples were stomached for 2 min with 0.1% peptone (100 ml), except stainless steel, where a rub-shake-rub method was used, and plated onto selective and non-selective media in triplicate experiments with duplicate replicates (n=6).
Results: Population decreases following the wet inoculation during drying varied depending on material and cleanliness (e.g., clean nitrile gloves showed a 3.41-log reduction and fouled cotton gloves showed a 0.31-log reduction). Population declines for wet inoculum inoculated surfaces, regardless of fouled or clean, exceeded 1 log, except for cotton rags (0.21-log reduction) and gloves (0.92-log reduction). Populations did not decline as rapidly following dry inoculations, but had lower starting populations than wet, with no rapid declines during drying. The trend for dry inoculum was similar, regardless of clean or fouled (e.g., stainless steel showed a 1.34- and 1.33-log reduction for clean and fouled, respectively). Population reductions following dry inoculation were much lower than those following wet inoculation over 8 h (e.g., clean rubber gloves showed a 4.15- and 0.50-log reduction and fouled cotton gloves showed a 1.61- and 0.22-log reduction for wet and dry inoculation, respectively).
Significance: Inoculation and surface cleanliness method both impact the survival of *L. monocytogenes* on field pack surfaces.

P1-193 Impact of Disinfection Treatments on Sprouting Alfalfa Seed Contaminated with *Salmonella* Revealed by Metabolomics

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Developing Scientist Competitor

Introduction: Alfalfa sprouts contaminated with *Salmonella enterica* have been the source of many outbreaks of foodborne illness in North America. Previous research has shown that *S. enterica* can proliferate on germinating seeds, but the influence of different disinfection treatments on the behavior of *S. enterica* remains unclear.
Purpose: The goal of this study was to understand how *S. enterica* infection and different disinfection treatments affect metabolites released by sprouting alfalfa seeds.
Methods: Uninoculated alfalfa seeds (IM), seeds inoculated with *Salmonella enterica* Agona and *Salmonella enterica* Typhimurium were subject to three different disinfection treatments, including: (i) a non-treated control (CTL), (ii) soaking in a 5,000-ppm chlorine solution (CLO), and (iii) a treatment compliant with organic production practices, consisting in a 50°C hot water dip followed by treatment with a solution of 2% hydrogen peroxide and 0.1% acetic acid solution (HPA). Metabolites recovered by rinsing seeds germinated for 24 h were identified by ultra-performance liquid chromatography/tandem mass spectrometry (n=4). Differences in the abundances of metabolites (P≤0.05) were determined by pair-wise Student's t test comparisons between treatments and the control.
Results: Our results revealed that almost all of the 535 identified compounds were affected by one or both of the disinfection treatments. Specifically, CLO diminished the levels of 44.5 to 46.9% of compounds, possibly via oxidative destruction, while HPA diminished or increased roughly equivalent amounts of compounds. Notably, HPA treatment increased the levels of several phosphatidylethanolamines, predominant *Salmonella* membrane phospholipids, in the IM samples by four- to 20-fold, but decreased by up to 25-fold in the samples exposed to *Salmonella* samples, especially in *Salmonella* Agona. All the metabolic pathway classes were affected by the treatments.
Significance: This study provided new insight into the ecology of *Salmonella* on germinating seeds, an important consideration in the development of better strategies to lower the risk associated with sprouted vegetables.

P1-194 Effect of Commercial Sanitizers in the Inactivation of *Salmonella enterica* Biofilms on Cherry Tomatoes

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Introduction: Due to their recent association with outbreaks of salmonellosis, disinfection of fresh cherry tomatoes requires special attention. It is well-known that foodborne pathogens such as *Salmonella* develop biofilm on vegetables surfaces that could affect the efficacy of disinfectants.
Purpose: The aim of this study was to evaluate the efficacy of disinfectants to reduce *Salmonella enterica* cells adhered and immersed into biofilms formed on the surface of cherry tomatoes.
Methods: Tomatoes were inoculated with a cocktail of five strains of *S. enterica* (8 log CFU/fruit) resistant to rifampicin and disinfected for 5 min with sodium hypochlorite (200 ppm), peracetic acid (80 ppm), and a citrus seed product (0.2%). The biofilm production of *S. enterica* was induced by inoculating the tomato (5 log CFU/fruit) and storing it at 25°C for 72 h; subsequently, the fruits were disinfected as previously described. Population of *Salmonella* was enumerated on triptic soy agar supplemented with rifampicin (200 ppm). Data was subjected to analysis of variance and the means were compared by Tukey's HSD test.
Results: The application of sodium hypochlorite, peracetic acid, and citrus seed product reduced the population of *S. enterica* adhered to the fruit by 5.4, 4.7, and 2.5 log CFU/g, respectively. When disinfection was applied to biofilms, the effectiveness of sodium hypochlorite and peracetic acid decreased, showing reductions of 3.8 and 2.8 log CFU/fruit, respectively. In contrast, the citrus seed disinfectant was more effective, with a reduction of 3.2 log CFU/fruit.
Significance: Sodium hypochlorite and peracetic acid, two of the most commonly used disinfectants in the fresh produce industry, efficiently reduced the number of *Salmonella* cells adhered to the surface of cherry tomatoes; however, their efficacy was reduced in the presence of biofilms. New alternatives are needed for the decontamination of cherry tomatoes.

Poster

P1-195 Growth and Survival of *Listeria monocytogenes* on Broccoli and Cauliflower Held at Varying Storage Temperatures

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Undergraduate Student Award Competitor

Introduction: The growth and survival of *Listeria monocytogenes* has been studied on several types of fresh produce stored in a variety of conditions. However, there is limited data on the behavior of *L. monocytogenes* on broccoli and cauliflower, vegetables that are commonly consumed raw.
Purpose: The objective of this study is to determine the growth and survival of *Listeria monocytogenes* on broccoli and cauliflower stored at different temperatures observed along the supply chain.
Methods: Fresh broccoli and cauliflower florets were inoculated with 20 µl of a five-strain nalidixic acid-resistant cocktail of *L. monocytogenes* and stored at 22, 10, 4, and -23 °C. The final inoculum for each sample was 3 log CFU/g for broccoli and 4 log CFU/g for cauliflower. Samples (n=6) were plated on tryptic soy and modified Oxford agars in duplicate and enumerated for *L. monocytogenes* using U.S. Food and Drug Administration Bacteriological Analytical Manual methods. Florets were sampled on days 1, 2, and 3 (22°C); days 1, 2, 3, and 7 (10 and 4°C); and days 1, 3, and 7 (-23°C) post-inoculation. JMP software was used to perform statistical analysis using Tukey's multiple comparison test (P<0.05).
Results: No significant population differences (P<0.05) were observed for broccoli and cauliflower samples held at frozen storage conditions. For broccoli and cauliflower samples held at 4°C, there was a significant increase (0.5 and 1 log CFU/g, respectively) in populations between days 0 and 7. Similarly, broccoli and cauliflower samples held at 10°C showed significant population increases (1.6 and 1.7 log CFU/g, respectively) between days 0 and 7. At 22°C, populations on broccoli and cauliflower significantly increased only by day 3 (1.8 and 2.1 log CFU/g).
Significance: These findings suggest that growth of *L. monocytogenes* is supported on broccoli and cauliflower at 4, 10, and 22°C, as well as survival at frozen temperatures. Good agricultural practices and good manufacturing practices are necessary to limit contamination with *L. monocytogenes*.

P1-196 Investigating the Prevalence and Persistence of *Listeria* spp. and *Listeria monocytogenes* in Produce Packinghouses

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Developing Scientist Competitor

Introduction: *Listeria monocytogenes* has emerged as a food safety concern for a number of produce commodities. While *L. monocytogenes* contamination can occur throughout the supply chain, contamination from the packinghouse environment represents a particular challenge and has been linked to recalls.
Purpose: This project aimed to study the prevalence, persistence, and contamination patterns of *Listeria* species and *L. monocytogenes* in produce packinghouses through the development of environmental monitoring programs.
Methods: A longitudinal study was performed in 11 produce packinghouses (commodities included micro-greens, peaches, apples, tomatoes, and broccoli) in three states from July to December 2017. Initially, packinghouses were visited to select sampling sites representing zones 2 through 4 (sites per packinghouse ranged from 34 to 44 swabs) and were subsequently sampled three to four times. Environmental samples were processed for *Listeria* species and *L. monocytogenes* using the U.S. Food and Drug Administration's Bacteriological Analytical Manual method. Isolates were confirmed by PCR for the *sigB* gene.
Results: Among the 1,413 samples tested (105 to 188 per packinghouse), the prevalence of *Listeria* species and *L. monocytogenes* was 4.4 and 2.1%, respectively. *Listeria* species and *L. monocytogenes* prevalence in a given packinghouse ranged from 0.0 to 13% and 0.0 to 12%, respectively. Seven of 11 packinghouses had a higher prevalence of *Listeria* species compared to *L. monocytogenes*, while only two of 11 packinghouses had higher *L. monocytogenes* prevalence. Interestingly, there were only five instances of *Listeria* species or *L. monocytogenes* repeat isolation (site testing positive >2 times). Additionally, nine of 11 packinghouses had at least one sample positive for *L. monocytogenes*, with the majority of *L. monocytogenes*-positive samples found in zone 3 (e.g., drains, floors, forklift wheels).
Significance: Data show *Listeria* prevalence in produce packinghouses is low, as sites were selected to find *Listeria*. Additionally, while there were limited cases of *Listeria* species or *L. monocytogenes*; further subtyping is needed to determine whether packinghouse control measures are effective at limiting resident *Listeria*.

P1-197 The Effect of Biological Soil Amendments and Indigenous Bacteria on *Salmonella* Newport Survival and Growth in Soil

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Introduction: Salmonella Newport has been implicated in several foodborne illness outbreaks in produce. Produce contact with contaminated soil containing *Salmonella* Newport is a potential route of contamination. Little is known about survival of the pathogen in soils, especially those containing biological soil amendments (BSAs).
Purpose: To determine the effect of BSAs and the presence of indigenous microorganisms on growth of *Salmonella* Newport in soil.
Methods: Soil (fine, loamy, mesic Aquic Hapludults) were either amended (A) with 6% (weight/weight) of a commercial BSA (3-2-3, nitrogen-phosphorus-potassium) or left unamended (U). Soil was mixed 1:2 (volume:volume) with sterile water, centrifuged, and supernatants (extracts) were collected. Extracts (A and U) were filtered (0.2 micron) to remove microorganisms (sterile [S]), while others remained unfiltered (not sterile [NS]) before inoculation with 2.7 to 3.2 log CFU/ml *Salmonella* Newport and incubation at 25°C. *Salmonella* Newport populations were determined at 0, 4, 8, 24, 30, 48, 72, and 96 h in AS, ANS, US, and UNS extracts. A least squares means model (P<0.05) was used to determine differences in growth of *Salmonella* Newport based on soil extract type.
Results: AS extracts supported significantly greater (P<0.05) populations (4.58 log CFU/mL) of Salmonella Newport than other extract types. ANS extracts supported significantly greater populations (3.46 log CFU/mL) than either US (2.36 log CFU/mL) or UNS (1.47 log CFU/mL). Populations reached 6.5 to 8.6 log CFU/ml and 3.9 to 5.3 log CFU/ml in amended and unamended extracts, respectively, after 96 h. Growth phase constants for populations were 0.71, 0.45, 0.29, and 0.09 h^{-1} in AS, ANS, US, and UNS, respectively.

Poster

Significance: Nutrients from BSAs supported the growth of *Salmonella* Newport populations in soil extracts with and without indigenous microorganisms present; however, little growth in extracts occurred in the presence of indigenous microorganisms without BSA nutrients. Survival of *Salmonella* Newport is dependent upon the nutrients and indigenous microorganisms present in soil.

P1-198 Comparison of Genotypic and Phenotypic Antibiotic Resistance Patterns in *Citrobacter* spp.

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Introduction: *Citrobacter* spp. are commonly found in water, soil, food, and the intestinal tracts of warm-blooded animals. Their presence on leafy greens has led to questions regarding the food safety and public health significance of these bacteria.

Purpose: The objective of this study was the genomic and phenotypic characterization of leafy greens associated *Citrobacter* spp. to determine their potential as foodborne pathogens.

Methods: Eight *Citrobacter freundii* isolates and four *Citrobacter amalonaticus* isolates were obtained from fresh produce samples that tested false-positive for *Salmonella* and *Escherichia coli* O157:H7 on the affinity-based mini-VIDAS system. Whole genome sequencing (WGS) was performed on the *Citrobacter* isolates on an Illumina MiSeq platform with 300-bp paired-end libraries and 30X coverage. The raw reads were assembled using the A5 pipeline, and sequences were analyzed for the presence of virulence genes using the Virulence Factor Database for antibiotic resistance genes using CARD and ResFinder, and for plasmids using PlasmidFInder. The *Citrobacter* isolates were phenotypically characterized for antibiotic resistance using the VITEK 2 system.

Results: C. freundii isolates were resistant to β-lactam antibiotics including cefazolin, cefuroxime axetil, and cefoxitin, while *C. amalonaticus* isolates were resistant to cefazolin, cefuroxime, and cefuroxime axetil. Phenotypic resistance correlated with the genotypic analysis, which revealed the presence of the *blaCMY-70* gene in seven *C. freundii* genomes and the *blaCFE-1* gene in one genome. Eleven of 12 *Citrobacter* genomes encoded efflux pump genes *mdtB* and *mdtC* efflux, conferring antibiotic resistance against novobiocin and deoxycholate. One *C. freundii* genome also encoded a multidrug efflux pump *acrB* and the quinolone resistance gene *QnrB28.* No plasmids or virulence genes were observed within the *Citrobacter* genomes.

Significance: The presence of antibiotic-resistant *Citrobacter* spp. on fresh produce is of potential public health concern, as these isolates may act as reservoirs to spread resistance determinants to foodborne pathogens.

P1-199 *Salmonella* Isolation Not Associated with *Escherichia coli* concentration in Agricultural Water Samples Collected from New York Streams

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❖ ***Developing Scientist Competitor***

Introduction: The quality of surface water used for produce production has emerged as a focal point of produce safety programs. However, interpretation of water quality tests is complicated by the fact that the relationship between indicators and pathogen presence varies widely.

Purpose: To characterize the relationship between *Escherichia coli* concentration and *Salmonella* presence in agricultural water samples.

Methods: Five New York streams were each sampled more than 30 times during the 2017 growing season for a total of 166 samples. At each sampling, 10 L of water was collected and tested for *Salmonella* presence. Data on *E. coli* levels and environmental conditions were collected. The relationship between *Salmonella* presence in the sample and the log most probable number of *E. coli* per 100 mL was characterized using logistic regression. Bootstrapping was used to estimate the predictive accuracy of the U.S. Food and Drug Administration's Food Safety Modernization Act Produce Safety Rule (PSR) water standard (geometric mean <126 CFUs/100 mL and statistical threshold value <410 CFUs/100 mL) in identifying water sources with a high or low risk of *Salmonella* presence. Random forest analysis (RFA) was used to identify additional factors associated with *Salmonella* isolation from water samples.

Results: Salmonella was detected in 46% (77 of 166) of samples. As expected, *Salmonella* presence and *E. coli* levels varied widely across time and between streams. According to logistic regression, there was a borderline association between the *E. coli* concentration and *Salmonella* presence (P=0.086). Application of the PSR standard had poor ability to predict water contamination status with respect to *Salmonella* (sensitivity=0.47, specificity=0.63). According to RFA, turbidity and rain within 48 h of sampling were associated with *Salmonella* isolation.

Significance: These findings suggest that *E. coli* concentration was not a reliable indicator of *Salmonella* presence for the sampled streams, particularly when the PSR water standard was applied. Hence, alternative approaches are needed to identify surface water with increased risk of *Salmonella* presence.

P1-200 Prevalence, Distribution, and Serotypes of *Salmonella* in Public Access Watersheds Near Leafy Green Growing Regions in Central California during 2011 to 2016

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Introduction: Fresh produce is responsible for nearly half of food-related illness outbreaks in the United States. Avenues of preharvest contamination include water and wildlife, and public access waterways are a central reservoir for *Salmonella* contamination.

Purpose: We report the prevalence and ecology of *Salmonella* in public access watersheds in California's Central Coast from a five-year study conducted in 2011 through 2016.

Methods: A total of 2,979 Moore swab samples collected bi-monthly from up to 30 sites in lakes, streams, ponds, and rivers over a 500-square-mile region during October 2011 through September 2016 were enriched for *Salmonella*, subjected to immunomagnetic separation, and plated on selective media (modified semisolid Rappaport-Vassiliadis, Rappaport-Vassiliadis soya broth, and xylose lysine deoxycholate). PCR targeting the *invA* gene was used to identify presumptive positives as *Salmonella*. Isolates were differentiated with a combination of rep-PCR and pulsed field gel electrophoresis (PFGE), and selected isolates were sent for serotype analysis.

Results: Of 2,979 samples from five interconnected watersheds, 57% were positive for *Salmonella*, resulting in more than 1,700 isolates. Statistical analysis indicated that *Salmonella* was less prevalent in the fall (September through November), but there was no difference in prevalence between the five watersheds. More than 50 different serotypes were identified, with the top three being the monophasic 6,8:d:- (which includes a monophasic Muenchen), Give, and Typhimurium. Other prominent serotypes included Oranienburg, Infantis, Enteritidis, and Heidelberg. Some of the serotypes showed location bias among the watersheds. Comparisons of PFGE profiles of *Salmonella* strains isolated from this and surveys of the region done prior to 2011 indicate persistence of some pulsotypes over seven years in the region.

Poster

Significance: The data indicate that *Salmonella* is widespread and persistent in watersheds of this leafy growing region. These waters could serve as a source of preharvest contamination of produce.

P1-201 Evaluating the Effect of Green Manures on Populations of *Listeria* spp. and *Escherichia coli* in Soil and on Lettuce Crops

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Introduction: Green manure (GM) cover cropping improves soil structure and adds nutrients to soil. However, our previous work showed that cover crops may enhance survival of soil *Escherichia coli* and *Listeria innocua*. The effects of GM on microbial safety of produce crops are unknown.

Purpose: Determine the fate and dispersal of *E. coli* and *Listeria* spp. in soils and on lettuce grown on different cover crop green manures.

Methods: Late summer-planted hairy vetch (HV), winter wheat (WW), rye (R), and hot mustard (HM) cover crops, grown in a randomized complete block design, were tilled into the soil in spring to form GM. Raised beds formed over GM were amended with liquid dairy manure two days before transplanting green leaf (Tropicana), red leaf (Vulcan), and romaine (Green Forest) lettuce seedlings. Lettuce and soil samples were collected weekly for six weeks. Samples were homogenized and 3M Petrifilms used to enumerate *E. coli*. Diluted homogenates were enriched in a mini-most-probable-number procedure to approximate populations of *Listeria* spp.

Results: In year one, *Listeria* spp. persisted at higher populations on red and green leaf than Romaine lettuce (P<0.01). *Listeria* counts were higher on green leaf lettuce on all GM compared to bare ground (P<0.01), and in soil in HV and R plots compared to WW or bare ground (P<0.01). In year two, *Listeria* levels increased over time (P<0.01), and were higher in soil than on lettuce (P<0.01). In HM plots, *Listeria* was higher relative to bare ground (P<0.01) in soil and on red leaf lettuce. In WW plots, romaine lettuce harbored *Listeria* populations equal to bare ground. All other GM supported lower populations (P<0.01). *E. coli* populations decreased over time in soil (P<0.01) and were only detected sporadically on lettuce.

Significance: Green manure can affect the persistence of *Listeria* spp. in soil and on lettuce, as well as the dispersal of *Listeria* from soil onto crops.

P1-202 Surface River Waters on the Maryland Eastern Shore are a Reservoir for Antibiotic-resistant *Salmonella enterica*

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Introduction: Some outbreaks of salmonellosis associated with produce have been traced back to farms on the Delmarva Peninsula on the East Coast of the United States, with a few specifically linked to irrigation water, suggesting an environmental reservoir for *Salmonella enterica* in this region. A high diversity of *S. enterica* in Delmarva surface water used for irrigation has been reported, but further research on *S. enterica* ecology in this region is needed.

Purpose: Evaluate antimicrobial resistance (AMR) among *S. enterica* isolates collected from rivers on the Maryland Eastern Shore.

Methods: A total of 157 Salmonella isolates were recovered from 23 sites along the Choptank, Nanticoke, Pocomoke, and Wicomico Rivers in the fall and the spring using modified Moore Swabs. Swabs were selectively enriched for the presence of *S. enterica*. Presumptive salmonellae were serotyped and antimicrobial susceptibility testing was performed with the Sensititre microbroth dilution system using the National Antimicrobial Resistance Monitoring System custom plate CMV1AGNF. Resistance breakpoints were obtained from the Clinical and Laboratory Standards Institute.

Results: *Salmonella* diversity was high and a total of 18 serotypes were recovered. Twenty five isolates (16%) from nine water samples were resistant to at least one class of antibiotics. Only one *Salmonella* Typhimurium isolate from the fall sampling showed AMR. Of the 25 isolates, 52% (13) were multidrug resistant (MDR, resistant to at least two classes of antibiotics), including nine isolates that were resistant to five classes of antibiotics. *Salmonella* Typhimurium was the serotype most commonly found to be antibiotic-resistant. Resistance to sulfisoxazole was the most prevalent, followed by tetracycline. AMR to amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftriaxone, and streptomycin was also elevated.

Significance: The prevalence of MDR *Salmonella* on the Maryland Eastern Shore is a significant public health concern, as the majority of farms in this region use surface water for at least some of their irrigation needs.

P1-203 Effect of Irrigation Water on the Microbiological Quality of Commercially Produced Fresh Spinach from Farm to Retail

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❖ ***Developing Scientist Competitor***

Introduction: Leafy greens can become contaminated with antibiotic-resistant pathogens from animal and human sources during agricultural production, harvesting, and minimal processing. In South Africa, surface water sources used for irrigation have increasingly been reported to be contaminated with enteric bacterial pathogens, resulting in increased risk of foodborne illness outbreaks following consumption of fresh produce.

Purpose: To investigate the prevalence and dissemination of potential human pathogenic antimicrobial-resistant *Enterobacteriaceae* isolates from spinach commercially produced using irrigation water from a river prone to microbial contamination.

Methods: Water (n=33), soil (n=2 composite samples), fresh produce (n=60), and contact surface (n=36) samples were analysed throughout the spinach supply chain. Coliforms/*Escherichia coli* and *Enterobacteriaceae* were enumerated. *E. coli*, *Salmonella* spp., and presumptive extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* were isolated using standard microbiological methods. Isolate identities were confirmed using matrix assisted laser desorption ionization-time of flight mass spectrometry and characterized using phenotypic (antibiotic resistance) and genotypic (resistance genes, DNA fingerprinting) analysis.

Results: River water *E. coli* counts exceeded the 1996 South African Department of Water Affairs guidelines of 0 CFU/100 ml irrigation water for RTE crops to be eaten raw. No *E. coli* was enumerated from any of the spinach (bunches and RTE) samples in the supply chain from harvesting in the field to the retailer when river water stored in a reservoir dam was used for irrigation. When river water was directly applied to spinach fields using overhead irrigation, *E. coli* was enumerated from the spinach samples, contact surfaces, and from the river, irrigation, and wash water throughout the supply chain. Presumptive positive ESBL-producing *Enterobacteriaceae* were isolated from irrigation water, spinach, and contact surfaces and included *E. coli* (n=5 isolates), *Serratia* spp. (n=17), *Rahnella* spp. (n=4), *Klebsiella* spp. (n=9), and *Salmonella* spp. (n=2).

Significance: Surveillance data will contribute to the development and implementation of crop-specific supply chain food safety risk-mitigation strategies.

Poster

P1-204 Public Private Partnership in Self-Monitoring of Water Quality

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Introduction: Water is the basic element used by farmers, caterers and food industries for washing, irrigating or even as an ingredient in food processing. Dr. Ewen TODD et al. showed in a study done in 2015 on waterborne diseases in MENA region, that 68-80% of people exposed to spring water, fall ill with bacterial or infectious diseases. Stakeholders in developing countries are encouraged to test for contaminants in water throughout the value-chain and should be aware of international norms for each parameter tested.

Purpose: The purpose of this subject matter is to showcase the importance of "self-testing" the water used across value chains with simple and efficient technologies in order to prevent diseases occurring from water contamination.

Methods: For regular monitoring of water quality, MEFOSA partnered with Palintest, a company that offers water analysis technologies. To better place these technologies, Lebanese NGO's were engaged to include these test as part of their objectives. For instance, Concern Worldwide and Oxfam were supplied by: tablets, multi-meters and turbimeters, in order to test pH, electric conductivity, turbidity, nitrite, nitrate and phosphate. Multiple water sources from different Lebanese areas were tested for preventive control and/or for corrective actions.

Results: Oxfam test results from 2017, showed high levels of Nitrate (>45 mg/L) in 68.57% of the samples taken from Boreholes mostly (source of drinking water), Concern also was able to isolate septic water tanks that were found highly contaminated. NGO's were able to prevent diseases occurring from contaminated water in rural settings. Other NGO's results are pending.

Significance: Easy to use testing kits helped Lebanese Public and Private sector identify which water source is better for drinking, for washing and which water they cannot use at all. NGO engagements set new PPP and allowed preventing the occurrence of several diseases related to water contamination.

P1-205 Improving the Safety of Strawberry Irrigation Water Using a Hexadecyltrimethylammonium Bromide Modified Zeolite Filtration System

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❖ ***Developing Scientist Competitor***

Introduction: The U.S. Food and Drug Administration's Food Safety Modernization Act (FSMA) has required that all irrigation water must be safe for use on produce, as a strategy to reduce foodborne illness. A surfactant modified zeolite (SMZ) filtration system could provide produce farmers with a sustainable, low-cost system for high quality and safe irrigation water.

Purpose: Evaluate the effectiveness of 20% (volume/weight) hexadecyltrimethylammonium bromide (HDTMA-Br) to develop a SMZ filtration system capable of removing *Escherichia coli* from irrigation water used on strawberry plants.

Methods: The SMZ 20% HDTMA-Br sand filtration system was tested in a strawberry field operating at 25 gallons per minute that was compared to two controls, one containing only agricultural sand in the filtration system and one with no filtration system. An *E. coli* non-pathogenic surrogate was used to spike pond water that naturally contain fecal material to 6 log CFU/ml of *E. coli*. Samples of 100 ml of water and a composite sample (~300g) of strawberry samples that were picked during January, February, and March of 2017. Bacterial counts were determined using *E. coli*/coliform petri-film.

Results: The water samples with the SMZ filtration system reduced *E. coli* counts >5 log CFU/ml in the irrigation water, whereas the sand filter reduced *E. coli* <1 log CFU/ml in the water. There was a significant difference (P<0.05) between the SMZ treatment and the control; however, the non-modified sand filtration system did not have a statistical difference from the control. There was no significant difference with the strawberry samples in any of the treatments.

Significance: SMZ modified with 20% HDTMA-Br filtration system is a viable solution for farmers to comply with new FSMA regulations and provide a way to reduce foodborne outbreaks.

P1-206 Prevalence of Fecal Indicator Bacteria in Surface and Recycled Water: A Conserve Study

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Introduction: Surface and recycled water can be used for irrigation of fresh produce crops. Investigation of microbiological water quality will aid in the mitigation of foodborne disease risk.

Purpose: Determine the impact of water type, season, and physicochemical parameters on the prevalence of fecal bacterial indicators (total coliforms [TC], *Escherichia coli*, and *Enterococcus* spp.) in surface and recycled water in Maryland.

Methods: Water samples (n=119) were collected from 11 sites (two ponds [PW], two tidal rivers [TRW], four non-tidal rivers/creeks [NTRW], and three recycled water sites [RW], quenched with 10% sodium thiosulphate) over a 12-month period. Fecal indicators were enumerated using standard membrane filtration, specifically Environmental Protection Agency method 1604 on MI agar for *E. coli* and TC and method 1600 on mEI agar for enterococci. Various water physicochemical parameters (temperature, pH, turbidity, salinity, nitrate, and dissolved oxygen [DO]) were measured using a ProDSS meter.

Results: Levels of *E. coli*, TC, and enterococci ranged from undetectable 4.1, 1.3 to 5.9, and undetectable 4.6 log CFU/100 mL of water, respectively. TRW and NTRW harbored higher levels of *E. coli* and enterococci compared to PW and RW (P<0.001). *E. coli*, TC, and enterococci counts in water were strongly significantly correlated to each other (Spearman ρ ranged from 0.40 to 0.71; P<0.001). A significant negative correlation was detected between both *E. coli* and enterococci with pH and DO (Spearman ρ ranged from -0.27 to -0.30; P<0.01). Enterococci levels were positively correlated with water turbidity (Spearman ρ=0.42; P<0.05) and *E. coli* were negatively correlated with salinity (Spearman ρ=-0.38; P<0.05). Seasonal variation, with higher bacterial counts during the summer and autumn months, was detected for PW and NTRW (P<0.05), but not for RW and TRW, except for TC in the latter. However, no correlations with temperature were revealed.

Significance: River water supported higher levels of bacteria than pond and recycled water. Characterization of various surface water types is needed before water is used for irrigation purposes.

Poster

P1-207 Pathogenicity of *Aeromonas* spp. Isolated from Surface and Recycled Water and Transfer Potential to Lettuce: A Conserve Study

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Introduction: Surface and recycled waters are reservoirs for many known and under-recognized human pathogens. Using these water sources for irrigation of fresh produce crops requires determining levels of microbial contamination and persistence, transfer potential of pathogens from water to crops, and virulence potential of those pathogens.

Purpose: Identification of the under-studied taxon *Aeromonas* spp. in surface and recycled water, characterization of pathogenic traits, and investigation of transmission to lettuce following irrigation.

Methods: *Aeromonas* spp. isolates (n=119) were recovered from water samples (n=119) from two ponds (PW), two tidal rivers (RW), four non-tidal rivers/creeks, and three recycled water sites in Maryland over 12 months. Pathogenicity of *Aeromonas* spp. was assessed by amplification of four virulence genes: heat-stable enterotoxin (*ast*), cytotoxic enterotoxin (*act*), aerolysin (*aer*), and flagellin (*fla*A). *In vitro* attachment was examined through biofilm formation on abiotic surfaces. Transmission of *Aeromonas* to loose leaf lettuce (cv. 'Mascara') was investigated by repeated overhead irrigation with PW and RW over a six-week period.

Results: *Aeromonas* carrying virulence genes were identified in 37, 32, 61, and 44% of pond, tidal river, non-tidal river, and recycled water, respectively. Higher levels of *Aeromonas* carrying virulence genes were observed in non-tidal river water (x^2=7.69, P=0.05) compared to other water types. The prevalence of *Aeromonas* carrying virulence genes in non-tidal river (x^2=23.36, P<0.01), tidal river (x^2=11.12, P<0.05), and recycled water (x^2=13.75, P<0.05) varied by season. *Aeromonas* isolates possessing *ast*, but not *act*, attached in lower populations to abiotic surfaces (P<0.05) compared to isolates not carrying virulence genes. Overhead irrigation of lettuce with both pond and river water indicated the propensity of *Aeromonas* spp. to transfer to greenhouse-grown lettuce (P<0.05).

Significance: Surface and recycled water support *Aeromonas* spp. that may carry virulence genes and can be transferred from irrigation water to food crops.

P1-208 Reduction of Enteric Pathogens in Irrigation Water by Zero-valent Iron and Sand Filtration

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✦ ***Undergraduate Student Award Competitor***

Introduction: The use of surface and nontraditional water sources for irrigation can reduce demand on critical groundwater resources. Treatment may be necessary when adopting these alternative water sources in order to reduce risk associated with microbial pathogens. Additionally, it is essential to understand how these irrigation sources may influence the microbiological safety of fresh produce and overall comply with the irrigation water quality standards set by the U.S. Food and Drug Administration's Food Safety Modernization Act.

Purpose: The efficacy of a zero-valent iron (ZVI) sand filter was assessed for the reduction of *Listeria monocytogenes* and *Escherichia coli* in surface water.

Methods: Water recovered from an agricultural pond was inoculated with *E. coli* TVS353 and an environmental *L. monocytogenes* isolate at 7 log CFU/mL and horizontally filtered over a three-month period through a PVC pipe filter, sized at 61 by 5.1 cm diameter, filled with 35:65 (volume:volume) ZVI:sand or sand alone. A total of 180 L was filtered, collected in two-pore volume fractions (2.8 L), and 10-mL aliquots were enumerated for *E. coli* and *L. monocytogenes* using tryptic soy agar supplemented with rifampicin and Brilliance Listeria Agar, respectively, and the data were averaged.

Results: Significant reductions (P<0.001) in populations of *E. coli* and *L. monocytogenes* were observed following ZVI sand filtration. *E. coli* populations (7.4±0.26 log CFU/mL) in surface water were reduced to 5.1±0.19 log CFU/mL by ZVI-sand filtration and 5.9±0.26 log CFU/mL after sand filtration. *L. monocytogenes* populations (7.4±0.16 log CFU/mL) in surface water were reduced to 4.6±0.38 log CFU/mL by ZVI-sand filtration and 5.2±0.33 log CFU/mL after sand filtration.

Significance: ZVI sand filters show promising results as a water mitigation step by reducing both *E. coli* and *L. monocytogenes* populations in pond water over a three-month period. This experiment will conclude following six months of filtration. Bacterial decay will be analyzed on lettuce irrigated with ZVI sand and sand-filtered water.

P1-209 Evaluation of Microbiological Quality of Agricultural Water and the Effect of Water Source, Sample Storage Conditions, and Methods of Analysis

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Introduction: The U.S. Food and Drug Administration's (FDA) Food Safety Modernization Act Produce Safety Rule requires agriculture water must be safe and sanitary quality for its intended use. The FDA has recognized several methods that are appropriate for testing. Among them, some methods require the sample to be delivered to the laboratory in 6 h and to be processed within 2 h of receipt.

Purpose: This study examined the effect of water source, storage temperature, and time between sample collection and analysis on levels of generic *Escherichia coli* levels using several FDA-recognized water testing methods.

Methods: Water samples were collected from a pond and a well located at the Louisiana State University AgCenter Botanic Gardens. The levels of generic *E. coli* naturally present in the pond water and inoculated in well water (ATCC 11775 at 5 CFU/mL) were examined at 0, 6, 12, 24, and 48 h after collection during storage at 4, 13, and 25°C. Samples were analyzed by five FDA-recognized testing methods: 1603, 1604, 1103.1, 10029, and IDEXX Colilert test. The data was analyzed by analysis of variance using SAS software.

Results: No significant difference (P>0.5) in the levels of generic *E. coli* was detected between the five testing methods. Generic *E. coli* levels in the water samples stored at 4 and 13°C remained similar throughout the storage time (48 h). However, a significant difference (P<0.05) in generic *E. coli* levels was observed in the pond water stored at 25°C. The initial generic *E. coli* levels of pond water (410 CFU/100 mL) stored at 25°C decreased to 86 CFU/100 mL after 48 h. Under the tested conditions, the source of water did not show a significant effect on the outcome of test results.

Significance: Our study suggests that water samples can be examined during the first 48 h of collection if maintained at 4 or 13 °C utilizing any of the five methods evaluated.

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P1-210 Development of User-friendly *Escherichia coli* Water Testing Method for Iowa Produce Farmers to Enhance Food Safety

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❖ ***Developing Scientist Competitor***

Introduction: Environmental Protection Agency (EPA) method 1603 has been approved as one of nine water testing methods for produce growers under the U.S. Food and Drug Administration's (FDA) Food Safety Modernization Act Produce Safety Rule. This method has a strict six-hour sample holding time between collection and initiation of analyses and a high price ($50-60) for the results. Long distances between the farm and laboratories make this problematic for produce growers within the Midwest.

Purpose: To determine if there is a significant microbial difference between six-hour and 24-hour sample holding times for the EPA 1603 water testing method

Methods: EPA 1603 (commonly known as membrane filtration method) was used to determine *E. coli* contamination in water samples. A total of 80 samples were collected (57 well water, 21 surface water, and two municipal water) from random fresh produce farm locations in Iowa. Samples of 200 mL were collected for each water type and stored below 10°C in cooler bags during transport. Temperature (°C) of each water source was recorded at time of sampling.

Results: No statistical difference (P>0.05) was observed between numbers of *E. coli* cells at 6 and 24 h. A total of 21 samples (17 surface water, four well water) had *E. coli* colonies, and seven of those samples (all surface water) were contaminated above the FDA maximum allowed limit of 126 cells/100 mL (average counts of 7,985 cells/100mL). Surface water was more likely to be contaminated, followed by shallow wells (<60 feet). No correlation of temperature (R^2=0.04) was observed to *E. coli* contamination levels.

Significance: This research provides evidence that the EPA 1603 method holding time can be extended to 24 h. This holding time will allow produce growers to analyze samples in more realistic time frames and will result in enhanced produce safety on farms by encouraging water testing.

P1-211 Differential Growth Dynamics among *Salmonella* Serovars in Surface and Reclaimed Waters Affect Transfer Potential onto Tomatoes

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Introduction: Surface waters are a known reservoir for *Salmonella enterica*; however, bacterial population dynamics in these irrigation sources are not well-studied. Growth dynamics may affect transfer potential of *Salmonella* onto crops during irrigation.

Purpose: Determine whether persistence in various water types impacts the potential for *Salmonella* to colonize tomato fruit.

Methods: In order to assess *Salmonella* population dynamics in various water types, filter-sterilized non-tidal river, tidal river, pond, and reclaimed water samples were inoculated with *Salmonella* Heidelberg, Javiana, Typhimurium, Newport and serovar 4,5,12:i:- and enumerated over 30 days. A crystal violet attachment assay was conducted to investigate abiotic surface attachment. To assess transfer potential onto tomatoes, *Salmonella* Javiana and Heidelberg were pre-incubated in water overnight before inoculation onto var. 'Red Robin' tomato fruit for retrieval and quantification the next day.

Results: S. enterica differentially persisted in water with respect to water type and serovar (P<0.05), with *Salmonella* Typhimurium having the slowest decline in non-tidal river water (P<0.05). *Salmonella* Heidelberg had the largest rate of decline over time in non-tidal river and pond water, but along with *Salmonella* Javiana exhibited significantly less decline than other serovars in reclaimed water (P<0.05). Attachment strength varied significantly by water type; pond water supported the strongest attachment across serovars (P<0.05). *Salmonella* Heidelberg exhibited the weakest attachment in all water types. *Salmonella* Typhimurium, Newport, and Javiana presented the strongest attachment (P<0.05). Both *Salmonella* Javiana and Heidelberg exhibited significantly higher transferability from all water types to tomatoes than from tryptic soy broth (P<0.05), but *Salmonella* Javiana better colonized tomato fruit than *Salmonella* Heidelberg (P<0.05).

Significance: Salmonella serovars demonstrated differential persistence in water and attachment to abiotic surfaces. *Salmonella* Heidelberg appeared less adapted to water environments and tomato surface colonization than *Salmonella* Javiana. Investigating the impact of previous habitats on *Salmonella* colonization of crops is necessary to better assess preharvest produce safety risks.

P1-212 Prevalence of Generic *Escherichia coli* in Mid-Atlantic Surface and Recycled Irrigation Water Sources and Comparison to Food Safety Modernization Act Water Quality Standards: A Conserve Study

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Introduction: Limited availability of groundwater has led to increased interest in the use of alternative water sources in agriculture. However, the microbiological safety of these alternative water sources (e.g., recycled water, processing plant water, pond water, brackish rivers) must be established to ensure they meet federal standards. The U.S. Food and Drug Administration's Food Safety Modernization Act Produce Safety Rule (PSR) states that all irrigation water in direct contact with produce must meet quality standards: geometric mean (GM) of 126 CFU of generic *Escherichia coli* per 100 ml and statistical threshold value (STV) of 410 CFU/100 ml. However, limited monitoring of potential alternative water sources for irrigation has been conducted in the Mid-Atlantic states.

Purpose: To assess surface and recycled water sources for the presence of *E. coli* and compare associated GM and STV values to PSR irrigation water standards.

Methods: Water samples (n=212) were collected on 22 separate occasions from September 2016 to December 2017 from 12 sites in the Mid-Atlantic: three recycled water sites (RW), one processing plant (PP), two ponds (PW), four freshwater rivers (NF), and two brackish rivers (TB). Per Environmental Protection Agency method 1604, 100 ml of each sample were collected and filtered, and *E. coli* was quantified on MI agar. GM and STV values were calculated from available data.

Results: Of the 12 sites tested, five had a GM above the 126 CFU/100ml PSR threshold, whereas eight had a STV above the 410 CFU/100 ml PSR threshold. Of all water sources analyzed, only four sites (two PW and two RW) had *E. coli* levels under both specified thresholds. TB and NF sources were characterized by significantly higher levels of *E. coli* than PW or RW (P<0.0001).

Significance: To comply with the PSR, 67% of tested sites would require mitigation to markedly decrease *E. coli* populations prior to use in direct contact irrigation.

P1-213 Prevalence of Generic *Listeria* spp. and *Listeria monocytogenes* in Surface Waters in the Mid-Atlantic Region of the United States

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Introduction: The ubiquitous nature of *Listeria monocytogenes* results in the presence of this pathogen in a variety of surface waters, and it can also transfer onto produce if contaminated waters are used for irrigation. Therefore, in addition to compliance with the U.S. Food and Drug Administration's Food Safety Modernization Act water quality standards, it is important to estimate the actual prevalence of *L. monocytogenes* in these irrigation sources.

Purpose: To evaluate prevalence *Listeria* spp. and *L. monocytogenes* in surface pond and river waters (PW and RW) in Maryland and Pennsylvania during produce growing season (March to July 2017).

Methods: RW samples (500 ml) were collected weekly from March to July 2017 from six sites spread over a 50-mile reach of the river. PW was collected in two ponds on five different dates (June to July 2017) from 50 (23 and 27) sites on the pond-specific same spatial grids. At every sampling, water temperature, turbidity, and flow rate (for RW) were determined. *Listeria* detection was conducted by selective enrichment in buffered *Listeria* enrichment broth, followed by streaking onto RAPID'*L.mono* and ALOA agars. Presumptive *Listeria* positives were confirmed using VITEK MS.

Results: Overall, 27% of PW (n=120) samples were positive for *Listeria,* of which 80% were *L. monocytogenes*. In RW samples (n=124) the prevalence of *Lm* was 98.5% and all samples were positive for *Listeria*. The incidence of *L. monocytogenes* in RW was dramatically greater than in PW, and was not affected by the flow rate or water temperature. Generic *Listeria* spp. detected in water samples were *L. seeligeri*, *L. welshimeri, L. innocua*, *L. ivanovii*, and *L. grayi.* Interestingly, *L. grayi* was found in RW and *L. seeligeri* in PW only.

Significance: The baseline information on the prevalence of *L. monocytogenes* in surface waters is essential in order to understand how these irrigation sources may influence the microbiological safety of fresh produce and to evaluate the feasibility of bacterial fecal indicators in the assessment of water quality.

P1-214 Presence of Viral, Bacterial, and Chemical Indicators in Recycled, Surface, and Processing Water Used for Crop Irrigation

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❖ ***Developing Scientist Competitor***

Introduction: *Escherichia coli* is the predominant indicator for food safety risks associated with agricultural water and has known limitations. Caffeine is consumed in relatively high amounts by humans, up to 10% cannot be processed by the body and is excreted. Pepper mild mottle virus (PMMoV) is a plant virus which humans can shed in concentrations of 10^9 virions per fecal gram. Use of PMMoV and caffeine as indicators should be explored in comparison to *E. coli* to determine water contamination and potential risk to agricultural crops.

Purpose: This study was performed to detect and connect viral, bacterial, and chemical indicators of contamination in non-traditional agricultural waters.

Methods: Recycled, processing and surface waters were sampled from April-October 2017 and tested for *E. coli* (n=24), caffeine (n=24), and PMMoV (n=11). *E. coli* was detected according to EPA method 1604 from 100 mL samples. Caffeine levels were determined from 1 L samples using HPLC/MS. PMMoV was detected in 20-40 L samples processed using NanoCeram filters followed by concentration with 100 kDa Centricon centrifugal filter units. PMMoV RNA was extracted and quantified by RT-qPCR using a standard curve.

Results: *E. coli* was detected at variable levels up to 4595 CFU/mL, in all recycled (n=12) and surface (n=6) samples as well as 50% (n=3) of processing water samples. PMMoV was detected in all samples, surface (n=4), recycled (n=4), and processing (n=3) water samples. Caffeine levels in recycled water were significantly (P<0.004) higher (129.40 ng/L) compared to those in surface water which had an average of (12.33 ng/L).

Significance: The use of caffeine and PMMoV as water quality indicators may provide greater insight to the source of contamination compared to *E. coli.* Though caffeine levels are significantly higher in recycled water compared to surface water, the prevalence of PMMoV and *E. coli* across samples may reflect variable human and non-human contamination.

P1-215 Levels of *Listeria monocytogenes* and Bacterial Fecal Indicators in Surface Waters in the Mid-Atlantic Region of the United States

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Introduction: Surface waters are commonly inhabited by *Listeria monocytogenes*, which originates from a variety of natural reservoirs besides fecal contamination. Water quality standards set by the U.S. Food and Drug Administration's Food Safety Modernization Act rely on bacterial fecal indicators (BFI). The correlation between the levels of BFI and *L. monocytogenes* in surface irrigation waters is unknown.

Purpose: To concomitantly evaluate population levels of *L. monocytogenes*, *Escherichia coli*, and *Enterococcus* spp. in surface waters.

Methods: Water was collected weekly from July to December 2017 from a river in Pennsylvania. Samples (two each of 100 ml and 500 ml) were collected from six sites spread over a 50-mile reach of the river and water flow rate and stage were recorded. *E. coli* and *Enterococcus* spp. were enumerated using a standard membrane filtration method on MI agar (*E. coli* and total coliforms) (n=150) and mEI (*Enterococcus*) agar. *L. monocytogenes* populations were quantified through a modified most probable number procedure in buffered *Listeria* enrichment broth using a dilution scheme of 5×100 ml, 8×10 ml, 8×1 ml, with a lower limit of detection of 0.0021 MPN/ml. Presumptive *L. monocytogenes* positives were confirmed using VITEK MS.

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Results: Overall, 98.9% (*n*=150) samples were positive for *L. monocytogenes*. Population levels of *E. coli* and *Enterococcus* spp. in water samples ranged from 4.5 to 46,500 CFU/100ml and 3 to 65,000 CFU/100mL, respectively. *L. monocytogenes* levels in water samples ranged from ≤0.0021 MPN/ml to 56.1 CFU/100ml. *L. monocytogenes* counts significantly correlated with *E. coli* and enterococci (R2=0.38 and 0.41; *P*<0.001), respectively. *L. monocytogenes* population levels were also weakly positively correlated with water flow rate (R2=0.15; *P*<0.01).

Significance: This new quantitative data on *L. monocytogenes* levels in surface waters in conjunction with BFI levels is essential for the validation of microbiological quality standards proposed for irrigation waters under the Food Safety Modernization Act.

P1-216 Suitability of *Escherichia coli* as an Indicator of Human Pathogens in Irrigation Water

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Introduction: This project sequenced the genomic makeup of irrigation water samples in order to validate the use of a previously quantified non-pathogenic surrogate (i.e., *Escherichia coli*).

Purpose: Advances in laboratory methods (e.g., sequencing) have raised concerns about the appropriateness of current foodborne pathogen tracking methods. Bacterial indicators of fecal contamination, such as generic *E. coli*, have not undergone extensive validation for estimating the occurrence of microbial pathogens in irrigation water. The aim of this study is to support or refute the use of *E. coli* as an indicator of irrigation water quality.

Methods: A total of 248 samples were sourced from irrigation canals located in the Southwest United States from 2014 to 2015. Samples were filtered (100 mL per sample, 0.45 µm) and DNA was extracted. The microbial profiles of the samples were evaluated using 16S rRNA metagenomics. The 16S results were examined for sequences matching potentially pathogenic genera, including *Legionella*, *Clostridium*, *Aeromonas*, *Acinetobacter*, *Mycobacterium*, and *Enterococcus*.

Results: Of the 248 samples, 220 had previously detected *E. coli* by most probablly number enrichment method (≥1 MPN per 100 mL). 16S sequencing detected low levels of *E. coli*, relative to the total prokaryotes observed in each sample. Of the 220 samples with previously quantified *E. coli*, 88% also contained 16S sequences identified as *Aeromonas*, *Clostridium*, *Legionella*, or a combination of the three. The results of the initial screening will be used to direct further analysis on a subset of samples using shotgun metagenomics.

Significance: A holistic blueprint of the microbiome of irrigation water can provide insights into the populations present and provide researchers with the data needed to determine if current markers from drinking water are fit for use as measures of quality in irrigation water. Future research will attempt to identify new pathogenic indicators in irrigation waters and determine if they are correlated to the presence of foodborne pathogens.

P1-217 Prevalence of *Salmonella* spp., *Listeria monocytogenes,* and *Escherichia coli* in Irrigation Water Sources in the Mid-Atlantic United States: A Conserve Project

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Introduction: Surface and recycled water sources can augment irrigation water without consuming potable water supplies. However, these sources must be evaluated for enteric pathogens that may contaminate crops intended for human consumption.

Purpose: To evaluate six surface and recycled water sources in the Mid-Atlantic United States for prevalence of *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli*.

Methods: Water was collected from six sites: one non-tidal freshwater (NF), one tidal brackish (TB), two pond water (PW), one recycled water (RW), and one processing water (PP) by traditional methods or filtered through modified Moore swabs. Sampling occurred on 22 different dates between September 2016 and December 2017 (*n*=112 samples). *E. coli* populations (CFU/100 mL) were determined by Environmental Protection Agency method 1604 and Moore swabs were analyzed for *Salmonella* and *L. monocytogenes* populations through a modified most probable number (MPN) procedure using volumes of 10, 1, and 0.1 L. One-way analysis of variance on recovered pathogen populations was performed using JMP software.

Results: *Salmonella* populations (±0.49 MPN/100mL) at the PP site were significantly (*P*<0.05) greater than at NF, TB, RW, and PW (0.01 to 0.10 MPN/100mL) sites. *L. monocytogenes* populations (±0.51 MPN/100mL) at the NF site were significantly greater than those at the other five sites (0.003 to 0.06 MPN/100mL). *E. coli* populations (±5.6 CFU/100mL) were significantly greater than those at PW and RW sites (5.46 to 17.6 MPN/100mL). Overall, PW and RW sites had similar levels of *Salmonella*, *L. monocytogenes*, and *E. coli*. The NF site had significantly greater populations of *L. monocytogenes* and *E. coli* compared to the RW and PW sites. Precipitation events after 24 h and 7 d were associated with increased recovery of *E. coli* and *Salmonella* populations, respectively.

Significance: These results indicate that pond and recycled water may provide irrigation water with lower levels of foodborne pathogens than non-tidal fresh water sources in the Mid-Atlantic United States.

P1-218 Occurrence of *Salmonella* and *Listeria monocytogenes* in Alternative Irrigation Water Sources on the Eastern Shore of Maryland: A Conserve Study

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Introduction: According to the Centers for Disease Control and Prevention, almost half of all foodborne illnesses in the United States are attributable to human consumption of fresh produce. Irrigation water has been identified as one possible source of this contamination. Moreover, water scarcity due to climate variation is placing stress on traditional irrigation water sources. Thus, identifying alternative irrigation water sources for use on fresh produce has become an area of great interest.

Purpose: This study investigated six nontraditional water sources on Maryland's Eastern Shore for the presence of *Salmonella* and *Listeria monocytogenes*.

Methods: Water samples (*n*=306) were collected from two recycled water (RW, *n*=66) and four surface water (SW, *n*=240) sites on the Eastern Shore of Maryland from October 2016 to December 2017. Samples were filtered using modified Moore swabs. Filters were subjected to primary enrichment in universal preerichment broth (UPB) followed by secondary enrichment in tetrathionate broth and Rappaport-Vassiliadis broth for *Salmonella* and buffered *Listeria* enrichment broth for *L. monocytogenes*. UPB-enriched samples were quantified by real-time PCR using a modified most probable number (MPN) procedure using volumes of 10, 1, and 0.1 L. Secondary enriched samples were isolated on media selective for *Salmonella* and *L. monocytogenes*, and culture-positive results were confirmed through real-time PCR.

Results: For both *Salmonella* and *L. monocytogenes,* MPN values varied among sites over the course of the study; this variation depended on the water source. For *Salmonella*, these values averaged 1.23 and 2.65 MPN/L for RW and SW, respectively. For *L. monocytogenes*, average MPN/L values were 0.02 and 0.753 for RW and SW, respectively. Overall, *Salmonella* populations for all sites were greater than those of *L. monocytogenes*.

Significance: The results of this research show that *Salmonella* and *L. monocytogenes* are present in irrigation water sources on the Eastern Shore of Maryland and show appropriate risk mitigation for their potential use for safe irrigation of food crops.

P1-219 Foodborne Pathogens in Fish Product Samples and Their Inactivation with Nisin and Ethylenediaminetetraacetic Acid

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Introduction: Despite improvements in recent years, food safety continues to be a growing concern. In South Africa, a dreadful listeriosis outbreak recently caused at least 36 deaths.

Purpose: The purpose of this work was to determine the microbiological quality of seafood in South Africa and to inactivate *Listeria monocytogenes* and *Vibrio metschnikovii* with a combination of ethylenediaminetetraacetic acid (EDTA) and nisin.

Methods: Samples of frozen fish, smoked fish, and sushi were collected from various retail outlets in the Durban area. A total of 60 samples were analysed for total plate count, aerobic and anaerobic spore forming count, and significant pathogens (*Escherichia coli, Vibrio* spp., *L. monocytogenes, Salmonella* spp., and *Staphylococcus aureus*). The effect of nisin at different concentrations (500, 1,000, and 2,000 IU/g) and when used alone or in combination with EDTA (250 ppm) was determined against the survival of *L. monocytogenes* ATCC 7644 and *V. metschnikovii* in Norwegian salmon (commonly used in sashimi products) at different temperatures (4, 7, 30°C).

Results: Mean total plate counts for frozen fish, smoked fish, and sushi were 3.88, 3.95, and 5.27 log CFU/g, respectively. Pathogens such as *Vibrio spp.* and *L. monocytogenes* were present in all three food categories. Nisin (1,000 and 2,000 IU/g) in combination with EDTA (250 ppm) against *L. monocytogenes* at low temperatures (4 and 7°C) showed a 4-log reduction after 24 h; however, they were not as effective against *Vibrio* spp., with only a 2.5-log reduction.

Significance: The presence of pathogens in RTE fish implies a possible public health risk. Also, nisin (1,000 and 2,000 IU/g) in combination with EDTA (250 ppm) could be used to control or inactivate *L. monocytogenes* in seafood.

P1-220 Prevalence and Virulence Genes of *Salmonella* Recovered from Seafood

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Introduction: *Salmonella* causes 1.2 million cases of non-typhoidal salmonellosis and 450 deaths annually in the United States (U.S). During recent decades, per capita seafood consumption has increased in the U.S. It has also been reported that *Salmonella* may be associated with seafood, especially shrimp, catfish, and tilapia. However, adequate information is not available about the prevalence and the presence of virulence genes in *Salmonella* recovered from seafood.

Purpose: The objective of this study was to investigate the prevalence and virulence genes of *Salmonella* recovered from seafood obtained from four retail stores located on the Eastern Shore of Maryland.

Methods:A total of 468 frozen catfish, shrimp, and tilapia (156 of each category) samples were analyzed for *Salmonella* using standard methods. Presumptive isolates were confirmed using BAX PCR. One isolate from each positive sample (*n*=127) was tested for the presence of *Salmonella* virulence genes *inv*A, *pag*C, *spv*C, and *spvR* by PCR.

Results: Twenty-eight percent of catfish, 27% of shrimp, and 26% of tilapia were positive for *Salmonella*. In these three types of seafood, the average log MPN/g of *Salmonella* ranged from 2.5 to 2.7. Seventy-three percent, 76%, and 71% of isolates recovered from catfish, shrimp, and tilapia were positive for *Inv*A, respectively. *Pag*C was detected in 59%, 71%, and 74% of catfish, shrimp, and tilapia, respectively. Sixty-eight percent of catfish, 88% of shrimp, and 74% of tilapia were found to contain *Spv*C. *Spv*R was found in 59% of catfish, 51% of shrimp, and 48% of tilapia. There were no significant differences (*P*>0.05) in the prevalence of *Salmonella* among samples and the presence of virulence genes in *Salmonella*.

Significance: The results suggest that *Salmonella* isolates recovered from seafood can possess virulence genes and thus have the potential to cause salmonellosis. Potential food safety hazards associated with seafood warrant further large-scale studies.

P1-221 Growth and Histamine Production of *Photobacterium* Species at Refrigeration Temperatures

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Introduction: Recently, histamine-producing bacteria (HPB) *Photobacterium* spp. capable of growth and histamine production at refrigeration temperatures were identified. Understanding the time/temperature combinations that result in accumulation of histamine above U.S. Food and Drug Administration (FDA) guidance levels is important to reduce the risk of scombrotoxin fish poisoning.

Purpose: To identify HP *Photobacterium* spp. of concern and determine the time resulting in 50 and 500 ppm histamine levels in yellowfin tuna.

Methods: Photobacterium angustum, *aquimaris*, *kishitanii*, and *phosphoreum* were inoculated in triplicate at 10^6 CFU/ml into LSW-70 broth containing 1% histidine (LSW-70B+) and incubated at 4, 10, and 20°C for 3 d, 3 d, and 20 h, respectively. *P. kishitanii* was inoculated in triplicate at 10^2, 10^4, and 10^6 CFU/ml into homogenized yellowfin tuna and incubated at 4 and 10°C for 14 d. Samples were analyzed for histamine by modified AOAC fluorometric method and histamine-producing bacteria (HPB) counts by most probable number real-time PCR.

Results: P. angustum, *P. aquimaris*, *P. kishitanii*, and *P. phosphoreum* produced 0.0, 3.5, 356.2, and 52.8; 2,058.3, 1,537.6, 1,748.5, and 1,340.6; and 2,920.5, 1,336.3, 820.3, and 225.2 ppm histamine in LSW-70B+ after 3 d, 3 d, and 20 h at 4, 10, and 20°C, respectively. Histamine reached FDA guidance levels of 50 and 500 ppm after 6-7, 4-5, and 2-3 d and after 8-9, 5-6, and 3-4 d in tuna inoculated with 10^2, 10^4, and 10^6 CFU/ml of *P. kishitanii* at 4°C and 1-2, 1-2, and 0-1 d, and after 2-3, 1-2, and 1-2 d at 10°C, respectively. *P. kishitanii* counts were 5.8-6.2, 6.6-7.2, and 6.4-7.6, and 6.6-7.1, 7.1-7.2, and 7.9 log MPN/g at 4°C and 5.0-7.4, 6.3-8.6, and 6.1-7.9, and 7.4-7.8, 6.3-8.6, and 7.9-8.9 at 10°C when histamine levels of 50 and 500 ppm were reached, respectively.

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Significance: ***P. kishitanii*** was a more prolific HPB at low incubation temperatures, whereas *P. angustum* was more prolific at higher temperatures. Additionally, results indicate that at high *P. kishitanii* concentrations, guidance levels of histamine are reached within 2 to 4 days at 4°C in yellowfin tuna.

P1-222 Characterization and Control of Histamine-producing

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Introduction: ***Erwinia*** and *Pluralibacter* spp. capable of producing toxic histamine levels were recently isolated from ingredients commonly used in tuna salad. The characterization and control of these histamine-producing bacteria (HPB) are necessary to prevent illness from tuna salad.

Purpose: The objective of this study was to characterize two *Erwinia* and one *Pluralibacter* spp. for growth and histamine production as they relate to temperature, pH, and salt and to examine the effect of dried vinegar powder on growth and histamine production in tuna salad.

Methods: Maximum growth rates were determined in duplicate in tryptic soy broth (TSB) using a Bioscreen turbidimetric instrument at 5 to 40°C. Histamine production was determined in triplicate in TSB containing 1% histidine inoculated with 10^6 CFU/ml *Erwinia* or *Pluralibacter* spp. after 24 h incubation at pH values 3-10 (pH 6.2; 30°C), 0-7% NaCl (pH 6.2; 30°C), or 5-35°C (pH 6.2; 0.5% NaCl). Finally, *Erwinia* and *Pluralibacter* spp. were inoculated in triplicate into tuna salad preparation (tuna, mayonnaise, celery, onion; 9:3:0.5:0.5) with and without 2% vinegar powder at 4, 10, and 25°C for 3 d, 4 w, and 4 w, respectively. Samples were analyzed for histamine by a modified AOAC fluorometric method and HPB by most probable number real-time PCR.

Results: Optimum growth temperatures in TSB for *Erwinia* and *Pluralibacter* spp. were 30°C and 30-33°C, respectively, and growth was observed at 5°C for both species. Optimum histamine production in TSB was at 30 and 30°C, 0 and 1-2% NaCl, pH 5.0 and 5-6.0 for *Erwinia* and *Pluralibacter* spp., respectively. No significant growth or histamine production was observed in tuna salad stored at 4°C. However, HPB was 6-6.4 and 5.4-7.1 log MPN/g lower at 10 and 25°C in tuna salad containing 2% vinegar powder compared to the control, and no histamine production was observed.

Significance: Understanding optimum growth conditions and histamine production helps tuna salad manufacturers formulate products and adjust processing conditions to minimize hazards from these HPB. Addition of 2% vinegar powder to tuna salad may prevent histamine production in the event of temperature abuse.

P1-223 Histamine-related Quality Changes of Japanese Spanish Mackerel during Storage

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Introduction: Histamine is the causative agent of scombroid poisoning and is a foodborne chemical hazard. Japanese Spanish mackerel (JS mackerel) is an important offshore and commercial fish in Taiwan, with annual landing of more than 600 tonnes. The fish possess high levels of histidine in their white muscle, so scombroid poisoning may occurr due to consumption of JS mackerel in Taiwan. Moreover, there is no information concerning histamine formation in JS mackerel meats packaged with polyethylene packaging (PEP) or vacuum-packaging (VP) during storage at different temperatures.

Purpose: This research was to investigate the effects of PEP (in air) and VP on histamine-related qualities under controlled storage temperatures.

Methods: The effect of PEP (in air) and VP on aerobic bacterial count (APC), histamine formation, and total volatile basic nitrogen (TVBN) of JS mackerel meats stored at different temperatures (-20, 4, 15, and 25 °C) was studied.

Results: The results showed that the APC, TVBN, and histamine contents increased as storage time increased when the PEP and VP samples were stored at 25 °C. At below 15°C, the APC, TVBN, and histamine levels in PEP and VP samples were reduced, but the VP samples had considerably lower levels of APC, TVBN, and histamine than PEP samples. Once the frozen fish samples stored at -20 °C for two months were thawed and stored at 25°C, VP reduced the increase of histamine in JS mackerel meats as compared to PEP.

Significance: This result suggested the JS mackerel meats packed with VP and stored at below 4 °C could prevent deterioration of product quality and extend shelf life.

P1-224 Application of High-pressure Processing on Preservation of Tuna Muscle during Storage

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Introduction: Histamine fish poisoning is a worldwide foodborne intoxication caused by the ingestion of seafood that contain high levels of histamine. Fish species such as tuna, mackerel, bonito, and saury that contain high levels of free histidine in their muscle are often implicated in histamine fish poisoning incidents. Recently, high-pressure processing (HPP) is an emerging non-thermal technology used in the preservation of many food products.

Purpose: The aim of this study was to determine the effects of HHP treatment on the levels of aerobic plate count (APC), total volatile basic nitrogen (TVBN), and histamine in tuna muscle, resulting in the prevention of histamine fish poisoning.

Methods: Skinless tuna muscle was treated with different pressures (20, 300, 400, 500, and 600 MPa for 5 min) and then stored at 4 and 15°C. During storage, tuna samples were analyzed for color, APC, pH, TVBN, and histamine content.

Results: The APCs of tuna samples after HPP treatment (200 to 600 MPa for 5 min) decreased with increased pressure from 5.97 log CFU/g of control (untreated) to 2.15 log CFU/g of 600 MPa treatment. The HPP (>400 MPa) significantly delayed the APC increase in marlin meat, regardless of the storage temperature. At 4 and 15°C, TVBN levels in HPP samples were reduced as compared to control. The HPP (>300 MPa) significantly delayed the histamine increase in tuna sample, regardless of the storage temperature.

Significance: These results suggest that tuna muscle treated with HPP (>400 MPa, 5 min) and stored at below 15°C could prevent deterioration of product quality and extend shelf life. Overall, these results prove the usefulness of HPP in seafood processing while enhancing the preservation and safety of tuna fish consumption.

P1-225 Accumulation and Survival of *Salmonella enterica* in Live Oyster Shell Stock

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Introduction: The current microbiological criterion for *Salmonella enterica* in live oyster shell stock is set based on the assumption that the levels of the pathogen remain stable. However, there is very little data reporting the growth potential of *S. enterica* in naturally contaminated oysters.

Purpose: The purpose of this investigation was to determine the kinetics of *S. enterica* growth and survival in live oyster shell stock.

Methods: A natural contamination event was mimicked by exploiting the natural filter feeding mechanism of oysters. A slurry consisting of algae and a three-strain *S. enterica* cocktail was prepared and fed to Pacific oysters. Oysters were removed at timed intervals, shucked, and the bacterial populations were enumerated to determine the optimal feeding period. A bacterial survival study was then conducted wherein the oysters were removed from their tank after the feeding period and maintained at room temperature (20°C). Bacterial levels were determined daily by standard plate count methodology.

Results: The oysters accumulated detectable levels of *Salmonella* after 30 min and the levels stabilized after 60 min, which was deemed the ideal feeding time, allowing for a *Salmonella* accumulation in the target range of 3 to 4 log CFU/g. The median starting population of *S. enterica* on the oyster shell stock was 4.30±0.5 log CFU/g and decreased to 3.70±0.2 log CFU/g after seven days at room temperature. The levels of background microflora mirrored those of *S. enterica*, decreasing from 4.80±0.5 log CFU/g to 4.30±0.3 log CFU/g.

Significance: The level of *S. enterica* on live oyster shell stock is stable over seven days. Given the short shelf life of oysters, the levels of *S. enterica* are not expected to vary significantly between the point of contamination and the point of consumption.

P1-226 Evaluation of an Alternative Method for Detection of *Vibrio cholera*, *V. parahaemolyticus*, and *V. vulnificus* in Seafood Products Using Real-time PCR

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Introduction: ***Vibrio*** spp. are a leading cause of bacterial infections related to seafood. *V. cholera*, *V. parahaemolyticus*, and *V. vulnificus* are mainly linked to gastrointestinal illness and septicemia. Their absence is required especially on raw or undercooked products for food safety. While isolation by culture using regulatory testing can be a labor-intensive process, an alternative method based on a quicker enrichment and a real-time PCR could allow rapid qualitative detection.

Purpose: This study evaluated inclusivity/exclusivity, LoD95, and performance of the global method for detecting those three bacteria in different seafood products.

Methods: The test includes four steps: selective *Vibrio* enrichment broth, iQ-Check *Vibrio* (includes DNA extraction and quadriplex real-time PCR), and data interpretation. For inclusivity/exclusivity, 136 certified strains were tested, 51 belonging to *Vibrionaceae* with 14 *V. cholera*, 11 *V. parahaemolyticus*, and 10 *V. vulnificus*. The LoD95 was determined by using a 10-fold serial dilution of a single inoculation (*V. cholera*, *V. parahaemolyticus*, or *V. vulnificus* strain) or a multi-inoculation. Six different seafood matrices (shrimp, prawns, oysters, scallops, tuna, and codfish) at different states (fresh, frozen, cooked, salted) were inoculated at 5 CFU/ml (or not) for assessment. All samples were culture confirmed following the ISO 21872-1:2017 method. Cq values for *V. cholera*, *V. parahaemolyticus*, *V. vulnificus*, and internal control targets were analyzed.

Results: The assay demonstrated 100% inclusivity for *V. cholera*, *V. parahaemolyticus*, or *V. vulnificus* and 100% exclusivity for non-*Vibrio*. LoD95 results were confirmed to 716, 224, and 84 CFU/ml respectively for single infection with *V. cholera*, *V. parahaemolyticus*, and *V. vulnificus*. For assessment, concordance to confirmation on thiosulfate-citrate-bile salts-sucrose agar and chromogenic medium was 100% for all PCR assays on enrichment broth or colony.

Significance: This study indicates that the *Vibrio* enrichment broth and iQ-Check *Vibrio* test is a rapid and sensitive method for detecting *V. cholera*, *V. parahaemolyticus*, and *V. vulnificus* in seafood products prone to the presence of competing flora. No significant difference was observed when compared with the reference culture method.

P1-227 Genetic Characteristics, Heat Resistance, and Antibiotic Resistance of *Vibrio parahaemolyticus* Isolated from Seafood-related Environments

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❖ ***Developing Scientist Competitor***

Introduction: As seafood consumption increases, the risk of *Vibrio parahaemolyticus* infection may also increases. However, there are only few studies to isolate, identify genetic correlation, and evaluate heat resistance and antibiotic resistance of *V. parahaemolyticus* in variant seafood-related environments.

Purpose: This study isolated *V. parahaemolyticus* in variant environments, and analyzed genetic characteristics, heat resistance, and antibiotic resistance of the isolates.

Methods: One hundred sixty seven samples (seafood: 126 samples, and environment: 41 samples) were collected from two fishery auction markets, four fishery markets and three online markets in South Korea from March to September in 2017. The samples were analyzed by quantitative and qualitative methods for *Vibrio parahaemolyticus* identification. The presumptive colonies were analyzed by PCR to identify *V. parahaemolyticus,* and virulence genes for *hns, tdh* and *trh*. Genetic correlations among *V. parahaemolyticus* isolates were evaluated by pulsed-field gel electrophoresis (PFGE). The isolates were exposed to 50°C for 10 min to evaluate the heat resistance, and antibiotic resistance of pathogenic isolates were also evaluated.

Results: Twenty eight samples [(16.8%; 17 seafood samples (10.5%) and 11 environment samples (26.8%)] of 167 samples were *V. parahaemolyticus* positive, and 35 colonies were isolated from the positive samples. The highest detection month was September (51.9%) and among the 35 isolates, only three (8.6%) of them had virulence genes (*tdh* and *trh*). In addition, these three isolates were ampicillin-resistance. Genetic correlations with more than 90% among 35 *V. parahaemolyticus* isolates were not observed. Most isolates reduced by 2-3 log CFU/ml by heat treatment, but three strains of them were reduced only 1 log CFU/ml.

Significance: This result indicates that the proportion of *V. parahaemolyticus* contaminated is high in fishery auction markets, 8.6% of the isolates are pathogenic and ampicillin-resistance. Therefore, safety regulation in fishery auction market have to be established to control *V. parahaemolyticus.*

P1-228 Surveillance and Prevalence of *Salmonella spp.* and Sanitary Indicators in Wild Caught and Farm-raised Catfish *(Siluriformes)* Carcasses in the United States

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Introduction: Catfish inspections have transitioned from the U.S. Food and Drug Administration (FDA) to the United States Department of Agriculture (USDA). The USDA Food Safety and Inspection Service (FSIS) and Food Emergency Response Network (FERN) consider it important to assess the food safety risk associated with consuming catfish in the United States. Surveillance of farm-raised and wild-caught catfish for pathogens is relevant as a public consumption safety protocol.

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Purpose: To detect the presence of *Salmonella* spp. and evaluate sanitary indicator organisms in raw wild-caught and farm-raised catfish carcasses in the United States.

Methods: A total of 240 catfish samples were collected from retail markets in the southeast region of Louisiana. *Salmonella* spp. detection was conducted using the USDA Microbiology Laboratory Guidebook (MLG) method. MLG 4.08 was used to isolate and identify *Salmonella* spp. by PCR and cultural methods. Presumptive positives were confirmed using biochemical assay and serological characterization. Of the samples, 100 were quantified by enriching a 25±2.5-g sample in 225 ml phosphate buffered saline. The samples were analyzed for aerobic plate count (APC), coliforms, and *Escherichia coli* using 3M Petrifilms, and for *Staphylococcus aureus* and *Salmonella* spp. using Baird-Parker and XLD agar at 37°C for 48 h.

Results: Salmonella spp. was detected and confirmed positive on 6 wild caught and 0 farm raised catfish. APC ranged from 4.6 to 6.34 (wild) and 4.0 to 5.96 (farm-raise) log CFU/g. *E. coli* ranged from 2.3 to 2.6 (wild) and 2.0 to 2.3 (farm-raised) log CFU/g. Coliforms were found at 2.0 to 3.94 (wild) and 2.0 to 2.7 (farm-raised) log CFU/g. *S. aureus* was found at 2.0 to 2.6 (wild) and 2.0 (farm-raised) log CFU/g.

Significance: Detection of *Salmonella* spp. and other pathogens in catfish will extend the protection of USDA-FSIS-regulated foods.

P2-01 The Role of Safe Quality Food Certification in Food Production

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Introduction: Although federal regulations, inspections, and product category audits have been used to standardize food production, the birth of private food safety standards is fast becoming more reliable and acceptable in the food industry. Private or voluntary food standards are being initiated to complement food regulations or legislation and to attain premium levels of consumer acceptance.

Purpose: The purpose of this study was to investigate the role of the Safe Quality Foods (SQF) certification to aid the production of safe and quality foods, post-implementation by food processors. Administered by the Food Marketing Institute and recognized by the Global Food Safety Initiative, the SQF certification process closes the food production loop by certifying food manufacturing, distribution, retailers, and brokers to an effective and robust food safety and quality control program.

Methods: Qualitative data were collected through interviews with 35 SQF practitioners from different food facilities on their perceptions of participating in SQF since implementation at their respective facilities.

Results: The majority of participants had a positive perception of SQF towards improving the overall safety and quality of their products. A total of 85.71% of the participants noted that the SQF scheme provides credible food safety and quality guidelines necessary for continuous improvement through the SQF codes, and 80% of the participants stated that SQF provides a good roadmap to meeting customer or retailer requirements. Of the participants, 97.14% also noted that passing the SQF certification provides trust in the food manufacturing process.

Significance: Food producers who have not adopted this scheme can benefit from this holistic certification to enhance their food production capability. In addition, amid the myriad failures emanating from food operations, participating in third-party certifications like the SQF provides defense measures against food production failures. Participating in SQF certification can also help food producers to move beyond compliance and gain competitive advantage.

P2-02 Factors Associated with Food Safety Behaviors in Cancer Patients Seeking Treatment

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Introduction: More than 14 million Americans live with cancer, which puts them at a high risk for foodborne diseases due to weakened immune systems. However, food safety factors and practices that may lead to risky food safety behaviors are not well-understood. The objective of this study was to i) identify sociodemographic factors associated with food safety behaviors among cancer patients and ii) determine the level of food safety knowledge and risk awareness in patients undergoing chemotherapy and radiation treatments.

Purpose: This study aims to investigate these factors and determine food safety practices among cancer patients in order to create more effective food safety education.

Methods: This was a cross-sectional, survey-based study that recruited participants from three cancer-specific hospitals in central Ohio. A 173-item questionnaire assessed the risk perception, food preferences, and food safety knowledge and food acquisition practices among cancer patients in treatment. In addition, the questionnaire included sociodemographic information, food insecurity status, quality of life, disease characteristics, and treatment history.

Results: Participants (n=222) were mostly female (68.9%), older than 50 (78.64%), and non-Hispanic white (85.97%); the majority was married (63%) and almost one third had less than a high school degree (30%). Patients were on average aware of 80% (mean=0.8±0.15; 0.20 to 1.00) of the food safety risks. However, only 34% of the patients were aware that cancer puts them at a high risk for foodborne diseases. Food insecurity was associated with lower risk awareness (P<0.01), more common inadequate food preparation practices (P<0.01), increased inadequate food acquisition practices (P<0.01), and lower overall food safety knowledge (P<0.05). Those who faced food insecurity had similar food acquisition behaviors as those who did not face food insecurity (t=108, df=204, P=0.28).

Significance: Food safety interventions focusing on cleaning and adequate storage would have the highest impact. The findings can be used to develop effective food safety education programs for cancer patients and highlight the need for food safety education of patients during cancer treatment.

P2-03 Effects of Food Safety Training on Achieving Food Safety Knowledge and Practices in Restaurants in the Emirates of Dubai

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❖ ***Developing Scientist Competitor***

Introduction: This study evaluates the effectiveness of using demonstrations in training sessions to improve food safety knowledge and practices of food handlers. The study also evaluates current food hygiene practices in Dubai restaurants based on well-established current good practices (CGPs).

Purpose: The purpose of the study is to evaluate the effectiveness of using demonstrations in training sessions to improve food safety knowledge and practices of food handlers. As part of this aim, the study also evaluates the current food hygiene practices in Dubai restaurants based on well-established current good practices (CGPs).

Methods: To the best of the researchers' knowledge, limited studies have developed a valid and reliable instrument to evaluate the effect of demonstration in training of untrained food handlers on achieving good hygiene practices. This study aims to develop an instrument for identifying the effect of demonstration in achieving knowledge and good hygiene practices.

Results: The study concludes that training with demonstration techniques is an effective way of improving compliance with food safety guidelines. There is presence of food safety shortcomings in restaurants related to time and temperature control, improper hygiene, and cross-contamination.

Significance: Findings from this study aim to provide insights into a fairly new, but evolving, research area in the retail foodservice sector. The outcomes of this study are expected to have national and international implications for the enhancement of food safety education through the use of demonstrations.

P2-04 Using Interactive Learning to Educate Youth about Safe Handling and Preparation of Poultry and Eggs

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Introduction: Collectively, youth are not as knowledgeable about food safety issues and are more prone to mishandling foods than older adults. However, it has been shown that educating families and communities through youth programming is one of the best ways to disseminate information and change food habits.

Purpose: The purpose of this study was to i) evaluate the effectiveness of active learning modules, each focusing on different key messages regarding poultry and egg food safety and ii) document potential dissemination of the knowledge to families.

Methods: Six hands-on learning activities, i.e. Bacteria on a Stick, Bean Bag Refrigerator Toss, Microscope and Toy Bacteria, Pick the Right Thermometer, Bag Demonstration, and PEEP Videos, were created by agricultural education students. These were delivered over three days in conjunction with the Mobile Agricultural Education Trailer at the National Future Farmers of America convention. After finishing the activities, 247 students completed an online survey that included knowledge assessment, interest, and impact questions.

Results: The majority got all questions correct except for hand washing. Only half knew how long they should wash their hands. Ninety-five percent reported learning something new about poultry and egg safety, 93% were more willing to practice food safety procedures at home, and 91% agreed that they would share what they learned with their families. Participants' favorite learning activities were Bacteria on a Stick (M=86.68) and Bean Bag Refrigerator Toss (M=82.50).

Significance: Participants gained new knowledge that they intend to implement at home and share with their families. Therefore, interactive learning, especially including activities that allow participants to taste, feel, do, or see, is an effective dissemination tool for youth.

P2-05 BAC Fighters' Perception of Effectiveness of the "Don't Wing It!" Poultry Education Campaign

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Introduction: Educators have expressed a need for targeted, simple, and relevant food safety education messages, stating that they are most effective at improving risky behaviors. Providing these on a popular Web site in a useable form is also an advantage.

Purpose: This study intended to evaluate BAC Fighters (i.e., users of *www.fightbac.org*) materials, perceptions of usability, and effectiveness of tools included in the "Don't Wing It!" poultry education campaign, available on the Partnership for Food Safety Education website.

Methods: Educational tools (e.g., brochures, recipes, presentations, blogs, etc.; available in Spanish and English) related to safe poultry selection, handling, and preparation were developed, evaluated, revised, and posted. Availability of campaign materials was promoted through e-cards, on Facebook, and during two webinars. An initial participation invitation email was sent to ~14,000 registered BAC Fighters. Two follow-up emails were sent to encourage completion of the survey. Approximately 800 individuals clicked on the survey link and completed the initial questions, with 226 qualifying for full participation by having previously accessed the "Don't Wing It!" campaign. Results reflect respondents' program awareness and use, as well as perceived effectiveness of the campaign. Suggestions for improvement were also collected.

Results: Each of the 12 program tools was rated highly effective (93 to 100%) in promoting positive change in food safety practices. Respondents (10%) who had conducted their own behavior-based evaluations reported a positive impact of the campaign materials in improving knowledge and promoting potential behavior change (100%). Although campaign brochures were targeted towards millennial parents and older adults, they were used with and deemed appropriate for a variety of audiences and in many different settings. Suggestions for improvement included recipes in Spanish, more recipes, both in English and Spanish, and providing a printable magnet.

Significance: Further promotion of the online program is needed to increase awareness of and usage by educators.

P2-06 Evaluation of User-friendly Tools to Support Food Microbiology Practical Laboratory Classes

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Introduction: Undergraduate food microbiology laboratory classes provide basic knowledge of microbial analysis of food through detection, enumeration, and identification of foodborne pathogens and spoilage microorganisms. These procedures are essential in the evaluation of sanitation programs. However, the preparation of laboratory exercise materials is usually time-consuming and cumbersome. Although ready-to-use alternatives are available, they are scarcely used in academic settings.

Purpose: The goal of this study was to evaluate students' perceived usability of two ready-to-use plating alternatives for yeast and molds and compare accuracy, repeatability, and agreement.

Methods: Sixty undergraduate students were randomly assigned to two groups. Each student swabbed a floor tile and a stainless steel coupon. Group A performed dilution and plated on a PDA (potato dextrose agar) dry media already made in a shallow dish with an adhesive on top. Group B used a ready-made PDA containing a cold-water-soluble gelling agent. Surfaces were previously inoculated with *Candida tropicalis* and *Aspergillus niger* (final concentration 10^3 CFU/cm^2). Plates were incubated at 25°C and checked after two, three, and five days. Enumeration was performed on PDA as control. A questionnaire regarding perceived usability was completed by the students, and statistical analysis was performed to assess accuracy and precision of the methods and students' perceptions.

Results: There were no significant differences (P<0.05) in colony counts between the two ready-to-use plating alternatives (within the same surface type). Correlation between colony count reads was high and positive for both alternatives. There was a high positive correlation for both alternatives

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on plastic tiles; however, correlation was lower for stainless steel tiles. No significant differences (P>0.05) were found in students' perception regarding usability.

Significance: The results of this study demonstrated that both alternatives were beneficial for supporting students' learning. Equivalent microbial counts were obtained and products were comparable in terms of usability, accuracy, and precision.

P2-07 Blockchain Solutions for Food Safety

JESSE DOWDLE

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Introduction: You've heard of Bitcoin, but did you know the fundamental technology behind it has the potential to transform our food supply? This technology (called blockchain) can be used to establish trust, increase accountability, and clear the fog from the global food supply chain. A new era is about to begin in the quest to improve food safety.

Purpose: The purpose of this session is to educate attendees about blockchain technology, including how it solves the biggest pains in traceability and compliance, how some of the major players in the industry are utilizing blockchain today, and where blockchain for food safety is heading in 2018.

Methods: This educational session will provide an overview of blockchain technology, the difference between a public and private blockchain, and explain real-world examples of successful pilot programs of blockchain in the food industry.

Results: During a pilot program conducted by IBM and Walmart, trackability time for a case of mangoes was cut to two seconds, down from days or weeks.

Significance: More than 3,000 people die of foodborne illnesses in the United States every year. Food traceability is one of the largest challenges to effective recalls. Blockchain technology has the potential to trace products, hold suppliers accountable for quality standards, and ensure safety through the supply chain.

P2-08 Development of Add-on Training Materials Customized for the Western United States to Supplement the Standardized Curricula for the Food Safety Modernization Act's Preventive Controls for Human Food and Produce Safety Rules

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Introduction: The goal of the Western Regional Center to Enhance Food Safety (WRCEFS) is to build regional infrastructure to support the delivery of the U.S. Food and Drug Administration's Food Safety Modernization Act (FSMA) training, education, extension, outreach, and technical assistance to producers and processors in the western region (WR) of United States (U.S.). Although standardized U.S. training curriculums are available, the need for supplemental materials to reflect region-specific agricultural production and processing systems has been identified during training sessions with produce growers and food processors in the WR.

Purpose: To identify priority topics for add-on FSMA training materials that address WR-specific agricultural production and processing systems.

Methods: Representatives (n=52) from 15 WRCEFS regions, other regional centers, federal and state government agencies, and industry stakeholders attended three breakout sessions at the WRCEFS annual meeting in March 2017 in Honolulu, HI to: i) review 16 supplemental materials developed by the Southern Center (SC) and Produce Safety Alliance (PSA; seven randomized groups with six to seven people/group; 20 min); and ii) brainstorm (eight groups with four to eight people/group; 10 min) and prioritize (WRCEFS sub-regions groups; 10 min) topics relevant to WR, specifically addressing the preventive controls for human food (PCHF) and produce safety (PS) rules of FSMA.

Results: SC and PSA materials were recommended for use as-is or modified on an as-needed basis by WR instructors. Participants prioritized 11 of 25 PCHF and 14 of 39 PS topics, with five of 11 PCHF and three of 14 PS add-on projects funded and presently being developed. These include: sanitation basics, value-added processing in California, aquaponics in the Pacific Northwest (PNW), a digital repository of California-specific farming practices, cottage industry in Hawaii, agricultural water in Hawaii, production of breadfruit flour, and simplified PCHF training in the PNW.

Significance: Materials developed will provide targeted supplemental curricula addressing PCHF and PS applicable to the western U.S., with elements that can also be adapted to other regions in or outside the U.S. to enhance food safety trainings.

P2-09 Consumer Food Safety Education Needs across the State of Washington

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Introduction: Consumers play a major role in the food safety cycle, and their lack of knowledge, or lack of regularly utilizing food safety best practices, contributes to foodborne illnesses.

Purpose: We conducted a statewide consumer food safety assessment to assess consumer food safety practices within Washington state, with the intent of developing, creating, and delivering new and effective food safety programming.

Methods: A consumer food safety questionnaire was developed using general food safety information published by the Centers for Disease Control and Prevention, the U.S. Food and Drug Administration, and the United States Department of Agriculture. Additional questions were composed from other published studies on consumer food safety practices. Surveys were delivered to 375 consumers across the state of Washington, at farmers markets and fairs.

Results: The results of this study showed a lack of consumer knowledge across a broad spectrum, which was not significantly different across demographics. Most consumers were expressed concern that poultry (81%), meats (65%), and fish (61%) pose the highest food safety risk, despite a large number of vegetables being recalled over the previous year. Only 49% of respondents were concerned with poor general sanitation practices causing foodborne illness. More than half of respondents (54%) indicated that they had previously taken food safety training. When consumers were polled as to where they get their food safety information from, the majority of consumers obtained their information from the internet (51%), followed by family and friends (23%). Only 19% of consumers used food safety information from government agency sources, and only 11% reported obtaining food safety information from the Washington State University Food Safety Extension.

Significance: These results show that Extension needs to play a greater role in educating consumers about food safety, and social media and websites are more likely to drive consumer education. Statewide data was sometimes inconsistent with nationwide data, and geographical differences may play a role in local food safety knowledge.

P2-10 The Missing Ingredient: Food Safety Messages on Recipe Blogs

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✦ ***Undergraduate Student Award Competitor***

Introduction: Educating consumers on safe food handling behaviours is important to reduce foodborne illness. The most popular online recipe blogs draw over 5 million unique monthly visitors. Their authors are social media influencers who have the potential to spread food safety messages to large audiences.

Purpose: The purpose of this study was to evaluate food safety messages on popular recipe blogs, including safe endpoint cooking temperatures, behaviours to reduce cross-contamination, washing raw fruits and vegetables, and maximum storage times.

Methods: Recipes containing either meat or raw fruits and vegetables from 50 popular recipe blogs were analyzed using a structured and pre-tested coding form to determine the prevalence of various food safety messages. Messages were evaluated based on current government recommendations.

Results: Of 784 recipes that met selection criteria, 479 contained meats for which specific endpoint temperatures are recommended by national health authorities. The use of a thermometer to ensure adequate cooking was suggested in 16.9% (n = 81) of recipes. This recommendation was more likely for certain types of meats than for others (F(7,201) = 11.686, p < .001). Where endpoint temperatures were provided (n = 79), 60.8% were correct (n = 48). Endpoint temperatures were often paired with incorrect subjective doneness indicators. Among recipes containing fresh fruits and vegetables (n = 304), only 3.3% (n = 10) suggested washing produce intended to be consumed raw. Recommended storage times were provided for 4.0% of recipes (n = 31), 55% of which (n = 17) correctly corresponded with government guidelines.

Significance: Safe food handling practices are rarely recommended on popular recipe blogs, and when provided, they often do not correspond with current government recommendations. Efforts are needed to encourage the adoption and promotion of food safety messaging on recipes blogs, as they have the power to reach large audiences.

P2-11 Effect of a Training Intervention on Vomit and Diarrhea Clean-up Guidelines, Food Safety Manager Knowledge and Attitudes, and Organizational and Environmental Change

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Introduction: Proper cleanup of vomit and diarrhea in foodservice and retail food establishments can prevent transmission of viruses, such as noroviruses.

Purpose: To assess the effect of a new training intervention on improving participants' knowledge and attitudes and to promote organizational and environmental change.

Methods: Developed a cleanup practices module that can be incorporated into food safety manager training. Recruited educators and randomly assigned them to a treatment (delivered intervention) or a control (delivered standard training) group. Collected data at pre- and post-intervention. Conducted statistical testing to assess outcomes of interest between the treatment and control groups.

Results: In summer and fall 2016, 34 educators conducted 116 training sessions across the United States, training a total of 1,136 food safety managers. Of these, 681 completed the pre-survey and 161 completed the post-survey. Results indicate mixed effects of the training. Compared to the control group, managers in the intervention group were more confident that they could clean up vomit and diarrhea in the dining room when customers are present (P=0.02) and in the bathroom (P=0.002). Knowledge of appropriate vomit and fecal cleanup procedures did not improve significantly when comparing the two groups. There was no statistically significant difference between the groups in the number of respondents who said they have adopted the cleanup procedures or purchased a cleanup kit since taking the intervention.

Significance: The intervention bolstered managers' confidence in their staffs' ability to handle vomit and fecal cleanup but failed to improve knowledge or behavior. This may be due to small sample size or because the intervention was not sufficient to generate knowledge or motivate behavior change. Future research should consider expanding the intervention to include more time devoted to vomit and fecal cleanup, as well as hands-on training at establishments.

P2-12 The Composition of an Intervention Programme Based on the World Health Organization's Five Keys to Safer Foods and the Assessment of Hospice Food Preparation Surface Cleanliness

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Introduction: Appropriate food handling practices are crucial for preventing the spread of foodborne diseases during food preparation and distribution in hospices. Both developed and developing countries are at an increased risk of foodborne disease. Training of food handlers is important to ensure the safety of food served to patients in hospices. To reduce the burden of foodborne diseases, interventions are necessary throughout the "food safety continuum" from farm to table.

Purpose: The aim of the study was to assess the efficacy of food handler training based on the World Health Organization's (WHO) Five Keys to Safer Foods and to concurrently assess the cleanliness of hospice food preparation surfaces.

Methods: A pre-test and post-test quasi experimental study design was employed using the WHO Five Keys to Safer Food questionnaire. Training was given to all the food handlers that participated in the study (n=100) and the scores were compared before and after training. The study was carried out in two hospices located in the South African provinces of Free State and Eastern Cape. Concurrently, food preparation surfaces were analyzed for cleanliness before and after training using ATP Hygiena bioluminescence. Statistical analysis was done in terms of mean and standard deviation (SD). SPSS Statistics software (v. 18) was used for all the analyses.

Results: The knowledge, attitudes, and self-reported behaviours of food-handlers were found to be significantly different between pre-training and post-training assessments (P<0.01). Moreover, there was a statistically significant reduction (P<0.001) of the ATP readings obtained from hospice food preparation surfaces post-training. The mean SD score of knowledge was 6.1 (1.11) for pre-training and 7.81 (1.25) for post-training. The mean SD score of attitude was 5.85 (1.02) pre-training and 7.40 (1.30) post-training. The mean SD score of self-reported behaviour was 5.63 (1.18) pre-training and 7.01 (1.56) post-training.

Significance: Regular food safety training is encouraged to reduce food contamination risks by adjusting the practices of food handlers and improving their skills. The data in the study suggest that food safety training has the potential to improve food handler knowledge and practices.

P2-13 Consumer Knowledge, Perceptions, and Purchasing Behaviors Associated with Food Processing Technologies in the United States

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Introduction: Despite the numerous benefits attributed to food processing, consumers continue to perceive the term negatively. Consumer opposition to food processing technologies compromises food safety integrity and has created implementation barriers within the food industry for novel and effective intervention strategies.

Purpose: The purpose of this study was to assess consumer knowledge, perceptions, and purchasing behaviors associated with both conventional and emerging food processing technologies.

Methods: A nationwide phone survey was administered by Virginia Tech's Center for Survey Research, with a target sample of 505 completions. Items on the survey included those related to food processing, pasteurization, microwave technology, high pressure processing, irradiation, light technology, and exposure to gas as mechanisms to make food safer for consumption, using both fixed choice close-ended questions and open-ended responses. A random-digit dialing method was employed for a sample of randomly selected telephone numbers (85% cellular numbers and 15% landline numbers); more cellular numbers were selected to ensure a representative population of all ages and an optimal rate of response for the study.

Results: A total of 505 fully completed interviews were collected (sampling error of ±3.8%). Approximately 67% of participants expressed concerns towards the term "processed foods." Once the term "food processing" was defined for interviewees, 41% of participants either "somewhat disagreed" or "strongly disagreed" with the desire to purchase food that had been processed. More than half of interviewees acknowledged having concerns with foods that have been irradiated or treated with gas to improve safety and quality.

Significance: Educational interventions are needed to provide information to consumers and increase their acceptance of new and currently used processing technologies. It is essential that misconceptions associated with food processing are addressed so that the food industry can effectively target emerging issues related to food, while still creating safe, high-quality products.

P2-14 Food Safety Cognition of Parents with Young Children and the Potential Use of Online Parenting Communities to Obtain Food Safety Information

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Introduction: Due to immature immune systems, young children (<5years) have an increased risk of foodborne illness and are associated with increased incidence. Consequently, given the association of the domestic kitchen with sporadic foodborne illness, implementation of safe food handling and storage practices is essential when preparing food for children. Given increased popularity of online parenting communities, there is a need to determine the potential use of such platforms by parents to obtain information relating to food safety during formula feeding, weaning, and solid food preparation.

Purpose: To determine the food safety cognitions of parents and establish the use of online parenting communities in relation to food safety information.

Methods: Online self-complete questionnaires distributed using social media were completed by parents of children (*n*=78).

Results: Although knowledgeable of some aspects of domestic food safety when preparing food for young children, gaps exist and food safety malpractices were reported. The vast majority (95%) of parents in this study reported accessing online parenting communities. It was established that <60% reported using online parenting communities ("MumsNet" and Facebook parenting groups) to obtain information, and <32% believed information acquired from such sources to be trustworthy. Nearly half (48%) of parents reported that social media influenced their everyday opinions and practices. The majority (73%) reported that if they saw a comment advising a food safety malpractice in an online parenting-community, they were likely to correct the misinformation. Furthermore, 92% stated they would be more likely to correct the advice if it was given to a friend. However, fewer (74%) stated they would correct the advice if it came from a friend.

Significance: The potential role of online parenting communities in obtaining food-related information has been established. Completion of the study has determined the need for research to investigate the communication of food safety malpractices among peers on parental social media platforms and explore the potential for such platforms to promote food safety.

P2-15 Consumer Awareness of *Campylobacter* in the United Kingdom

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Introduction: Campylobacter is the most common cause of foodborne illness in the United Kingdom (UK), with an estimated economic cost of £900 million and a human cost of 100 deaths annually. Poor food safety practices in the home are believed to be a common cause of campylobacteriosis. Consequently, there is a need to determine consumer awareness of *Campylobacter* and explore associated consumer food safety practices.

Purpose: To investigate knowledge of *Campylobacter* and determine self-reported domestic food safety practices associated with the risk of campylobacteriosis.

Methods: An online questionnaire to determine consumer food safety knowledge, attitudes, and self-reported practices was completed by consumers in the UK (*n*=183).

Results: The majority (80%) were aware *Campylobacter* was a bacteria, and 62% indicated awareness that *Campylobacter* was associated with raw chicken. Only a fifth (20%) were aware of the Food Standards Agency's "Acting on Campylobacter Together" campaign. The majority (90%) indicated awareness that *Campylobacter* may cause complications that could cause death in vulnerable patients, and 69% perceived the home to be the most common cause of foodborne illness. Although 91% of participants reported that they always wash their hands using soap after handling raw chicken, it was determined that 10% would wash raw chicken, only 10% reported using a temperature probe at home to ensure thorough cooking of meat/poultry, and many reported conducting visual inspections. Consequently, 43% were aware of recommended cooking temperatures (>75°C), but 47% stated temperatures exceeding 100°C. Less than half (49%) reported that they "always" use separate chopping boards for raw meat/poultry and ready-to-eat foods.

Significance: Although findings indicate public awareness regarding *Campylobacter,* food safety malpractices were reported. However, it must be considered that self-reported data may not equate to consumer behaviour. Therefore, behavioural studies to observe consumer food safety practices in the domestic environment are required. Further studies are needed to investigate why consumers fail to adhere to recommended food safety practices.

P2-16 A Narrative Review of International Research Studies Detailing Food Safety Awareness of Professional Food Handlers and Practices in Catering and Manufacturing Environments

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Introduction: Foodborne illness outbreaks associated with manufacturing and catering environments remain a public health concern. Consequently, the need to assess the food safety of food handlers in such food production environments is of the utmost importance. However, utilized methods influence the type of data that can be measured. There is a need to consolidate international food handlers food safety data to assess cognition and behavior.

Purpose: To review the methods and measures utilized in research studies to assess the food safety awareness and practices of professional food handlers in catering and manufacturing environments.

Methods: Professional food handler food safety research studies (*n*=20) were identified and reviewed, and findings were summarized according to assessment of knowledge, attitudes, self-reported practices, and observed behaviors related to key components of food safety.

Results: The majority of studies (60%) were published between 2013 and 2017 and included North America, Europe, Asia, and Africa. Although all studies focused upon professional food handlers, the majority (70%) were from catering/retail establishments and fewer studies were conducted in manufacturing/processing environments (10%). Survey methods of data collection were widely utilized, including self-complete questionnaires (80%) and interviews (35%). Observation of behavior was less frequently used (30%). Consequently, the majority of findings were based on self-reported practices and knowledge. The most frequently covered topics in reviewed studies included awareness of when hand hygiene should be implemented and practices relating to cross-contamination. Analysis of data also determined that gaps in knowledge of some key food safety practices may exist among professional food handlers and malpractices were reported.

Significance: Completion of this narrative review has identified the need for an in-depth systematic literature review to further explore the topic. Given this study identified a lack of observational data, there is a need for research, particularly in manufacturing environments, to observe the food safety behaviors of professional food handlers; such methods can also be used to evaluate the impact and effectiveness of food safety training on food handlers.

P2-17 Public Worry Regarding Specific Food Safety Issues in Lebanon

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Introduction: Food safety in Lebanon is a major public health issue potentially associated with the country's unique public health infrastructure and political challenges regarding policy and strategy. Foodborne illness is reportedly widespread, but the absence of a proper disease reporting mechanism makes incidence difficult to quantify. Improvement of consumer food safety is critical to reducing health risks; however, little is known about consumer food safety concerns in Lebanon.

Purpose: To determine and explore Lebanese consumers' risk perceptions, concerns, and perceived adequacy of food safety information.

Methods: Qualitative face-to-face interviews (*n*=43) were conducted with consumers who approached a Lebanese University (Modern University for Business and Science) Health Day stand held in a shopping mall in Beirut, Lebanon. Interviews enabled exploration of food safety perceptions and concerns amongst consumers.

Results: The majority perceived overall personal risk of foodborne illness to be "very high" and "greater" when eating outside of the home. Some respondents reported avoiding consuming food prepared outside of the home due to perceived lack of food safety guidelines and audits: "restaurants… do not abide by the necessary guidelines of food safety." Consumers were concerned regarding accuracy of and adherence to expiry dates: "the expiry dates are changed on some products; they even change the food source on the label." Concerns unique to Lebanon included electricity interruptions, which were believed to be associated with unsafe food storage practices: "we do not have electricity 24/24 hours and this is a huge defect when storing food in fridges because the temperature of those fridges goes up." Other food safety concerns included water safety, food contamination, and crop irrigation. A lack of food safety information was reported, with a desire for more information.

Significance: The study highlights food safety concerns that are particularly unique to Lebanon and has identified the need for further research to determine Lebanese consumers' cognitive and behavioural influences related to food preparation and storage, as well as food-safety education.

P2-18 A Comparison of Food Safety Knowledge, Attitudes and, Training Experiences of Trainee Dietitians from a Welsh and a Lebanese University

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Introduction: Dietitians provide nutritional advice to vulnerable patients. Delivery of food safety advice by adequately trained registered dietitians can inform vulnerable patients of increased foodborne illness (FBI) risks and enable risk-reducing food safety practices. However, gaps have been identified in the food safety knowledge of practicing registered dietitians. Dietitians need appropriate and adequate knowledge and skills to deliver effective food safety advice, which can be gained as trainee dietitians. The approach to teaching traine dietitians regarding food safety varies between institutions.

Purpose: Compare knowledge, attitudes, and teaching approaches of trainee dietitians regarding food safety from two international universities.

Methods: Paper-based questionnaires were completed by trainee dietitians at Cardiff Metropolitan University, Cardiff, United Kingdom (*n*=34) and the Modern University of Business and Science, Beirut, Lebanon (*n*=25).

Results: All trainee dietitians had received food safety education as part of their degree course. Those in Cardiff participated in a one-day food safety training course, whereas those in Beirut attended food microbiology lectures. Significant differences were determined in food safety knowledge between institutions. Knowledge of recommended cooking temperature to indicate food safety was lacking; although 41% in Cardiff knew the correct temperature, significantly fewer ($P<0.05$) were aware in Beirut (24%). More than half (68%) in Cardiff were aware of recommended refrigeration temperatures; knowledge was significantly greater ($P<0.05$) and 100% were aware in Beirut. Although trainee dietitians at both institutions indicated confusion regarding date labelling, awareness that "use-by" dates indicate food safety was significantly greater ($P<0.05$) in Cardiff (62%) than Beirut (32%). The majority (76% Cardiff, 68% Beirut) felt the provision of food safety information should be standard for dietitians.

Significance: Trainee dietitians from both institutions indicated that food safety should be part of a dietitian's role when advising vulnerable patients. Differences in knowledge between institutions may suggest that teaching approach affects knowledge retention. However, the approaches taken at

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both institutions, although different, are not clinically applicable to enable trainee dietitians to inform and enable vulnerable patients to reduce FBI risks. Consequently, these findings identify the need for specifically targeted training for trainee dietitians.

P2-19 Food Safety Behaviors and Practices of Vendors at Mississippi Farmers' Markets

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Introduction: Mississippi farmers markets (FM) continue to grow as they benefit producers and consumers due to their contributions towards consumer health and the economy. State regulations have allowed for FM vendors to sell produce, dairy, eggs, honey, meat, and poultry, as well as, more recently, cottage food products in response to the demand for locally grown and fresh food products. Food safety and processing guidance exists for FM; however, a study has not been conducted to investigate vendor practices and behaviors at Mississippi FM.

Purpose: The purpose of this study was to assess the effectiveness of state and federal regulatory guidance materials by auditing FM vendor food safety practices and behaviors and to determine if additional training needs exist for FM managers and vendors.

Methods: Observations and audits were conducted at eight FM *(n=57* vendors) by reviewers (*n*=2). The audit checklists (*n*=8) were developed and derived from state and federal requirements based on commodity, general Mississippi FM requirements, and cottage food operation requirements to assess vendor food safety practices and behaviors. Data was collected until saturation was met.

Results: Farmers markets in general, regardless of size or location, had areas of non-compliance. Reviewers found incomplete cottage food product labeling with 79% of vendors observed (19 of 24), and confusion about products allowed under the law as prepared foods were being served at 50% of the FM visited (4 of 8). In addition, produce storage was not appropriate, as 78% of vendors observed (25 of 32) stored items on the ground. Additionally, eggs were not under proper temperature and refrigeration (3 of 3).

Significance: With continued growth and interest in FM, additional food safety education guidance and training is an integral need for managers and vendors. This study highlights gaps in vendor food safety training, practices, and compliance with state and federal requirements.

P2-20 The First Year of Implementing Food Safety Modernization Act Produce Training

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Introduction: The U.S. Food and Drug Administration (FDA) Food Safety Modernization Act (FSMA) is part of a large overhaul of the current food system aimed at reducing and preventing foodborne illnesses. One tier is the Produce Safety Rule, which focuses on safe handling of produce from preharvest to postharvest. The Produce Safety Alliance (PSA) is a collaborative effort providing trainings and grower outreach so that growers are in compliance by their expected deadline.

Purpose: During 2017, trainers implemented grower outreach and education with an approved seven-module curriculum. Determining the trainers' ability to present a nationally standardized curriculum, grower farming practices, and knowing how well each module were received will assess how states are meeting small to large growers' needs.

Methods: A validated post-test-only tool from the Association of Food and Drug Officials measured teaching effectiveness, farming practices, and demographics. The six-page survey included Likert scale, multiple choice, and fill-in-the-blank questions.

Results: A total of 66 respondents completed the evaluation. Respondents were categorized by years of farming, novice (0 to 20 years) and advanced (21+ years). The majority of respondents felt the instructors were familiar with the delivered content (97.1%). Trends were observed with novice respondents, agreeing that the presentations for Module 1-7 were helpful. Novice respondents did not have a written farm food safety plan (39.7%). Novice growers identified as female (36.8%), were between the ages of 26 and 40 years of age (26.9%), and were a farm-owner operation (38.2%). Their farms were 0 to 10 acres (27.9%), averaging less than $25,000 in sales over the previous 3 years (16.2%).

Significance: The results from Maryland shows that PSA outreach is meeting the needs of small to large growers. However, there may be a need to help small growers recognize whether they are truly exempt. Extension, farming associations, and the United States Department of Agriculture need to continue promoting and hosting the grower training.

P2-21 Consumer Response to "Don't Wing It!" Web Site on Safe-handling of Raw Poultry

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Introduction: Consumers play an important role in ensuring that meals made with raw poultry are safe to eat. The "Don't Wing It" Web site provides recipes and food safety information targeted to millennial parents of young children (<5 years) and older adults (65+).

Purpose: To estimate the impact of a simple "Don't Wing It" prototype Web site on consumer behaviors and to measure satisfaction.

Methods: We recruited participants in target populations (parents and older adults) from a Web-based panel and randomly assigned them to the treatment group (exposed to the "Don't Wing It" Web site) or the control group (alternative Web site). We collected data at baseline (*n*=3,937) and follow-up (*n*=2,363, four weeks after exposure). We used difference-in-difference (DiD) models to assess the impact of exposure to the Web site on participant behaviors.

Results: About 92% of treatment group participants (*n*=984) reported going to the Web site and reading all or most of the information. Most found the Web site very or somewhat informative, very or somewhat useful, easy to navigate, and easy to understand. About 80% reported learning at least one new thing, and 74% would refer to the Web site again. About 50% self-reported behavior changes after exposure to the Web site; most common behaviors were washing hands after touching raw poultry, placing poultry in a plastic bag at the grocery store, and improved storing practices for raw poultry and leftovers. Responses were similar for parents and older adults. Based on results of the DiD analysis, there were no statistically significant differences in behavior between baseline and follow-up when comparing the treatment and control groups.

Significance: Participant satisfaction with the "Don't Wing It" Web site was high. Although participants self-reported behavior changes, the changes were not statistically significant when compared with the control group. This may be because one exposure was not sufficient to motivate behavior change. Future research should consider expanding the intervention to include repeated exposure to food safety messaging.

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P2-22 Evaluating a Consumer-focused Intervention Designed to Identify Food Safety Hazards in Retail Food Stores

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Introduction: Previous research has shown that food safety hazards identified by experts are not congruent with consumer perceptions. Past research approaches include presenting consumers with simulated scenarios and employing surveys, interviews, and focus groups to gather attitude and self-reported behavior data. Missing from the literature is an evaluation method for food safety communication to measure consumer perceptions of food safety hazards at retail in real time.

Purpose: The purpose of this study was to test the effectiveness of a short video series for improving food safety hazard identification by consumers while grocery shopping using a novel ethnographic data collection approach. Also included in this approach are validity and reliability tests for the method.

Methods: Participants (*n*=69) were recruited for two shopping sessions in October and November 2017 in Wake County, North Carolina, where they collected citizen science-style data on their individual perceptions in one of three grocery stores using a mobile electronic survey. Observations conducted by a trained Environmental Health Specialist were used to represent expert assessment. Intervention group participants (*n*=30) received a series of five two-minute videos about retail food safety hazard identification prior to their second shopping session and participated in focus groups after their final shopping session to provide feedback about their experiences.

Results: A total of 66 participants completed both shopping sessions. More hazards were identified in agreement with expert assessment post-intervention (*n*=9, 82%) compared to baseline (*n*=2, 18%) across groups. Intervention participants agreed with expert assessment 17% of the time (*n*=7), compared to 12% of control participants (*n*=4) at post-intervention. On average, participants were most likely to identify at least one expert-identified hazard in the meat and poultry department (*n*=5 participants, 45%).

Significance: Videos about retail food safety hazard identification may help consumers improve their identification of potential food safety hazards while grocery shopping.

P2-23 Midwest Region Round Two Needs Assessment for the Food Safety Modernization Act's Produce Safety Rule

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❖ ***Developing Scientist Competitor***

Introduction: The U.S. Food and Drug Administration's Food Safety Modernization Act (FSMA) Produce Safety Rule was established in response to frequent foodborne illness outbreaks caused by raw produce consumption in the United States. Due to the complexity of this new rule, there is an immediate need to assess current grower knowledge of the law and to provide resources that help guide them into compliance.

Purpose: To assess knowledge and educational gaps of produce growers as related to the Produce Safety Rule in the Midwest and to provide guidance to extension educators on how to best fill these gaps.

Methods: A modified, two-phased Delphi approach was used to gather information from growers; results from round two are presented here. Paper and electronic questionnaires were distributed by educators and organizations (*n*=30) in 12 Midwest states. A five-point Likert rating scale was used to assess current knowledge and educational needs in areas of water testing and usage; biological soil amendment of animal origin requirements; crops from animal fecal contamination; and worker training. A similar scale was used to identify preferred types of educational resources. Respondents were asked what their two most preferred ways to learn new information were. The questionnaire was reviewed for content validity and clarity by extension and growers partners in the Midwest.

Results: Findings indicated respondents (*n*=410) least understood biological soil amendment of animal origin requirements, water testing and usage requirements, and protection of crops from animal fecal contamination. The most preferred educational resources were online videos, extension publications and fact sheets, and printed checklists. The most preferred way to learn new information was hands-on and experiential events, text-based information (e.g., books online and in print), and visual-based messages (e.g., posters).

Significance: This needs assessment tool can be modified for use in other regions by food safety practitioners. Our findings will guide curricula development for growers and identify the best approach to create and deliver educational resources.

P2-24 Investigating the Accuracy of Food Test Strips to Measure pH Values of Home-preserved Foods

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❖ ***Developing Scientist Competitor***

Introduction: Home-preserved foods have been linked to several outbreaks of foodborne illness, notably botulism. The cause of these outbreaks is usually improper processing or formulation. Improper formulation can result in products with elevated pH levels, potentially allowing for the growth of *Clostridium botulinum* and production of botulinum toxin.

Purpose: The purpose of this study was to determine if paper pH test strips designed for consumer use were able to accurately determine the pH levels of home-canned goods when compared to digital pH electrodes.

Methods: The pH levels from a total of 80 home-canned foods entered in the North Carolina State Fair Foods Preservation Competition were tested with both consumer pH strips and digital pH electrodes. Data data from both measurements were compared using SAS Studio analysis software.

Results: The mean pH value of preserved foods measured with the commercially available test strips was 4.01, while the mean pH value when measured with digital pH electrodes was 3.68. This represents a statistically significant ($P<0.01$) difference between the two methods. The Pearson correlation between the two methods was 0.63. The commercially available test strips measured the pH as 0.34 higher than the digital pH electrodes. One entry (0.01%) into the competition had a pH higher than 4.6 measured with both the test strip and digital electrode, high enough to support the growth of *Clostridium botulinum.*

Significance: Paper test strips on average rated the pH 0.34 higher than the digital electrodes. This positive association biased away from the null value would increase the rate of false positives. This could lead to home canners overestimating the pH values of their foods, decreasing the risk of consuming foods above 4.6 but increasing the chances of discarding safe foods.

Poster

P2-25 Strategies to Reach Television Chefs with Safe-handling Information

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Introduction: Cooking shows on television often model food handling practices that conflict with safe handling recommendations. Up to 60% of those who enjoy cooking shows indicate that they prepare dishes demonstrated by the television personality once a month. Half or more say they follow the same practices they see demonstrated on television. Further, many acknowledge that the chef often doesn't give them all the information they need to prepare the dish safely.

Purpose: The purpose of this project is to identify strategies to motivate chefs and show producers to adopt safe handling guidelines in food preparation programs.

Methods: A coalition of food industry representatives, consumer groups, and government agencies reviewed the literature documenting food handling by chefs and consumer response to food handling observed on popular television shows. A letter requesting an in-person meeting was prepared and addressed to the chefs targeted in the research paper that documented consumer response to the chef's practices. Chefs were asked to join the coalition and lead in the development and adoption of safe handling guidelines for television shows.

Results: A draft of safe handling guidelines designed after the cook, clean, chill, and separate messages promoted by the Partnership for Food Safety Education was prepared for the chefs to modify as needed to fit within the confines of a television show. Project challenges include administrative delays related to government agency approvals and reaching the chefs, since only one coalition member had previously contacted a chef targeted for this opportunity. Chefs will be reached via their Web pages, with in-person meetings scheduled for early spring.

Significance: Consumers view celebrity chefs as role models. This project will strive to present safe handling as a way for chefs to enhance their expertise and prestige among consumers and fellow professionals.

P2-26 Investigating Cross-contamination from Raw to Ready-to-Eat Foods during Consumer Meal Preparation Using MS2 as a Surrogate

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Introduction: Cross-contamination frequently occurs in consumer kitchens due to risky behaviors, some of which occur when handling raw meat and poultry products. Pathogens have been shown to move throughout a kitchen; however, the frequency and level of contamination during a meal preparation event have not been well-studied.

Purpose: This study was conducted to determine how frequently cross-contamination from a raw poultry product to ready-to-eat leafy greens occurs during consumer meal preparation, as well as the degree of contamination and whether being shown a video on proper thermometer usage impacts cross-contamination.

Methods: A meal consisting of turkey burgers inoculated with 10^8 PFU of bacteriophage MS2, a lettuce garnish, and vegetable salad was prepared by participants (*n*=383) in a test kitchen. One-half of the participants were shown the food safety video on proper thermometer usage before meal preparation (intervention group); the other half served as a control group. After preparation, sampling of lettuces was completed and immediately tested for enumeration of MS2 using an RT-qPCR-based assay. Statistical analysis of prevalence and concentration of MS2 cross-contamination was completed using R software.

Results: Lettuce samples, aggregates of the vegetable salad and lettuce garnish, were positive for MS2 33% of the time. The salad lettuce made up 87% of the positive lettuce samples. The average concentration of MS2 in these samples was 2.3×10^4 PFU/g (max=1.6×10^5 PFU/g; min=1.83×10^3 PFU/g). There was no significant difference between intervention and control groups.

Significance: This study provides information about the potential for cross-contamination of pathogens during food preparation, the efficiency of cross-contamination, and the impact of food safety messaging on preventing events. This information is important to future risk modeling and will aid in the design of better methods by which to reduce the likelihood of cross-contamination in household food preparation.

P2-27 Investigating the Impacts of a Media Campaign Targeting Food Safety Practices

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❖ ***Developing Scientist Competitor***

Introduction: Food safety related messages are most effective when they are meaningful to the intended audience, accurate in information, delivered repeatedly, and distributed at appropriate times.

Purpose: The purpose of this study was to gather in-depth information on impacted perceptions, food safety attitudes, and self-reported behavior changes following a pilot of a national food safety message campaign (160isgood.com). The campaign focused on safe handling, specifically the internal cooking temperature of grilled ground beef patties to reduce Shiga toxin-producing *Escherichia coli* (STEC).

Methods: The campaign was delivered over 14 weeks through radio, movie theater, and digital advertisements and resulted in 2.2 million impressions in the target community. A random-dial telephone survey (*n*=300) was administered prior to the campaign, focusing on perceptions of beef food safety, thermometer usage, and handling practices. Following the campaign, six, one-hour focus groups were held to measure effectiveness and opinions on the campaign.

Results: Out of 300, 24% (*n*=72) of post-test respondents recalled any beef safety advertisements that included the "160 is good" message. Additionally, 14% (*n*=44) of pre-test respondents and 16% (*n*=50) of post-test respondents reported using a food thermometer when cooking ground beef patties to determine when burgers are done. Very few interviewed respondents recalled any of the "160 is good" beef safety campaign messaging, and there was minimal increase in thermometer usage due to the campaign. The six focus groups show differing perceptions, such as positive feedback on including an animated movie theatre pre-roll due to the captive audience as well as skepticism about using a radio spot to drive consumers to a website to learn more about food safety.

Significance: The research provided expanded insight on the effectiveness of mass food safety messaging. There were mixed reviews on this campaign, suggesting a mass media campaign may not be the most effective method of communicating food safety practices.

Poster

P2-28 Development and Assessment of a Visual Educational Food Safety Tool for Farmers Market Vendors

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Introduction: Farmers markets are a popular source of fresh produce and local foods. However, the conditions of farmers markets and vendors' lack of food safety knowledge pose challenges to safe food, especially in ready-to-eat foods. Visual-based learning tools have been useful in conveying critical food safety messages to improve safe handling practices when targeted messages are tailored to a specific audience.

Purpose: The purpose of this study was to develop and assess attitudes and knowledge of Iowa farmers market vendors towards a minimal-text visual food safety poster.

Methods: Using the data collected from a pre-survey of farmers market vendor and employee food safety attitudes, a visual-based, minimal-text poster was developed and distributed to farmers market managers (*n*=6), focusing on four topics from the U.S. Food and Drug Administration's 2013 Food Code. A post-intervention survey was developed to assess food safety knowledge and attitudes of vendors and employees (*n*=27), including an open-ended question to elicit poster feedback.

Results: Twenty-seven vendors and employees completed the post-intervention survey. Most respondents had worked at farmers markets for three or more years (37% 3 to 4 years, 30% 5+ years) and over half (52%) had participated in food safety training. Results found participants had positive attitudes towards food safety (M=4.33/5, SD=0.38) and averaged seven correct knowledge questions out of a possible 10. Fifteen respondents recalled seeing the poster and were generally positive, commenting "Love it!", "It's great!", or "I like them". One respondent stated they would continue to use the poster. Ten respondents that saw the poster reported that the content was "very informative", "easy to understand", and a "helpful reminder".

Significance: This study demonstrated positive attitudes of farmers market vendors towards use of minimal-text, visual-based food safety educational learning tools. The posters can be used by farmers market managers and vendors in educating and promoting safe food handling practices.

P2-29 Needs Assessment for Exempt Home Food Operations and Home Bakeries in Iowa

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Introduction: Farmers markets and the variety of products sold at farmers markets have both been steadily increasing. In Iowa, there are regulations that allow vendors to make fresh and value-added products out of their homes without a food license or kitchen inspection. Developing a training relevant for these producers is important to reduce the risk of foodborne illness.

Purpose: The objective of this study is to analyze the current food safety knowledge, confidence in following Iowa regulations, and background information among Exempt Home Food Operations and Home Bakeries.

Methods: A needs assessment survey using mixed-methods was developed and distributed to target audiences via online and hard copy. Food safety knowledge questions were developed using the 2013 U.S. Food and Drug Administration Food Code and were in either multiple choice or true/false format. Personal confidence understanding and following Iowa regulations was assessed using a modified Likert scale. Demographic information was also collected.

Results: Descriptive statistical analysis shows that 63% of participants in the needs assessment (*n*=86) have already started selling foods that are produced out of their homes, with a mean of 5.91 years in production. Participants indicate high confidence in personal ability to follow Iowa regulations that are related to their products (4.3 on a 5-point scale). Overall food safety knowledge among the home kitchen producers had a mean of 73% correct answers. Of the participants, 96% correctly identified the Big 8 allergens, but only 52% of participants knew the correct height to store food off the ground.

Significance: The results show that producers have a high level of confidence in their personal ability to follow Iowa regulations; however, most producers barely passed the assessment on key food safety concepts. A training designed for these producers would be helpful in increasing food safety and knowledge of Iowa regulations, thereby making products safer for the public.

P2-30 A Comparison of Food Safety Training Methods and an Investigation of Factors Impacting Training Outcomes

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❖ ***Developing Scientist Competitor***

Introduction: Internal food safety training is often performed by food production companies to fulfill requirements of regulatory agencies or private industry. There are a variety of food safety training methods, yet little research has been performed to determine which methods are most effective. There is also not a complete understanding of how food safety culture affects food safety training outcomes.

Purpose: The purpose of this study was to evaluate the effect of various training formats and food safety culture on changes in knowledge and food safety intentions of employees.

Methods: Individualized e-module, group discussion-based virtual tour, and group e-module training programs were developed using the following learning theories: programmed instruction, social and situated learning, and direct instruction. The trainings were developed on four training topics: Introduction to Good Manufacturing Practices and Material Controls, Personal Responsibility and Communicable Diseases, Allergen Control, and Food Defense. The training programs were implemented at 66 dairy processing plants. Pre- and post-training evaluations were received from 793 employees at 22 of the 66 plants using a validated evaluation method.

Results: There was a significant increase in knowledge scores observed for employees as a whole. Of the three training formats evaluated, there was only a significant increase in knowledge for the group discussion-based virtual tour and the group e-module training ($p<0.05$). Additionally, only the group e-module training displayed a significant increase in employee intentions for food safety tasks ($p<0.05$). A regression did not show a significant effect of certain food safety culture constructs on employees' change in knowledge or intentions.

Significance: This data suggests that group food safety training may be more effective than individual training. This data also suggests that the use of certain learning theories can impact training program effectiveness.

Poster

P2-31 Effectiveness of On-line Versus Face-to-Face Produce Safety Training for Farmers and Farmers Market Managers

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Introduction: On-line courses may offer farmers and market managers flexible opportunities to learn best practices to enhance produce safety. This project converted existing curricula used in face-to-face training for on-line delivery.

Purpose: Objectives were to convert training materials for farmers and market managers for on-line delivery, to include interactive components, and to compare effectiveness with face-to-face training in improving knowledge of produce safety and motivating changes to enhance safety.

Methods: Voice-overs were addd to PowerPoint slides using Camtasia software (v. 2). Question and answer activities were interspersed for interaction. Fact sheets were included. Participants could enter and leave courses as needed, with the total length of training being two to three hours. Online evaluations were completed using a Qualtrics platform. Results were compared to face-to-face training results.

Results: For 140 farmers completing on-line training, 65% had farmed three years or less, compared to 41% in face-to-face trainings. Similar knowledge improvements were found for both delivery methods. The on-line course was less effective in motivating improvements related to inspecting facilities for hazards and keeping better records, but more effective than face-to-face training for improvements related to land and water use, irrigation methods and timing, water for washing produce, handwashing and toilet facilities, and training workers. For 108 market managers completing the on-line course, 75% had managed three years or less, compared to 61% in face-to-face trainings. Both delivery methods were similar for improving knowledge of the importance of asking questions about vendor practices, requiring certifications, providing training, and using cleanable or single-use containers. On-line training was less effective in motivating managers to improve several practices compared to face-to-face training.

Significance: On-line training may be effective in reaching and improving knowledge of beginning farmers and market managers, but may be less effective than face-to-face training in motivating improvements in practices. Additional research is needed with both methods to determine effects on actual behavior change.

P2-32 Investigating Handwashing Practices of Consumers during Meal Preparation: An Observational Approach

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✦ ***Undergraduate Student Award Competitor***

Introduction: Inadequate handwashing has been identified as contributing factor to foodborne illness, especially when preparing raw meats and poultry. Hands can become vectors to move pathogens around sites for foodborne pathogens found in raw meat/poultry and contribute to home acquired foodborne illnesses. However, the frequently and level of contamination has not been well studied.

Purpose: This study was conducted to determine the adequacy of handwashing events by consumers while preparing a meal consisting of raw ground turkey burgers and a ready-to-eat salad. Handwashing practices before the start of meal preparation as well as after handling raw poultry were also investigated.

Methods: A sample of 352 participants were recruited from various North Carolina communities, and asked to prepare and cook a meal in a simulated home kitchen while being observed by researchers. One half of the participants were shown an educational food safety video on thermometer use before meal preparation (intervention group); the other half served as a control group. Handwashing chances and attempts were coded and efficacy compared to public health messages were measured.

Results: Researchers observed 2195 cases in which a handwashing event was required; of these handwashing was attempted 20% of the time. Among handwashing events attempted, only 1.14% of attempts contained all steps of a correct handwashing event. Most common reasons for inadequate handwashing include: rinsing hands only; drying hands with a dish towel and/or used paper towel; drying hands on alternative surface (apron, shirts); and scrubbing with soap under 10s. There was no significant difference between intervention and control groups.

Significance: Currently, how pathogens move around a kitchen is not well known. The level of inadequate handwashing and non-attempts revealed in this study demonstrate hands are likely a vehicle for pathogen transfer from raw poultry products to the kitchen environment.

P2-33 Development and Implementation of a Culinary Science Course for Food Science Students

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Introduction: There is a gap in food safety curricula with respect to commercial kitchen preparation and training. Currently, students are taught high-level theory regarding food science concepts; however, no practical experience with food manipulation or behavior is included. A "culinary science" course with the capability of bridging the gap between theory and implementation of food safety practices is needed to enhance food safety in high-throughput culinary environments.

Purpose: This case study evaluated ease of implementation of an undergraduate course to introduce culinary fundamentals concurrently with adjacent topics in food science.

Methods: Over 15 days, culinary fundamentals were taught to students (*n*=5) in a kitchen laboratory and supplemented with food science lectures in a traditional classroom setting. Major topics included kitchen and microbiological safety, knife skills, emulsions, starch manipulation, and proteins/Maillard reactions. Student evaluations were administered based on key competencies, such as professionalism, preparedness, and organization. Daily quizzes extracted from lecture topics and a final exam also contributed to the students' final grades. Course effectiveness was determined by pre- and post-testing approved by the Institutional Review Board. Compounded means were calculated based on knowledge gained and self-identified skills gained.

Results: The average knowledge score (35 questions) improved from the pre-test (64.27%) to the post-test (94.49%), resulting in a knowledge gain of 30.22%. The self-identified skills score (17 questions) was 66.60% for the pretest and 94.32% for the post test, resulting in an increase of 27.72%.

Significance: With further implementation, this course could become a standard for food science departments that do not provide culinary training. This culinary science course would be beneficial for any student of food science, particularly those interested in product development.

Poster

P2-34 Alternative Methodologies for Quantifying and Understanding Food Safety Behavior Relationships among Restaurant Food Handlers

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Introduction: Human behavior plays an integral role in the five risk factors identified by the U.S. Food and Drug Administration that contribute to the occurrence of foodborne illness from commercial food establishments. Behavior studies can be limited by time and resource-intensive methodologies for collecting data. The use of novel methods of data collection and analysis, such as script-based covert recall and randomization tests, can offer advantages that can enhance our understanding of the complexity of food safety behaviors.

Purpose: Test alternative methodologies that address limitations of previously tested methods and use these methods to elucidate how handwashing frequency relates to demographic variables.

Methods: Food handlers (*n*=124) were selected from valid, permit-holding restaurants using a cluster sampling procedure of zip codes. Handwashing frequency was determined through script-based covert recall, where employees were interviewed about their propensity to wash their hands following five scenarios that necessitate handwashing. Handwashing frequency was calculated as a percentage of times participants mentioned handwashing over total handwashing scenarios. Randomized tests were coded through R statistical software that compared mean handwashing frequency between demographic variables such as work status, level of food safety training, and gender.

Results: Script-based covert recall was a resource-friendly means of acquiring handwashing frequency data from restaurant food handlers. Comparing mean handwashing frequency between full- and part-time workers using a randomized test of 1,000 repetitions, a statistically significant relationship was found (*P*=0.005). This was confirmed by a logistic regression model which indicated part-time workers were more likely to wash their hands after eating and before preparing food.

Significance: Alternative, scenario-focused methods are beneficial for obtaining handwashing frequency data. Randomized tests shed insight into how demographic variables such as work status may influence behavior execution. Industry professionals may consider more targeted behavior change interventions for full-time workers.

P2-35 Examination Delivery Methods for Food Safety Training – Does Phrasing Make a Difference?

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❖ ***Developing Scientist Competitor***

Introduction: An estimated 48 million people get sick from a foodborne illness annually, and 60% of all foodborne illness outbreaks are associated with restaurants. Pre-employment training on personal hygiene and food safety is the most effective way to prevent foodborne illness outbreaks associated with food service. Although the current accredited food safety trainings available in the United States cover critical topics, the manner in which these training programs are presented and tested tend to overlook the estimated 57% of food handlers with little to no education.

Purpose: The purpose of this study was to investigate whether the phrasing of food handler exams makes a difference for entry-level employees.

Methods: A between-subject experimental design was implemented through the use of two exams covering two versions of food safety questions in a different order. The questions consisted of traditional questions phrased similar to those currently used in food handler's exams and updated questions phrased with simplistic wording for entry-level employees. Participants (*n*=42) consisted of newly hired food employees from all service-level food establishments located in a university setting. The multivariate analysis of variance was selected as the analytical method to test the treatment effects of phrasing on food employees' performance on the food handler's exam.

Results: The current results indicated that there was no overall significant statistical difference in the respondents' performance (Wilks' Lambda=0.074>0.05) when the phrasing of food handler's exam used simplistic wording. However, since 40.5% of the participants identified as working towards a bachelor's degree, this study will be expanded to food establishments outside of a aniversity setting to include a more representative group of food employees.

Significance: These results demonstrate that the gap between food safety training programs and food employees is not correlated with the manner in which food handler exams are worded or phrased.

P2-36 Assessment of Microbiological and Chemical Quality of Bubble Tea Beverages Sold in Taiwan

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Introduction: Bubble tea (also known as pearl milk tea or boba milk tea) is a tea-based drink invented in central Taiwan in the 1980s. Most bubble tea recipes contain a tea base that is mixed or shaken with fruit or milk, to which chewy tapioca starch balls or fruit jellies are often added. In spite of the potential benefits offered by vendor bubble tea beverages, concerns over their safety and quality have been raised.

Purpose: This research was undertaken by testing 105 bubble tea beverages sold by vendors in Taiwan so that a better understanding of the safety and quality of the products could be accomplished in order to better protect consumers.

Methods: A total of 105 bubble tea beverages sold by vendorinof Taiwan were purchased and tested to determine the presence of food pathogens (e.g., *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*), aerobic plate count (APC), coliforms, and food additives (sweeteners, preservatives, maleic acid, and coumarin). In addition, we also inspected the origin labeling of the tea leaves to confirm whether the vendors comply with the policy of tea origin labeling in this study.

Results: This study showed that 51 samples (48.6%) for APC and 55 samples (52.4%) for coliforms exceeded the Taiwanese guidelines of 4 log CFU/ml and 10 MPN/ml, respectively. None of the samples contained *E. coli*, *Salmonella* spp., *S. aureus,* sweeteners, preservatives, maleic acid, or coumarin. However, 63 out of 105 bubble tea samples (60%) were not correctly labeled with the origin of the tea leaves and were therefore in violation of food labeling regulations.

Significance: These results showed that the bubble tea samples had satisfactory pathogen and chemical quality, with no *E. coli*, *Salmonella*, *S. aureus*, sweeteners, preservatives, maleic acid, or coumarin, but nearly half of the tested bubble tea samples had APC and coliforms levels greater than the Taiwanese guidelines.

Poster

P2-37 Retail Deli Employees' Food Safety Perceptions and Behaviors Align with *Listeria monocytogenes* Contamination Risks

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❖ ***Developing Scientist Competitor***

Introduction: *Listeria monocytogenes* is highly prevalent in some but not all retail delis. We hypothesized that managers and associates in delis with historically low *L. monocytogenes* prevalence have positive foods safety perceptions and behaviors compared to employees in delis with increased prevalence.

Purpose: This study investigated relationships among retail deli employees' food safety behaviors and perceptions with *L. monocytogenes* contamination risk and deep clean interventions.

Methods: A 44-question survey of food safety perceptions and behaviors was distributed to retail deli managers and associates from 50 retail delis among six U.S. states that participated in longitudinal *L. monocytogenes* prevalence study. The survey was distributed to all delis three times over 12 months; the distribution of surveys to the delis that were deep cleaned fell before, after, and follow-up deep clean. A generalized linear mixed model (SAS 9.4) was developed to evaluate associations among *L. monocytogenes* predicted risk and deep clean with food safety perceptions and behaviors (α=0.05).

Results: A total of 507 surveys were returned. Responses from 14/44 (31.8%) questions were significantly correlated with *L. monocytogenes* risk, participating in a deep clean, and their interaction (α=0.05). Increased levels of employee commitment to food safety programs were strongly associated with implementation of deep clean interventions (P=0.0112), and the interaction between deep clean and *L. monocytogenes* risk (P=0.0038). Associates and managers had more positive food safety behaviors and perceptions, and better perceived employee training programs in stores with low *L. monocytogenes* risk. Higher self-reported handwashing compliance was recorded post-deep-clean (P=0.0055). Associates and managers were more likely to agree upon a healthy food safety environment after deep clean (P=0.0198).

Significance: This study underscores that employee food safety perceptions and behaviors are a critical component of *L. monocytogenes* control in retail thus resources should be directed to support systems that help associates value food safety.

P2-38 Development, Implementation, and Evaluation of a Food Service Focused Handwashing Intervention: A Pilot Study to Indicate Effectiveness

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Introduction: Effective handwashing behaviour reduces the spread of foodborne illness. Compliance in food service businesses is difficult to manage and maintain due to time constraints and food handler cognitive influences. Graphic signs, tailored to individual food business sectors, may prove useful in encouraging handwashing behaviour and thereby reducing the associated risks.

Purpose: This study aimed to design and evaluate a bespoke, graphic, handwashing intervention intended to improve handwashing practices in a small food service operation. Evaluation of the intervention aimed to quantify potential short- and longer-term effectiveness.

Methods: A review of health education and handwashing literature and consultation with food service employees informed intervention development. A proxy indicator of handwashing frequency was determined by paper towel counts at the dedicated kitchen hand sink in a food service business. Paper towels were counted before, immediately following intervention, and six weeks post-intervention. Qualitative food handler (n=7) interviews at the end of the study explored food handler perceptions of the intervention and potential behavioural influence.

Results: The handwashing poster depicted typical food served at the business, customer expectations, and foodborne illness consequences. The shock tactics approach used photographic images and short, clear text relevant to food service employees. Post-intervention, food handlers expressed their understanding of the consequences of not washing hands as "severe", as well as "people can get ill" which "could kill someone". The design was described as giving a "clear" and "spot-on" message which was "eye-catching" and "made you think" about handwashing so that foodborne illness "never happens". Baseline findings indicated paper towel use at 59% per meals served, rising to 106% immediately following intervention and 101% six weeks later.

Significance: A tailored handwashing "shock tactics" visual cue was perceived as positive, supported by an increased frequency of paper towel use and thus handwashing action attempts. Sustained effectiveness of such intervention may be achieved as part of a broader handwashing strategy, including verbal explanatory training sessions.

P2-39 Comparison of Sanitary Inspection Results on Cutting Boards in Different Types of Children's Foodservice

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Introduction: Since the establishment of Changwon Center for Children's Foodservice Management (CCFSM) in Korea in 2011, center dietitians have been performing inspections on children's food service establishments based on Korea Food and Drug Administration (KFDA) sanitary checklist. Children's food service can be divided into two groups: institutional food service, which should obey the KFDA's Food Sanitation Act, and small foodservice, which are exempted. Cutting boards have been pointed out as a type of equipment with insufficient sanitary management in previous studies.

Purpose: The purpose of this study was to create a sanitary consulting strategy for cutting boards by comparing the results of simultaneously performed "inspection by checklist", "adenosine triphosphate (ATP) monitoring", and "aerobic plate count (APC)" between the two types of children's food service.

Methods: Five dietitians visited 225 food service establishments (95 institutional, 130 small) to examine whether they performed "control/sterilize cutting boards sanitarily" appropriately. In this visit, dietitians swabbed a 100 cm^2 area on each cutting board twice; once for obtaining ATP measurements, and the other for APC using 3M Petrifilm Plate. Chi-square test and Student's t test have been applied by SPSS Statistics 23.0 software.

Results: There was no significant difference between the two groups in ATP measurements (institutional=990.6±3446.3 RLU/100 cm^2, small=892.5±2753.7 RLU/100 cm^2) and APC (institutional=1.1±1.8 log CFU/100 cm^2, small=1.6±1.7 log CFU/100 cm^2). But in performance rate of "control/sterilize cutting boards sanitarily" and adequacy of APC based on KFDA standards (<2.7 log CFU/100 cm^2), institutional foodservice (84.2 and 84.2%, respectively) showed significantly (P<0.05 and P<0.01, respectively) higher rates than small food service (63.8 and 73.1%, respectively) and turned out to be performing better sanitary management. As small food service establishments were mostly not well-financed, CCFSM donated new cutting boards to those which substantially exceeded the APC standard.

Significance: Compared to the 77.8% of APC adequacy from the total subjected inspection, total ATP adequacy was only 62.8%. Therefore, more realistic guidelines shall be prepared to conduct ATP hygiene monitoring for food contact surfaces, including cutting boards.

P2-40 Consumers' Self-Reported and Objectively Assessed Knowledge and Risk Perception of Fresh-cut Produce

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Introduction: The Centers for Disease Control and Prevention (CDC) identified 713 fresh-cut produce-associated illness outbreaks between 1990 and 2005. However, no previous study has been conducted to investigate consumer knowledge, handling procedures, and risk perception about fresh-cut produce from shopping to eating. Additionally, the influences of self-reported and objectively assessed knowledge of fresh-cut produce handling on consumer risk perceptions toward fresh-cut produce are still unknown.

Purpose: The objectives of this study were to investigate: i) consumer knowledge, practices, and risk perceptions about fresh-cut produce, and ii) how self-reported, objectively assessed knowledge can influence consumer risk perceptions associated with fresh-cut produce.

Methods: A survey instrument was designed to collect consumer input on self-reported and objectively assessed knowledge of fresh-cut produce handling practices and risk perceptions of fresh-cut produce. A total of 1,043 respondents participated in this survey nationwide. Analysis of variance and linear and polynomial regression were used for data analysis of this study.

Results: Respondents lacked knowledge of storage order (capability ratio [CR]=9.5%), surface cleaning and sanitizing (CR=27%), and disposing of fresh-cut produce (CR=34%). Consumer risk perceptions were lowest when there was a high congruence between self-reported and objectively assessed knowledge (curvature=0.10, P<0.01). Also, in the condition of self-reported and objectively assessed knowledge congruence, there was a reverse U-shape relationship between consumers' knowledge level and their risk perceptions (curvature=-0.10, P<0.01).

Significance: Theoretically, the results showed that consumer risk perception is a psychological outcome that is determined by the interaction of self-perceived and objective knowledge level. Practically, the results indicated that more effective education materials related to fresh-cut produce should target low-compliance practices such as storage order, surface cleaning and sanitizing, and disposing of fresh-cut produce.

P2-41 Occurrence of *Listeria* spp. and *Listeria monocytogenes* on Avocados Acquired from Retail Establishments

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Introduction: Studies on the distribution of *Listeria* in different environments and foods are useful in understanding the ecology of *Listeria* species, including *L. monocytogenes*. Contamination of avocados with *Listeria* spp. may occur as a cross-contamination at different stages of the production chain. In Mexico, local markets are important points of sale for avocados where they are stored at ambient temperature, exposed to several environmental contamination sources, and largely handled by sellers and buyers. These conditions may favor its contamination and the survival of *L. monocytogenes*.

Purpose: To determine the occurrence of *Listeria* spp. and *L. monocytogenes* on avocados (*Persea americana* var. Hass) acquired from local markets.

Methods: Ripe avocados (200±50 g) with dark green or black epicarp (n=150) were obtained from 30 establishments located in 13 local markets in Guadalajara, Mexico. Each avocado was placed into 200 ml of UVM broth and then placed in an ultrasonic bath at 110 kHz for 1 min. The rinse was filtered and the membrane enriched following the United States Department of Agriculture technique. Isolation was performed in Modified Oxford (MOX) and Polymyxin Ceftazidime Oxford (PCOM) agar. Isolates were confirmed by biochemical tests, and then *L. monocytogenes* isolates were identified by PCR.

Results: Listeria spp. were isolated from 20% (30 of 150) and *L. monocytogenes* from 12% (18 of 150) of analyzed samples. The most common species were *L. monocytogenes* (18 of 30), *L. innocua* (16 of 30), *L. welshimeri* (3 of 30), and *L. grayi* (2 of 30). *Listeria monocytogenes* was individually isolated from 37% (11 of 30) of positive samples and together with *L. innocua* from 23% (7 of 30) of positive samples.

Significance: Listeria spp. including *L. monocytogenes* are present on the epicarp of avocados sold in local markets. It is important to reinforce good handling practices to verify that avocados are handled and stored as safely as possible to minimize risks of contamination with *L. monocytogenes*.

P2-42 Significance of Health Code Violations in Food Service and Retail Operations Located in Low- and High-income Communities

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Introduction: The incidence of foodborne illness is not typically tracked by race, income, or ethnicity; however, reported cases have shown an increased incidence of foodborne illness among minority and low-income populations. Studies have shown that low-income communities that are classified as food deserts have increased access to fast food restaurants compared to high-income neighborhoods. This reality may contribute to food safety concerns in low-income neighborhoods due to a significantly higher number of outbreaks associated with food service (68%) as compared to domestic kitchens (9%).

Purpose: The purpose of this study was to contribute to designing a framework to identify food safety challenges in food deserts that can be used by future researchers in other urban communities to address food insecurity.

Methods: Publicly available health department records from low- and high-income food service and retail operations were compared to quantify if there was any significant difference in sanitation practices and violations. These operations included fast food restaurants, dine-in restaurants, and grocery stores. The findings were tabulated by operation type, frequency of violation, and violation type, with an emphasis on severe health code violations.

Results: There were no significant indications that the number or type of violations were dependent on the community's income; however, there was a higher incidence of repeat violations in grocery stores. In some cases, the same retail store continued to demonstrate repeat violations over the course of several months. Severe violations such as improper holding of time-temperature controlled food and failing to meet the minimum internal cooking temperature were also present, but no correlation to the community's level of income were found.

Significance: The data collected has the potential to construct a framework and study design applicable to different urban settings which could be used to identify trends in food insecurity.

Poster

P1-43 Withdrawn

P1-44 Withdrawn

P2-45 Antimicrobial Resistance Patterns of *Enterococcus* and *Staphylococcus* Species Isolated from Grocery Store Shopping Carts

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Introduction: Grocery carts are exposed to a variety of food products and environments, and children riding in them could be at a higher risk of exposure to pathogens that have antimicrobial resistance (AMR) due to touching and sucking on cart handles and other surfaces.

Purpose: The purpose of this study was to determine the AMR patterns of *Enterococcus* and *Staphylococcus* species from the base and handles of grocery carts.

Methods: Swab samples (*n*=50) were collected from 10 different grocery stores in Lubbock, Texas during February 2017. Samples were taken from the base of grocery carts and the handles of the cart, using a pre-moistened sponge with 25 ml buffered peptone water. Samples were streaked onto *Enterococcus* agar for detection of *Enterococcus* spp. and Baird Parker agar for *Staphylococcus* spp., with one isolate randomly selected and streaked onto blood agar plates for susceptibility testing. AMR was analyzed using the micro-broth dilution (Sensititre) susceptibility MIC plates and following the National Antimicrobial Resistance Monitoring System protocol. Resistance and susceptible breakpoints were determined from the Clinical and Laboratory Standard Institute.

Results: Enterococcus spp. were present in 42% (*n*=21) of the grocery cart samples. From those, 4.7% (*n*=1) expressed multi-drug resistance (MDR, three or more antibiotics), 71.4% (*n*=15) were resistant to at least one antibiotic tested, and 23.8% (*n*=5) were pan-susceptible. *Staphylococcus* spp. were present in 34% (*n*=17) of samples. Of those, 17.6% (*n*=3) had MDR, 35.3% (*n*=6) had resistance to at least one antibiotic, and 47.1% (*n*=8) were pan-susceptible.

Significance: AMR is a growing global concern and grocery carts are a public surface that can spread potentially harmful pathogens which could have AMR. This study shows that cleaning and disinfection of carts is warranted due to the potential public health risk, especially to minors and toddlers.

P2-46 Ability of Foodborne Pathogens to Survive in Kitchen Grease

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Introduction: Cooking grease may contaminate countertops and equipment in commercial and retail kitchens. Its presence may decrease the efficacy of sanitizers, attract pests and insects, and harbor disease-causing microorganisms. Past evidence suggests that pathogens such as *Salmonella* are able to persist in matrices with low water activity; however, the ability of this and other pathogens to survive in kitchen greases has been left mostly unexplored.

Purpose: The purpose of this study was to evaluate the ability of foodborne pathogens to survive in salted butter and shortening, which were chosen to mimic greases that may contaminate surfaces in food preparation spaces.

Methods: Overnight cultures of *Listeria monocytogenes* and *Salmonella enterica* were centrifuged and resuspended in 0.1% peptone before being inoculated into 25.0±0.5-g samples of salted butter or shortening. Samples were stomached for 2 min to ensure even distribution of the bacteria throughout the food matrix. Uninoculated butter and shortening were used as controls. At 0, 2, and 7 days, 100 ml of tryptic soy broth supplemented with lecithin and polysorbate 80 were added to the food samples and stomached for 1 min. Serial dilutions were plated in duplicate. Two replicates of each treatment were performed.

Results: Salmonella was recovered from butter at 7.15, 7.2, and 7.1 log CFU/g on days 0, 2, and 7, respectively, while the recovery for *Listeria* was 6.8, 7.1, and 7.2 log CFU/g. *Listeria* exhibited slightly lower recovery in shortening of 6.8, 6.6, and 6.0 log CFU/g on days 0, 2, and 7, respectively, while *Salmonella* exhibited similar survival at 6.6, 7.1, and 7.2 log CFU/g, respectively.

Significance: This study shows that these pathogens are able to survive with very little reduction in viability for at least one week at room temperature and underscores the need to degrease countertops and equipment before sanitizing.

P2-47 Cooling Techniques: Characterizing *Escherichia coli* Population Changes in Low-sodium Marinara Sauce

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Introduction: The U.S. Food and Drug Administration has identified improper ("slow") cooling as a factor that contributes to foodborne illness outbreaks. School nutrition programs can cool leftover food products for reuse in future meals. Thus, research that characterizes the impact of a variety of cooling methods on foodborne pathogen populations is important for protecting public health.

Purpose: Characterizing *Escherichia coli* population changes in low-sodium marinara sauce subjected to cooling methods commonly used in school nutrition programs was the purpose of this research.

Methods: Canned, low-sodium marinara sauce was heated to 73.9°C (165°F), poured to 2- and 3-inch depths into commercial serving pans, and then cooled to 57.2 to 60.0°C (135 to 140°F) before inoculation with *E. coli* (target concentration of 10^4 CFU/g) as a surrogate for Shiga toxin-producing *E. coli*. All pans were stored uncovered or covered with one or two layers of aluminum foil in a commercial walk-in freezer (-20°C), or placed in ice water baths in a commercial walk-in refrigerator (4°C). MacConkey agar was used to enumerate *E. coli* populations at 0, 4, 8, 12, and 24 hours.

Results: Product depth (*P*<0.0001) and chilling time (*P*=0.0312) were statistically significant. An *E. coli* population difference of 0.40 log CFU/g was observed between 2-inch (4.20 log CFU/g) and 3-inch (3.79 log CFU/g) product depths. In regards to time, the largest increase in *E. coli* populations occurred between the 0- and 8-hour time points, with a difference of 0.21 log CFU/g.

Significance: Although significant, a marginal increase of 0.21 log CFU/g was more likely due to natural variation caused by inoculating and sampling large quantities of food rather than cooling failure. This combined with the lack of additional significant variables (i.e., cover) suggests that all cooling method combinations were effective at controlling *E. coli* populations in low-sodium marinara sauce.

Poster

P2-48 *Listeria* Controls at Retail: Nationwide Surveillance Results

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Introduction: A 2010 FSIS risk assessment found over 80% of listeriosis illnesses associated with ready-to-eat meat or poultry products (i.e., deli meat) were attributed to product sliced or handled at retail.

Purpose: Ongoing surveillance assesses whether there is a continued increase in retailers following recommendations from "FSIS Best Practices Guidance for Controlling *Listeria monocytogenes* (*Lm*) in Retail Delicatessens".

Methods: During routine surveillance of retail delis (n≈1750/year), FSIS Compliance Investigators observe and document whether retailers are following 33 recommendations from the guidance. Observations were evaluated based on the percentage of recommendations followed by category and by the top 8 recommendations FSIS identified as most important in preventing *Lm* contamination.

Results: The percentage of recommendations followed increased from FY16-Q2 to FY18-Q2 in all categories. Product handling increased from 85% to 90%, cleaning and sanitizing 80% to 83%, facility and equipment controls 96% to 98%, and employee practices 95% to 96% (n=127,258 practices observed). The percentage of delis following all top 8 recommendations increased from 58.5% to 65.3%. Recommendations least likely to be followed include (i) clean and sanitize equipment every 4 hours; (ii) cover RTE foods promptly; and (iii) separate RTE products from raw products. The percentage of delis following all top 8 recommended practices is highest in jurisdictions which have adopted more recent versions of the Food Code. In states under 1995, 1997, or 1999 Food Codes, only 55.6% of delis (n=288) were following all top 8 recommendations, compared with 69.5% in states which had adopted the 2013 Food Code (*n*= 811).

Significance: Increasing adoption of those recommendations found to significantly decrease the likelihood of illness in the "Interagency Risk Assessment – *Listeria monocytogenes* in Retail Delicatessens" would help protect public health by increasing retailers following the FDA Model Food Code and reducing risk of listeriosis from RTE meats handled at retail.

P2-49 Quality Changes in Abalone and Seaweed Rice Porridge Product for Infants after Addition of *Lactobacillus plantarum* Ln1 during Accelerated Storage

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Introduction: The accelerated test is widely utilized for setting expiration dates of products.

Purpose: The study tested the changes of chemical, microbial, sensory, and antioxidant qualities of an abalone and seaweed rice porridge product for infants by addition of *Lactobacillus plantarum* LN1.

Methods: The samples were stored at 35 and 65°C in incubators for 24 days for the tests of the chemical properties (pH, acid value, Hunter's color, viscosity, salt density), sensory properties, antioxidant qualities (DPPH radical scavenging activity, reducing power), and microbial quality (plate count agar [PCA], coliforms, *Bacillus cereus*).

Results: The sensory qualities of the sample in terms of overall satisfaction, color, flavor, texture, and taste were reduced with increasing days in storage, especially when stored at 65°C, while the L, a, b values of Hunter's color and salt density of the samples were constantly maintained during 24 days at 35 and 65°C. The pH values of the samples increased with storage. The microbial qualities showed that only coliforms (among PCA, coliforms and *B. cereus*) according to the Food Code of Korea for infant products were detected in the sample stored for 24 days at 65°C. DPPH radical scavenging activity of the samples with *L. plantarum* showed higher antioxidant activity than those without *L. plantarum* and decreased with storage days.

Significance: Based on the study, the abalone and seaweed rice porridge product with *L. plantarum* could be a stable for one year under room temperature distribution, and the addition of *L. plantarum* is helpful for increasing antioxidant capacity.

P2-50 Trends in *Salmonella* Infection Rates in Urban and Rural Counties in North Carolina and the Impact of Urbanization, 1997 to 2014

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❖ ***Developing Scientist Competitor***

Introduction: Rates of *Salmonella* infection in the United States (U.S.) have not changed over the past 20 years. There are distinct geographical trends in *Salmonella* infection rates, with the southeast reporting the highest rates of illness. *Salmonella* is an important foodborne pathogen and environmental contamination resulting from various human activities, such as animal production and urbanization, may contribute to the public health burden.

Purpose: We aimed to determine if there are differing *Salmonella* infection rates in urban compared to rural settings in North Carolina and to understand the impact of urbanization.

Methods: Salmonella infection rates for North Carolina counties (*n*=100) were obtained for 1997 through 2014. Infection rates were calculated using U.S. Census intercensal estimates, and the percent of the population residing in urban settings was compared between the 2000 Census and 2010 Census. Poisson models were used to determine the impact of urbanization on *Salmonella* infection rates.

Results: Sixty-six counties representing 84% of North Carolina's population saw increases in the percent of the population living in urban areas, and twelve counties representing 5.4% of the population shifted from being either completely rural to mostly rural or mostly rural to mostly urban between 2000 and 2010. As urbanization increased, *Salmonella* infection rates increased, with areas in transition having the highest rates of infection. Mean *Salmonella* infection rates were lowest in completely rural counties, higher in counties that were mostly rural or mostly urban, and highest in urbanizing counties. For each percentage point increase of the population living in urban areas, *Salmonella* infection rates per 100,000 population increased by 2% (*P*<0.05).

Significance: Urbanization represents increased risk of *Salmonella* infection, which could be due to more routes of transmission. Understanding how transmission routes vary between urban, rural, and transitional settings warrants further study.

Poster

P2-51 Reactions of Broiler Sera to *Salmonella* Flgk and Flid Flagellar Proteins

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Introduction: *Salmonella*, a gram negative bacterium, is the leading foodborne pathogen. It causes human acute bacterial gastroenteritis worldwide. Poultry products are considered one of primary reservoirs of this bacterium. Because the bacterial flagellum is involved in motility, adhesion, and other virulence activities, the flagellum may be the targets for inducing immune responses in chickens. Two flagellar proteins (FliD and FlgK) were selected for the study due to their exposure to the environment and involvement in colonization in the intestinal mucosa.

Purpose: In this communication, we expressed and purified these two proteins and used them as probes to evaluate if broiler sera collected from the selected areas contained antibodies against these proteins.

Methods: The *fliD* and *flgK* genes were amplified by PCR, and the proteins were over-expressed in an *Escherichia coli* expression system. The recombinant proteins were purified by a nickel-chelating affinity chromatography and confirmed by SDS-PAGE analysis and the His-tag detection. Enzyme-linked immunosorbent assay (ELISA)was applied to detect whether broiler sera had anti-FlgK and anti-FliD antibodies.

Results: The recombinant FliD and FlgK proteins were purified and had a respective, relative mobility of relevant sizes and positions in SDS-PAGE. Sera from the FlgK- and FliD-immunized broilers reacted strongly to FlgK and FliD, respectively, indicating that these proteins are immunogenic. Further, we used FliD and FlgK as probes to survey prevalence of anti-*Salmonella* antibodies in broilers. The ELISA showed 66% of broiler sera reacted to FlgK, while about 38% reacted to FliD. The results implicated that these anti-FlgK antibodies may be prevalent in the poultry population.

Significance: These results provide a rationale for further evaluation of these proteins as vaccine candidates to induce protective antibodies in broilers against *Salmonella* so that food safety for poultry can be improved.

P2-52 Feel the Dragon's Breath Burn: Investigation of Liquid Nitrogen Exposure after Consuming a Dessert from a Local Fair in Florida

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Introduction: Liquid nitrogen is used in food processing to quickly freeze foods. Liquid nitrogen has not been recognized as safe by the U.S. Food and Drug Administration and there is a hazard to consumers if the product is not handled appropriately.

Purpose: On October 23, 2017, the Florida Department of Health in Escambia County (DOH-Escambia) received complaints of burn injuries after consuming a dessert called Dragon's Breath at a local fair. The dessert is a combination of cereal and liquid nitrogen.

Methods: DOH-Escambia performed an assessment of the vendor with the Department of Business and Professional Regulation (DBPR) on October 26, 2017. An overview of the product and directions given to consumers regarding consumption was reviewed. DOH-Escambia interviewed fair attendees who reported being injured after consuming the dessert, collected medical records, and performed active case finding. A case was defined as someone who developed a burn-like injury after consuming or touching the Dragon's Breath dessert at the Pensacola Fair from October 20 to 23, 2017.

Results: The vendor was unlicensed prior to the visit by DOH-Escambia and was given a temporary license by DBPR for the remainder of the event. Three people met the outbreak case definition. Injuries associated with the cases included a burned thumb and burns to the inside of the mouth. All three cases sought medical attention for their injuries. Two cases reported that instructions were given on how to consume the dessert, but all cases reported not being fully aware of the risk associated with consuming the dessert.

Significance: This investigation into the use of liquid nitrogen as a food additive highlights the risk of consuming the product if not handled properly. This was a novel dessert to DOH investigators, and the manager of the event stated that a vendor offering this or a similar item would not be allowed to operate at future events.

P2-53 Food Poisoning Outbreaks and Climate Change in Korea over the Past Two Decades

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Introduction: Outbreaks of food poisoning in Korea are increasing on the whole. The reasons for the increase are the increase in the use of group meals and eating outside the house due to social changes such as increase in nuclear and dual-income family and an aging population. In addition, food safety risk factors may have increased due to climate change. However, there are few studies on the fluctuation of food poisoning outbreaks in Korea due to climatic factors.

Purpose: The purpose of this study was to evaluate the long-term effects of climate factors on variation in outbreaks of food poisoning of Korea.

Methods: The officially collected data on the number of reported cases of food poisoning were analysed for the years from 1996 to 2015. Climate data measured by the Korea Meteorological Administration's automatic weather stations were used. Correlation and regression analyses were used to establish the relationship between meteorological factors and fluctuation in the occurrence of food poisoning.

Results: The annual outbreaks of food poisoning were found to be significantly associated with average annual temperature, lowest annual temperature, number of days with precipitation, and humidity ($P<0.05$). The number of food poisoning incidents caused by *Campylobacter jejuni*, pathogenic *Escherichia coli*, norovirus, and protozoa increased significantly ($P<0.05$). The outbreaks of *C. jejuni* food poisoning were affected by average annual temperature and humidity. Pathogenic *E. coli* food poisoning was affected by average annual temperature and the lowest and highest annual temperatures. Norovirus food poisoning was impacted by average annual temperature, lowest annual temperature, and humidity. Protozoa food poisoning was affected by average annual temperature.

Significance: These data indicate that there is a strong association between climate variability and food poisoning outbreaks in Korea. Climate change, especially warming climate, has affected the pattern of food poisoning outbreaks.

P2-54 Features of Norovirus Food Poisoning Outbreaks in Korea

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Introduction: Norovirus is a common virus that causes water- and foodborne illness. In Korea, there have been sporadic cases of norovirus infection from the past, but it has been a problem since the early 2000s. In 2005, there were about 10 cases of norovirus food poisoning, but cases started to increase rapidly in 2006.

Purpose: The purpose of this study was to investigate the epidemiological characteristics of norovirus food poisoning outbreak in Korea in the last 10 years.

Methods: Food poisoning outbreak statistics from Korea's Ministry of Food and Drug Safety and data from the national norovirus outbreak surveillance network of the Korea Centers for Disease Control and Prevention were used. A five-year moving average of the number of outbreaks and cases was calculated during the past 10 years (2006 to 2015).

Results: In 2008, there were food poisoning outbreaks caused by norovirus throughout the year. From that point on, in most years, norovirus was the most common cause of food poisoning outbreaks. From the five-year moving average, outbreaks of norovirus food poisoning tended to increase. The number of outbreaks was more than one-third and the number of cases were more than half in the winter (December to February). Food frequently incriminated was fish and shellfish/seaweed. Also, there were more outbreaks in school and group meals using groundwater. In 2015, outbreaks increased in kindergartens and daycare centers. A recent study on norovirus outbreaks in Korea reported that genotype GII.17 was prevalent, but GII.47 has prevailed since 2014.

Significance: These results indicate that norovirus is the most common cause of foodborne illness in Korea and that it will remain an important pathogen in public health in the future. This epidemiological analysis suggests that norovirus infection has been indigenized in Korea and that large-scale contamination of food and groundwater are increasing.

P2-55 Presence of *Campylobacter* spp. in Food Stuffs, Animal Feces, and Rivers of East Tennessee

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❖ ***Developing Scientist Competitor***

Introduction: Campylobacter spp. are one of the main causes of bacterial gastroenteritis in the United States. Campylobacteriosis is commonly associated with the consumption of poultry, meat, and raw milk. East Tennessee counties experience a higher prevalence of illness compared to other regions of the state based upon surveillance performed by the Tennessee Department of Health.

Purpose: The purpose of this study was to determine the presence of *Campylobacter* spp. in food stuffs, animal feces, and rivers in East Tennessee to better understand possible vehicles for campylobacteriosis in the region.

Methods: Samples (n=258) were collected from local farmers markets, processing facilities, and rivers. A 25-g food or fecal sample was diluted in 225 ml Mueller-Hinton broth with supplements and incubated at 37°C for 4 h then 42°C for 44 h in a microaerophilic environment (5% O_2, 10% CO_2). Water samples (100 ml) were vacuum-filtered through a 0.45 µm membrane filter. Food stuffs, feces, and filters were plated onto Campy CHROMagar and incubated in a microaerophilic environment (5% O_2, 10% CO_2) for 4 h at 37°C and then 44 h at 42°C. All presumptive positives *(n=46)* were confirmed using PCR with targets for *Campylobacter* spp., *C. jejuni*, *C. coli*, and *C. lari*.

Results: From the 46 presumptive positive samples, 42 were confirmed as *Campylobacter* spp. Samples associated with *Campylobacter* spp. positives included microgreens (n=5 of 5; 100%), sprouts (n=2 of 3; 66%), Tennessee River water (n=8 of 8; 100%), Hiwassee River water (n=6 of 7; 86%), cattle feces (n=5 of 6; 86%), swine feces (n=12 of 13; 92%), and geese feces (n=4 of 4; 100%). Twelve pork fecal samples were confirmed as *C. coli*, and one goose and one cattle fecal sample were confirmed as *C. jejuni*.

Significance: These data indicate that *Campylobacter* spp. are present in surface waters, animal feces, and sprouted seeds in East Tennessee. Further speciation of positive samples will clarify what implications these findings have for food safety.

P2-56 Estimating the Burden of Foodborne Illness for *Campylobacter*, *Salmonella*, and *Vibrio parahaemolyticus* in Japan, 2006 to 2015

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Introduction: In Japan, it is mandatory to report the numbers of food poisoning incidents and cases; however, these do not exactly reflect the real burden of foodborne illnesses due to the passive nature of this surveillance. We have been estimating the real burden of foodborne diseases for *Campylobacter*, *Salmonella*, and *Vibrio parahaemolyticus* in Japan since 2006.

Purpose: Estimating the burden of foodborne illnesses associated with three pathogens in Japan from 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 2015. The physician consultation rate and the stool submission rate were estimated from telephone population surveys conducted for all of Japan and for Miyagi prefecture alone. We merged the telephone survey data conducted in 2016 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 all of Japan were 5.5 to 13.6 million, 1.2 to 2.8 million, and 49,000 to 438,000 during 2006 to 2015, respectively. Those estimated for all of Japan based on data from Miyagi prefecture were 0.64 to 1.6 million, 78,000 to 190,000, and 7,000 to 63,000 during 2006 to 2015, respectively. The numbers of reported foodborne illnesses per year in Japan during 2006 to 2015 for *Campylobacter*, *Salmonella*, and *V. parahaemolyticus* were 1,600 to 3,100, 440 to 3,600 and 50 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. Continuing active surveillance systems to complement the present passive surveillance is strongly suggested in order to identify and prioritize food safety measures more precisely and to monitor the effectiveness of risk management options.

P2-57 Epidemiology of Foodborne Norovirus Outbreaks in the United States, 2009 to 2016

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Introduction: Noroviruses are the leading cause of foodborne disease in the United States (U.S.). About one in five reported norovirus outbreaks are spread through food, which presents opportunities for prevention.

Purpose: To describe the epidemiology of foodborne norovirus outbreaks in the United States.

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Methods: Foodborne outbreaks occurring between August 2009 and December 2016 with norovirus reported as a single confirmed etiology to the National Outbreak Reporting System (NORS) were matched by outbreak identification numbers with outbreaks reported to CaliciNet, a U.S. laboratory norovirus surveillance network. We analyzed matched outbreaks by genotype for epidemiologic characteristics.

Results: Overall, 573 foodborne norovirus outbreaks were reported in both NORS and CaliciNet. The most commonly reported norovirus genotypes were GII.4 (49%), GII.2 (8%), and GII.6 (8%). Compared to non-GII.4, GII.4 outbreaks had higher rates of outpatient visits (6.9 versus 5.5 per 100 cases, $P<0.01$) and hospitalizations (13.4 versus 6.3 per 1,000 cases, $P<0.01$); non-GII.4 outbreaks had higher rates of emergency department visits (4.6 versus 3.3 per 100 cases, $P<0.01$). An implicated food was identified in 208 (36%) outbreaks, among which foods with multiple commodities (102 outbreaks, 59%) were most frequently reported and only molluscan shellfish (11 outbreaks, 5%) were implicated more often in non-GII.4 outbreaks than GII.4 outbreaks (9 versus 3%, $P=0.04$). Of the 287 outbreaks reporting at least one contributing factor, food workers were implicated as the source of contamination significantly more often among non-GII.4 outbreaks (82%) compared to GII.4 (72%, $P=0.04$).

Significance: Foodborne norovirus outbreaks reported in the U.S. are most commonly caused by GII.4 noroviruses, which are associated with higher rates of outpatient visits and hospitalizations. Food workers are commonly implicated as the source of contamination, particularly in non-GII.4 outbreaks. These findings can help guide food safety interventions, including worker hygiene, improved food handling and preparation, and further development of norovirus vaccines.

P2-58 Risk Factors Associated with *Campylobacter* Prevalence in Livestock Raised on Small-scale Diversified Farms in California

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❖ ***Developing Scientist Competitor***

Introduction: Diversified farms are often small-scale and raise a combination of livestock and produce or multiple livestock species with the intent of selling specialized animal products directly to consumers. The increasing number of small-scale diversified (SSD) farms reflects growing consumer interest and demand for sustainably produced local foods, including humanely raised animal products such as meat and eggs. However, livestock naturally harbor *Campylobacter* and other zoonotic foodborne pathogens that can cause severe human illness.

Purpose: The objective of this study was to assess the association between risk factors and the prevalence of *Campylobacter* in livestock raised on SSD farms.

Methods: Twenty California SSD farms were enrolled in a cross-sectional study and livestock fecal samples were collected twice in 2015 to 2016. Cattle, swine, and small ruminant fecal samples (n=1,009) were cultured for *Campylobacter* using selective media, with PCR confirmation of presumptive positive isolates. Farms completed a questionnaire regarding livestock health, biosecurity procedures, demographics, and management practices. To identify risk factors that may be associated with the prevalence of *Campylobacter* on SSD farms, farm management practices were analyzed using generalized linear multilevel models.

Results: Sixty-five percent (13 of 20) of the farms raised more than one livestock species and 85.0% (17 of 20) kept poultry. *Campylobacter* was found on 65.0% (13 of 20) of the farms at least once during the study. The overall *Campylobacter* prevalence was 10.8% (109 of 1,009), including isolates from poultry (11.3%), swine (12.6%), cattle (15.9%), goats (7.5%), and sheep (8.6%). Fifty-nine isolates were *C. jejuni*, 28 were *C. coli*, and 28 were not speciated. Livestock species, season, sample type, and farm size were significantly associated with the presence of *Campylobacter*.

Significance: As the number of SSD farms continues to grow, evaluating risk factors and management practices that are unique to these operations will help identify scale-appropriate food safety mitigation strategies to keep food safe from farm to fork and protect California's agricultural economy.

P2-59 *Clostridioides* (*Clostridium*) *difficile* in the Human Diet: Systematic Review and Meta-Analysis to Assess Ingestion Risk

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✦ ***Undergraduate Student Award Competitor***

Introduction: Clostridioides (Clostridium) difficile is a spore-forming bacterium that may produce toxins upon replication in the intestinal tract, causing inflammation, primarily colitis, often with severe complications in susceptible individuals. The main source of infection has long been attributed to health care facilities, with spill over into the community. However, recent studies have shown that approximately 70 to 75% of cases are not due to health care transmissions. There has been a growing interest in quantifying the extent to which these infections may be due to foodborne exposure.

Purpose: The objective of this study was to conduct a systematic review of the scientific literature reporting the presence of *C. difficile* in foods for subsequent quantification of the risk factors associated with the prevalence using meta-analysis and meta-regression.

Methods: This study was conducted following the Cochrane Handbook for Systematic Reviews of Interventions. An initial search of studies retrieved 1,923 records from PubMed and the Web of Science using a consensus keyword algorithm. Google Scholar search was used to identify grey literature. Seventy-one relevant studies passed inclusion criteria for itemized and cumulative meta-analysis.

Results: The earliest study was published in 1981, with an exponential increase over the last decade. Geographically, studies were conducted in Africa (n=3), Asia (n=13), Australia and New Zealand (n=3), Europe (n=17), North America (n=34), and South America (n=1). Together, the generated dataset represents microbiological protocols from 22 different countries, 216 scientists, and 207 itemized food products of plant and animal origin, including beef (n=37), pork (n=33), poultry (n=31), seafood (n=25), and vegetable (n=24) items. The overall prevalence (%) of *C. difficile* across all tested food items was 10.7±17.2%, with a mean sample size per item of 95.6±137.6 (mean±SD).

Significance: This work will help appraise existing evidence and determine global prevalence of *C. difficile* in foods intended for human consumption as a risk factor for *C. difficile* infections.

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P2-60 Prospective Whole Genome Sequencing for *Salmonella* Has Highlighted Problems with Frozen Breaded Chicken Products in Canada

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Introduction: In May 2017, Canada began prospective whole genome sequencing (WGS) for *Salmonella*. Prior to this, clusters of enteric illness were assessed based on serotype and/or pulsed field gel electrophoresis patterns. More recently, the National Microbiology Laboratory (NML) also began routine sequencing of all non-clinical *Salmonella* isolates identified through FoodNet Canada's sentinel site surveillance system.

Purpose: To present how prospective WGS highlighted the issue of *Salmonella* in frozen breaded chicken products.

Methods: Multijurisdictional WGS clusters of *Salmonella* isolates are identified by the NML. Clusters are assessed and epidemiologic information is obtained to determine the possible source of the illnesses. Additional information on non-clinical isolates is obtained from FoodNet Canada to help inform hypothesis generation.

Results: Between May and December 2017, a total of 156 multijurisdictional *Salmonella* WGS clusters were assessed, with a total of 2,161 clinical isolates and 157 non-clinical isolates. Twenty-three of these clusters included one or more non-clinical isolates obtained from frozen breaded chicken. These 23 clusters represent a total of 874 clinical isolates with a range of one to 171 clinical cases within clusters. Epidemiological investigations were initiated for 13 of these 23 clusters, and 37% of cases with exposure information reported consuming or probably consuming frozen breaded chicken during their exposure period. Two investigations led to recalls of various frozen breaded chicken products.

Significance: The implementation of prospective WGS has changed the way *Salmonella* clusters are assessed and investigated. It has provided increased ability to differentiate enteric illness clusters by identifying clusters of illness associated with frozen breaded chicken exposure and highlights the products' significant burden on reported salmonellosis in Canada. The ongoing and collaborative work of federal, provincial, and territorial public health partners to gather and summarize evidence will be used to support regulatory and risk assessment activities.

P2-61 The Impact of Prospective Whole Genome Sequencing for *Listeria monocytogenes* on Outbreak Detection and Response: A Canadian Perspective

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Introduction: In January 2017, Canada began prospective whole genome sequencing (WGS) for clinical isolates of *Listeria monocytogenes*, replacing pulsed field gel electrophoresis (PFGE) as the standard molecular typing technique used for surveillance of *L. monocytogenes*. WGS provides enhanced discriminatory power and has been demonstrated to be superior to PFGE in identifying true clusters of illness.

Purpose: This abstract describes how the transition from PFGE to WGS affected outbreak detection and response activities for multijurisdictional *L. monocytogenes* clusters in Canada.

Methods: Multijurisdictional *L. monocytogenes* clusters identified by the National Microbiology Laboratory are reviewed with federal epidemiologists and prioritized for further investigation. The number of PFGE clusters and investigations from January 1, 2014 to December 31, 2016 was compared to the number of WGS clusters and investigations from January 1 to December 31, 2017. These investigations included information requests to partners and the review of case-level information collected through the Enhanced National Listeriosis Surveillance Program.

Results: Epidemiological investigations were initiated for 21 out of 34 multijurisdictional PFGE clusters identified between 2014 and 2016. Retrospective WGS was completed for 15 of these PFGE clusters, and only one was confirmed to be genetically related. This was also the only cluster for which a source was identified. Three multijurisdictional *L. monocytogenes* clusters were detected by WGS in 2017, but none resulted in epidemiological investigations, as they did not meet case count thresholds for follow-up (greater than two cases).

Significance: WGS has demonstrated greater discriminatory power than PFGE for the detection of *L. monocytogenes* clusters in Canada. Previous unsuccessful attempts to identify sources of illness within PFGE clusters were likely due to the investigation of genetically unrelated cases. Implementation of WGS has resulted in the detection of fewer, more genetically related clusters of LM, resulting in fewer epidemiological investigations and more efficient use of resources for outbreak response.

P2-62 Treatment Failure in a Patient with Multidrug-resistant *Shigella* Linked to Attending a Wedding in Ireland, Tennessee, 2017

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Introduction: Shigellosis is a major diarrheal disease in the United States (U.S.) and is transmitted primarily person to person. Challenges for clinicians include choosing antibiotics empirically to treat resistant strains. We reviewed an outbreak of *Shigella sonnei* linked to a wedding in Ireland in 2017. A Tennessee patient who attended the wedding reported that there were multiple ill attendees from the U.S. and Ireland.

Purpose: Documentation of treatment failure among healthy individuals with *Shigella* infections.

Methods: Data were collected using standard and supplemental case report forms and medical record review. Data were shared with Centers for Disease Control and Prevention and other state partners and health officials in Ireland. Ireland conducted a cohort study to identify suspected vehicles. Teleconferences were held to facilitate the exchange of epidemiological and laboratory information. Isolates from cases underwent whole genome sequencing (WGS) and antimicrobial susceptibility testing by broth microdilution.

Results: The patient in Tennessee was a healthy young woman who attended the wedding two days before her illness began. A total of 50 out of 100 attendees were interviewed and 21 (42%) of the 50 reported illness. Eight U.S. cases were identified from five states. No suspected food items were

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identified. Eight *S. sonnei* clinical isolates from Wisconsin (2), Tennessee, and Ireland (5) were closely related by WGS (0 to 2 single nucleotide polymorphisms). Six stool isolates (from five Irish patients and one Tennessee patient) were resistant to four classes of antibiotics, including the recommended treatment agents, trimethoprim-sulfamethoxazole and ciprofloxacin. The Tennessee patient failed treatment with cefixime, despite *in vitro* susceptibility to other β-lactams.

Significance: Emergence of multi-drug resistant *S. sonnei* among healthy persons is a growing public health concern and can rapidly spread internationally, as evidenced by this outbreak. Tennessee's case highlighted the potential threat of treatment failure with oral cephalosporin use. Collaboration between state, federal, and international colleagues helped to monitor the outbreak's progress and facilitate exchange of information.

P2-63 Modulating Effect of ZnO Nanoparticles on Immunological and Histopathological Alterations Induced by Chlorpyrifos in Rats

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Introduction: Chlorpyrifos is a widely used organophosphate insecticide. Zinc ocide nanoparticles (ZnO) showed effective adsorbing properties for insecticides *in vitro*.

Purpose: This study aimed to estimate the potential effect of ZnO against chlorpyrifos toxicity *in vivo* in male rats.

Experimental design: Animals were divided into one control and three experimental groups. The first group received drinking water containing 75 mg/L chlorpyrifos (a concentration nearly equivalent to one-twentieth of the LD50 for rats). The second group received drinking water containing 75 mg/L chlorpyrifos and 200 mg/L ZnO, and the third group received drinking water containing 200 mg/L ZnO. All groups received the treatments for 9 weeks.

Methods: Every three weeks, blood samples were taken randomly from each group to estimate macrophage and neutrophil phagocytic activity, as well as concentration of lysozyme in serum. At the end of the experiment, specimens from the liver and spleen were collected for histopathology.

Results: Neutrophils and nitric oxide activity of macrophage phagocytic activity were reduced in the chlorpyrifos-intoxicated group compared to the control group, while other groups showed increased activity throughout the experiment. Serum lysozyme activity was reduced in the chlorpyrifos-intoxicated group compared to the control group, while other groups showed no significant difference from the control group throughout the experiment. Moreover, chlorpyrifos induced marked histological alterations in the liver and spleen. These findings suggested that the ZnO nanoparticles have a high affinity for adsorbing chlorpyrifos and can effectively modulate its toxicity in rats.

Significance: ZnO nanoparticles may offer a novel, effective, and inexpensive approach for the preventive management of chlorpyrifos toxicity in animals.

P2-64 Production of Aflatoxin B_1 and B_2 during the Production of Wheat Malt for Use in Craft Beer Production

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Introduction: Aflatoxins B_1 (AFB_1) and B_2 (AFB_2) are secondary metabolites of filamentous fungi that cause various toxic syndromes and possess carcinogenic potential. These mycotoxins may be present in wheat craft beer if the wheat grains are infected with toxigenic fungi and those fungi produce aflatoxins during storage or malting.

Purpose: This study evaluates the production of AFB_1 and AFB_2 during the malting of wheat grains for use in craft beer manufacture.

Methods: The malting was performed at 14.5±0.5°C according Central European Commission for Brewing Analysis procedures. Steeping includes: water immersion (5 h), aeration (19 h), water immersion (4 h), and aeration (44 h). Aflatoxin-free, toxigenic fungi-free wheat grains (600 g) were inoculated in the first water immersion step by immersing in a suspension of *Aspergillus flavus* (5 log spores/ml) of a strain known to produce AFB_1 and AFB_2 originally isolated from wheat grains. After steeping, germination was performed at 95 to 98% relative humidity for 96 h (turning twice daily), followed by kilning (16 h at 50°C, 1 h at 60°C, 1 h at 70°C, and 5 h at 80°C). Rootlets were removed after kilning. Samples were collected during steeping (n=4), germination (n=4), and kilning (n=2). AFB_1 and AFB_2 were determined (dry basis) in each sample by high-performance liquid chromatography (detection limits of 0.03 µg/kg [AFB_1] and 0.02 µg/kg [AFB_2]; quantitation limits of 0.09 µg/kg [AFB_1] and 0.07 µg/kg [AFB_2]).

Results: AFB_1 and AFB_2 were produced during steeping and detected during malting. The lowest levels of AFB_1 (229.35±20.77 µg/kg) and AFB_2 (5.65±0.20 µg/kg) were detected during the germination and steeping stages, respectively. The highest levels of AFB_1 (455.67±12.86 µg/kg) and AFB_2 (13.06±0.18 µg/kg) were observed in the beginning of kilning. Both AFB_1 (240.46±11.16 µg/kg) and AFB_2 (6.36±0.29 µg/kg) were detected in the wheat malt.

Significance: Aflatoxins can be produced during the malting of wheat grains. This should be considered by craft beer manufacturers using malted wheat.

P2-65 Deposition of Copper in Cattle and Buffalo Tissues Slaughtered in Assiut Province, Egypt

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❖ ***Developing Scientist Competitor***

Introduction: Chronic copper poisoning may occur in animals under natural grazing conditions, as a consequence of excessive consumption of copper-containing salt licks or mixtures, unwise use of copper-containing drenches, contamination of feeds with copper compounds, and from agricultural or industrial sources. Abnormally high copper levels are characteristic of a number of diseases in humans, such as Mediterranean anemia and cirrhosis.

Purpose: Detection of copper toxicity in the tissue of cattle and buffalo.

Methods: A total of 168 samples of liver (part of the caudate lobe), kidney, and muscles (part of the diaphragm) were collected from 23 male cattle and 33 male buffaloes (2 to 3 years old) slaughtered in abattoirs in Assiut, Egypt. Each sample weighed approximately 50 g and was individually placed in polyethylene bags and labeled with the date, type, age, and sex of each animal. The collected samples were immediately taken to the laboratory in an ice box where they were kept deeply frozen at -20°C until preparation, digestion, and analysis. In accordance with AOAC methods (1975), the prepared samples were prepared for measurement by using the atomic absorption/flame emission spectrophotometer (Shimadzu model AA 630-02), using an air-acetylene flame and hollow-cathode lamp. The Mann-Whitney U test was used to compare any two groups with skewed data.

Results: Egyptian permissible limits for copper are 15 mg/kg. No samples of cattle muscle contained copper levels higher than the permissible limit, whereas 1 and 26% of cattle kidney and liver samples, respectively, did contain levels higher than the permissible limit. For buffalo, 3, 3, and 25% of samples of buffalo muscle, kidney, and liver contained copper at levels higher than the permissible limit, respectively.

Significance: Monitoring programs must be carried out periodically to measure heavy metals in soil and plants intended for animals in order to assess their load of heavy metals and avoid their hazards on animals and humans. Strict enforcement is needed for regulations on sewage waste pollutants which discharge onto farm land.

P2-66 Rapid Classification of Aflatoxin Levels in Single Corn Kernels by UV-Vis-NIR Spectroscopy

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❖ ***Developing Scientist Competitor***

Introduction: Corn is a major crop in the United States, and aflatoxin contamination poses threats to consumer food safety and grower economic stability. Current industrial methods for aflatoxin management in corn focus on bulk aflatoxin concentration, which can lead to either acceptance of contaminated corn kernels (a consumer food safety risk) or rejection of mostly harmless corn kernels (grower economic loss, food waste) due to the skewed nature of aflatoxin distribution.

Purpose: This research aims to investigate the potential of using a custom-built ultraviolet-visible-near infrared spectroscopy system (UV-Vis-NIR) to classify single corn kernels by aflatoxin concentration. Advantages of this method over previous spectroscopic methods include a broader spectral range and higher resolution at speeds theoretically fast enough for in-line use.

Methods: Single kernels from a cob inoculated with aflatoxin-producing *Aspergillus flavus* (240 kernels) and an uninoculated cob (240 kernels) were: i) scanned individually for reflectance at a wavelength range from 304 to 1086 nm by an increment of 0.5 nm; ii) ground; and iii) measured for aflatoxin by enzyme-linked immunosorbent assay. Using the spectra and the respective aflatoxin concentration, a random forest model was trained on 80% of the kernels to classify single corn kernels above or below 20 ppb of aflatoxin and was tested on the remaining 20% of the kernels.

Results: Among 480 kernels, 374 kernels had <20 ppb of aflatoxin and 106 kernels had ≥20 ppb of aflatoxin. The random forest algorithm achieved a training sensitivity of 87% with a specificity of 98%, and a testing sensitivity of 86% with a specificity of 97%. Regions around 390, 550, and 1050 nm were shown to significantly influence classification accuracy.

Significance: In this study, UV-Vis-NIR spectroscopy showed considerable potential in classifying single corn kernels by aflatoxin level. Ultimately, this technique can be upscaled and utilized to rapidly sort single corn kernels in industrial applications.

P2-67 Growth and Ochratoxin A Production by *Aspergillus fresenii* and *Aspergillus sulphureus* on Niger Seeds at 0.82 and 0.86 Water Activity at 37°C

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Introduction: Niger seeds are used as cattle and bird feed, and are also pressed for cooking oil. Fungal growth and mycotoxin contamination of niger seeds poses a high risk for human and animal health. To control ochratoxin-A (OTA) production on niger seeds, it is necessary to know the range of water activities of the seeds supporting mold growth and OTA production. Our previous results revealed that *Aspergillus fresenii* and *A. sulphureus* grew rapidly and produced high quantities of OTA on niger seeds with 0.94 and 0.90 aw, respectively, at 37°C.

Purpose: This study determined the growth and OTA production of *A. fresenii* and *A. sulphureus* on niger seeds at low water activity (aw=0.82 and 0.86, respectively) at 37°C.

Methods: Sterilized ground niger seeds (2.5 g) at different aw values (0.82 and 0.86) were placed in separate petri plates. Each plate was spot inoculated with 10 µl spore suspension (10^6 to 10^7 conidia/ml). Plates were then sealed with parafilm, placed in a closed glass jar, and incubated at 37°C for 30 days. Growth on each plate was determined by measuring the area covered by mycelium. After 5, 10, 15, and 30 days of incubation, OTA production in each inoculated plate was determined using enzyme linked immunosorbent assay and high-performance liquid chromatography.

Results: The two fungi showed similar growth rates, which were 2.9 to 3.3 mm/day at 0.86 aw and no growth at 0.82 aw. From seeds (0.86 aw) inoculated with *A. fresenii*, 23 and 13 mg/kg of OTA were detected after incubation for 15 and 30 days, respectively. From seeds (0.86 aw) inoculated with *A. sulphureus,* 286 mg/kg OTA was measured after 30 days of incubation. No OTA was found from seeds with *A. sulphureus* growth for 5, 10, or 15 days.

Significance: The data collected from this study can be used to establish standards for the storage of niger seeds.

P2-68 Studies of Aflatoxin B1 (AFB1) Production by *Aspergillus parasiticus* on Niger Seeds

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Introduction: Niger seeds are used as cattle and bird feed and pressed for cooking oil. Fungal growth and mycotoxin contamination of niger seeds pose a high risk to human and animal health. Previous studies reported that niger seed cake supports fungal growth and increases the risk of aflatoxin contamination of milk when it is fed to dairy cows. To control aflatoxin production on niger seeds, it is necessary to know the range of water activities of the seeds supporting fungal growth and AFB1 production.

Purpose: This study determined the growth and AFB1 production of *A. parasiticus* on niger seeds at water activity (0.86, 0.90, 0.94, 0.98 a_w) at 20, 27 and 35°C.

Methods: Sterilized ground niger seeds (2.5g) at different water activity (a_w) values (0.86, 0.90, 0.94, 0.98) were placed in separate petri plates. Each plate was spot inoculated with 10 µl spore suspension (10^6-10^7 conidia/ml). Plates were then sealed with parafilm, placed in a closed glass jar, and incubated at different temperatures for a total of 30 days. Growth on each plate was determined by measuring the colony diameter. After 5, 10, 15, 20 and 30 days of incubation, AFB1 productions in each inoculated plate was determined using high performance liquid chromatography (HPLC)

Results: Our results show that niger seeds support not only fungal growth but also AFB1 production by *A. parasiticus*. High level of AFB1 production (over 200 µg/kg) was observed at all the four a_w used in this study depending on the incubation period and temperature. At 0.90, 0.94 and 0.98 a_w, the highest AFB1 (252, 206 and 265 µg/kg, respectively) was produced in 5 days incubation period and at 27°C while at 0.86 a_w, the highest AFB1 (267 µg/kg) was determined in 30 days incubation period and 35°C. Overall, the interaction of water activity, temperature and incubation period played important role on the production of AFB1.

Significance: The data collected from this study can be used to establish storage standards of niger seeds to minimize the production of AFB1.

Poster

Poster

P2-69 A Comparative Study of Heavy Metal Exposure Risk from the Consumption of Some Common Varieties of Cultured and Captured Fishes in Bangladesh

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❖ ***Developing Scientist Competitor***

Introduction: Food toxicity and health risks due to exposure to heavy metals through fish consumption have become major concerns in the present era. It has become necessary to assess the content of heavy metals such as iron (Fe), copper (Cu), zinc (Zn), arsenic (As), mercury (Hg), and lead (Pb) in commonly consumed cultured and captured fish in Bangladesh.

Purpose: The purpose of this study was to compare the possibly carcinogenic, non-carcinogenic, and other health risks in cultured and captured fish in Bangladesh.

Methods: Fish samples (*n*=14) from seven fish species in both captured and cultured categories were collected, washed, separated into flesh and bones, minced, and oven-dried for 12 to 24 hours at 80°C, then ground into a fine powder and made into circular pellet weighing 0.1 g each and 7 mm in diameter. Each pellet was compiled in an x-ray fluorescence spectrophotometer for 1,000 seconds to determine heavy metal content. Risk assessment was performed using formulas in Microsoft Excel and SPSS software.

Results: The assessment revealed that among all other identified metals, Zn was most common in the fish samples, followed by Fe, Cu, and others. The HRI of Pb in almost all the samples exceeded the permissible limit (<1) for both adults and children set by the United States Environmental Protection Agency (EPA) and the Food and Agriculture Organization of the United Nations. For Hg, maximum non-carcinogenic risk was estimated at 859.72 for adults and 7,523.57 for children from the consumption of cultured *Puntius sarana*, which is very alarming. Among the carcinogenic risks of Pb, bones of captured *Anabas testudineus* and flesh of cultured *Puntius sarana* showed maximum values of 1.51×10^{-2} and 1.57×10^{-2}, respectively, in children; this exceeds the allowable risk of 1.00×10^{-6} recommended by the EPA (1999). Summarizing the results, the toxicity scores showed substantial rate of possibility to be exposed to heavy metals through fish consumption, especially cultured fish.

Significance: Human health can be at risk due to chronic exposureto heavy metals through fish consumption over the years.

P2-70 Assessing Cumulative Dietary Organophosphate Pesticide Exposure from Fruit and Vegetable Consumption in the United States

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Introduction: Organophosphate pesticides (OPs) are insecticides widely used in agriculture. Since OPs share a common toxicological mechanism of action, dietary risk assessment from OP exposure often uses cumulative approaches considering toxicological equivalencies, known as relative potency factors (RPFs), among different OPs; RPFs are often calculated from the benchmark dose (BMD) or no adverse effect level (NOAEL) of individual OPs. The process to determinate RPFs is subjective and is influenced by the choice of toxicology dosing regimens and the selection of appropriate toxicity endpoints.

Purpose: The purpose of this study was to assess the influence of common methods to establish OP RPFs (BMD and NOAEL) and a novel method (lethal dose 50 [LD50]) on cumulative estimated dietary exposures to OPs and to examine the advantages and disadvantages of each method.

Methods: Cumulative dietary exposure estimates for OPs were developed using three different methods of determining RPFs (BMD, NOAEL, LD50) and using three different OPs as Index Chemicals (methamidophos, disulfoton, azinphos-methyl). RPFs were calculated as the potency ratio between the Index Chemicals and the toxicity endpoints selected. A probabilistic method was used to estimate cumulative dietary exposure considering food consumption and residue data. In this study Lifeline 5.0 modelling software was used to estimate cumulative exposure. Residue data were obtained from the 2014 USDA Pesticide Data Program.

Results: Findings differed slightly between the methods used to establish RPFs and the specific choice of reference OP, but were all found to be far below the acute reference dose (RfD) of all of the reference OPs at the 99.9th percentile of exposure.

Significance: Results suggest that all three methods to establish RPFs yield comparable findings. The choices of RPF method and reference OP may be governed by the availability and quality of toxicological data. Exposure estimates suggest insignificant dietary risk to consumers in the United States.

P2-71 Comparison of the *Aspergillus flavus* Spores Reduction on Stored Doenjang with Gamma and Electron Beam Irradiation

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Introduction: Aspergillus spp. are a predominant mold in meju (dried fermented soybeans), and *A. flavus* is a main pathogenic mold that can produce aflatoxins. Doenjang (fermented soybean paste) is a Korean traditional food that uses meju, and *A. flavus* transmission through raw materials is a risk to the human body. Irradiation can be applied as a non-thermal sterilization method to food.

Purpose: This study investigated the effects of gamma and electron beam irradiation (1 to 3 kGy) on the reduction of *A. flavus* in doenjang, physiochemical properties, and sensory evaluation changes.

Methods: Doenjang inoculated with 5 log *A. flavus* was stored at 10 and 25°C for up to 30 days in order to confirm a decrease in fungi spores as a result of temperature. After that, the environment with low fungus reduction was selected and gamma and electron beam irradiation were performed to reduce *A. flavus*, followed by physiochemical (pH, moisture, and Hunter color) and sensory evaluation (seven-point hedonic scale).

Results: The population of *A. flavus* stored at 10 and 25°C were significantly decreased ($P<0.05$; 5.50 to 4.39 at 10°C and 5.51 to 0.00 at 25°C). After gamma and electron beam irradiation (1 to 3 kGy) on doenjang stored at 10°C, *A. flavus* were significantly reduced ($P<0.05$) to 1.54 to 4.97 and 1.26 to 4.93 log CFU/ml, respectively. Moisture, pH, and sensory (colour, appearance, flavor, texture, and overall acceptability) evaluation were not significantly changed ($P>0.05$) after gamma and electron beam irradiation (1 to 3 kGy). However, there were significant changes ($P<0.05$) in Hunter color after gamma and electron beam irradiation (1 to 3 kGy).

Significance: This study suggests that gamma and electron beam irradiation on doenjang may be effective in reducing *A. flavus* without physiochemical or sensory changes.

P2-72 Detection of Chitinase and β-1, 3 Glucanase Genes against *Aspergillus flavus* in Transformed Peanuts

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Introduction: Infection of peanut seeds with *Aspergillus flavus* and *Aspergillus parasiticus* is a serious problem in Georgia. There is no direct action peanut farmers can take to control fungi that produce aflatoxin. Transgenic approaches are being undertaken to prevent invasion by *Aspergillus* fungi or to prevent biosynthesis of aflatoxin. Hydrolytic enzymes, rice basic chitinase, and β-1, 3 glucanase, have been established to be active against various pathogens, especially fungal diseases, with particular reference to *A. flavus*.

Purpose: In our initial study, somatic embryos from zygotic peanut embryos were successfully generated via tissue culture techniques. Current investigation confirms the presence and expression of chitinase and β-1, 3 glucanase genes via *Agrobacterium* mediated transformation.

Methods: A total of 100 zygotic embryos of peanut were separated from the seeds. Murashige and Skoog (MS) medium was supplemented with growth regulators for root and shoot initiation of calli. Somatic embryos were transformed by co-cultivating with *Agrobacterium tumefaciens* (pCAMBAR-Glu 289 and pCAMBAR Chi11). Genomic DNA was isolated from the putatively transformed plants and amplified by PCR using gene-specific primers. Expression of the pathogen-related (PR) proteins, chitinase and glucanase, was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoblotting.

Results: Efficient and successful *Agrobacterium*-mediated transfer led to the production of more than 50% of the transgenic explants. The specific primers confirmed successful gene inserts into the transgenics. While the chitinase gene was detected at ~700 bp, the glucanase was amplified at ~1,200 bp in explants. Coumassie blue stains highlighted band sizes of ~12 and ~31 KD for glucanase and chitinase, respectively.

Significance: Because the chitinase and glucanase genes were stably inserted into our transformed peanuts, the specific proteins expressed can be studied further to develop transgenic varieties which may prevent *Aspergillus* contamination and aflatoxin production.

P2-73 Effectiveness of Dry Purging for Removing *Salmonella* from a Contaminated Lab-scale Auger Conveyor System

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Introduction: Current sanitation and cleaning methods in low-moisture food processing environments range in application strategy. Use of wet sanitation methods is typically avoided, as they may promote microbial hazards. However, the efficacy of dry cleaning methods, such as dry purging, on reducing microbial loads on processing equipment have not been researched.

Purpose: The objective was to determine the efficacy of dry purging as a microbial reduction technique in a lab-scale auger conveyor system.

Methods: Whole wheat flour was inoculated with a cocktail of five *Salmonella* serotypes and then equilibrated to a water activity of 0.43±0.02. One-hundred grams of inoculated product was fed by a hopper into a sterile, lab-scale auger conveyor system constructed of 1.5-in standard 304 stainless steel sanitary tubing and a 55-cm 304 stainless steel dry-product transport auger. The auger conveyed flour through the system at a constant 50 g/rev. After the contaminated product was pushed through the system, 500 g of uncontaminated whole wheat flour was conveyed through the system at the same rate. Flour (25 g) was sampled at intervals of 25, 50, 100, 200, and 400 g. A representative amount of flour (~2 g) from each interval was mixed with buffered peptone water and enumerated by serial dilution on trypticase soy agar with 0.6% yeast extract.

Results: The original *Salmonella* population of inoculated wheat flour was 8.65±0.31 log CFU/g. The population of *Salmonella* transferred to the first 25 g of push-through flour was 4.95±1.37 log CFU/g. Subsequent samples of push-through flour asymptotically arrived at a cell density of 3.50±0.22 log CFU/g after 400 g.

Significance: Development of a scalable experimental design of low-moisture microbial hazard propagation provides industry-applicable results to further assist risk assessments during processing. This study indicates that *Salmonella* may not be completely removed from a contaminated powder conveyor system using dry purging alone.

P2-74 Survival of *Salmonella enterica* in Low-moisture Military Ration Products

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Introduction: Survival of *Salmonella enterica* in low-moisture foods (LMF) with low water activity (aw) have been increasingly implicated in foodborne disease outbreaks over the past decade.

Purpose: This trend has prompted the United States Army to investigate the potential long-term survival of *S. enterica* in LMF, since they are frequently included in military rations and are required to have a three-year minimum shelf life at 25°C.

Methods: Simulated commercial products with low aw such as peanut butter (0.21), mocha dessert bar (0.43), boil-in-bag eggs (0.091), chocolate protein drink (0.34), cran-raspberry first strike bar (0.50), and freeze-dried beef stew (0.077) were prepared with a dry inoculum of *S. enterica*. A five-strain inoculum of *S. enterica* serovars (Agona, Enteritidis, Montevideo, Tennessee, and Typhimurium) was prepared and added to an ingredient in each product. Products were stored at 4, 25, or 40°C for 3 years and *S. enterica* was enumerated by dilution and plating on tryptic soy agar and selective Hektoen enteric agar.

Results: Results from storage studies to date indicated viability of *S. enterica* in peanut butter after 2 years and chocolate protein drink and boil-in-bag eggs after 1.5 years when stored at 4, 25, and 40°C. Mocha desert bar, which had the highest aw of the stored products, had approximately 0.5-log loss at 4°C, 1.5-log loss at 25°C, and lost viability at 40°C after 1 year. Cran-raspberry first strike bar had approximately 0.5-log loss at 4°C, 3-log loss at 25°C, and lost viability at 40°C after 6 months. Freeze-dried beef stew had no loss at 4 and 25°C and 1-log loss at 40°C after 3 months.

Significance: These data suggest that *S. enterica* can survive in low-moisture military ration products at elevated temperatures for extended periods of time, which indicates the need for additional safety protocols.

P2-75 Characterization of *Pedicococcus acidilactici* ATCC 8042 as a Potential *Salmonella* Surrogate in Toasted Oats Cereal

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❖ ***Developing Scientist Competitor***

Introduction: The presence of *Salmonella* in low water activity (aw) foods is a concern for the food industry. Because of the U.S. Food and Drug Administration's Food Safety Modernization Act, the need for validating thermal processes has become critical. Validation of an existing process in a food facility can be accomplished using a "generally recognized as safe" (GRAS) bacterium with similar heat tolerance.

Purpose: The objective of this study was to determine if *Pediococcus acidilactici* ATCC 8042, a GRAS microorganism, could serve as surrogate to *Salmonella* in toasted oat cereal for thermal inactivation.

Methods: A comparative study of five-strain *Salmonella* cocktail, *Enterococcus faecium* NRRL B-2354, and *P. acidilactici* ATCC 8042 was conducted. Strains were inoculated onto commercial toasted oats cereal (TOC) and equilibrated for a week at 0.33 aw. The thermal inactivation kinetics of the bacteria were measured at 80, 85, 90, and 95°C. Initial decimal reduction values were calculated using the Weibull model. Statistical significance was determined by the Tukey-Kramer HSD test.

Results: At 80°C, the rate of *Salmonella* cocktail inactivation (δ) was two-fold slower (122.5 min) than the values of *P. acidilactici, E. faecium* (60 and 62.4 min, respectively), but those differences were not significant (P<0.05). At 85°C, the δ values for *Salmonella*, *P. acidilactici*, *E. faecium* were 23.0, 30.9, and 39.54 min, respectively. At 90°C, the δ values were 22.7, 33.0, and 16.0 min, respectively. At 95°C, the δ values of the three bacteria were less than 25% different (4.2, 5.8 and 4.8 min, respectively). Statistical analyses at all temperatures resulted in no difference among the kinetic parameters of these bacteria.

Significance: This study suggests that *P. acidilactici* has similar heat tolerance as *Salmonella* and *E. faecium*, a previously recognized surrogate, and it could have potential as a GRAS-status surrogate.

P2-76 Comparison of the Thermal Resistance of *Salmonella enterica* Serotypes in Peanut Butter and Soy Protein Powder

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Introduction: Despite guidelines suggesting that organisms be selected based on the pathogen resistance to inactivation, few studies have evaluated the thermal resistance of individual *Salmonella enterica* serotypes, which is critical to determining appropriate serotypes for use in microbial challenge studies.

Purpose: The purpose of this project is to determine the thermal resistance of individual serotypes of *Salmonella enterica* in two different food matrices.

Methods: Creamy peanut butter and soy protein powder were inoculated at 8.3±0.4 log CFU/g with five individual serotypes of *Salmonella enterica (*Montevideo, Tennessee, Mbandanka, Enteritidis PT30, and Agona*)*. Approximately 1-g samples at ~0.25 aw were packed into thermal test cells, heated in an isothermal water or oil bath (90±0.2°C), then immediately cooled at specified time intervals. Samples were then diluted with 9 ml of buffered peptone water (BPW), stomached for 30 seconds, diluted again with BPW, and plated on modified trypticase soy agar supplemented with 0.6% yeast extract. Plates were incubated at 37°C for 24 h prior to enumeration.

Results: Thermal resistance for the serotypes tested varied between the strains (P<0.05), as well as between the two matrices. In both matrices, *Salmonella* Montevideo was found to be the most resistant of the strains tested (peanut butter $D_{90°C}$ = 16.67±3.04 min; protein powder $D_{90°C}$ = 7.75±1.14 min), while the least resistant strains varied, with *Salmonella* Enteritidis PT30 in peanut butter ($D_{90°C}$ = 11.03±1.11 min) and *Salmonella* Mbandanka in protein powder ($D_{90°C}$ = 6.78±0.67 min). Resistance of *Salmonella* Tennessee was also found to be significantly different (P<0.05) than for *Salmonella* Montevideo in peanut butter ($D_{90°C}$ = 12.09±2.23 min).

Significance: The results suggest that some serotypes of *Salmonella* are more thermally resistant than others and resistance may be product-dependent, which must be taken into consideration when selecting serotypes for a challenge study.

P2-77 A Review of Inoculation Techniques for Low-moisture Foods

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Introduction: Methodologies for the inoculation of low moisture foods remain limited with respect to food matrices. Instead, research has focused on individual food products. The characteristics of organism survival differ within low moisture foods. This study will evaluate the existing *Salmonella spp.* and *Listeria spp.* inoculation techniques used with low moisture foods and compare the survival patterns of microorganisms.

Purpose: The purpose of this study is to evaluate inoculation methods for products with intrinsically low moistures and water activities by comparing specific techniques and determining microorganism survival and recovery.

Methods: Powders were inoculated using derived from literature and Northland Laboratories. Dry inoculation of whey protein powder involved the desiccation of cells that were later homogenized with the product. Wet inoculation of silica beads was done by both spotting and soaking; both were then massaged with a spice matrix and dried. A direct inoculation of culture into the spice matrix was used and followed by drying. At Northland Laboratories, seasoning was spread over a large area, spray inoculated, dried and incubated at ambient temperature.

Results: Initial inoculation levels were achieved, but survival varied significantly based on antimicrobial properties of products, existing salt characteristics, manipulated water activities, and initial dilutions. However, characteristics needed for survival vary significantly as demonstrated through predictive modeling. Under acceptable conditions for both organisms, the time to observe one log reduction may vary by an average of 30 hours.

Significance: The data suggests that there is an underrepresentation of matrices in current literature regarding inoculation techniques of low moisture foods for *Salmonella spp., Listeria spp*. and other microorganisms. Further, the data between papers, and methods within the same matrices, needs further exploration for future applications.

Poster

P2-78 Survival of *Salmonella* spp., *Listeria monocytogenes*, Shiga Toxin-producing *Escherichia coli*, and *Enterococcus faecium* on Sunflower Kernels during Oil Roasting

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Introduction: Process validation aimed at addressing pathogens in sunflower kernels is an essential part of any food safety plan. However, insufficient data is available to demonstrate the reduction of pathogens on sunflower kernels during oil roasting. *Enterococcus faecium* has been determined to be a good surrogate for *Salmonella* spp. on almonds. Previous research indicated variations of heat resistance on various nut types. Therefore, heat-resistance testing was performed to determine if *E. faecium* is an appropriate surrogate for *Salmonella* spp., *Listeria monocytogenes*, and Shiga toxin-producing *Escherichia coli* (STEC) on sunflower kernels.

Purpose: The purpose of this study was to determine the survival of *Salmonella* spp., *L. monocytogenes*, STEC, and *E. faecium* on sunflower kernels during oil roasting. Additionally, the heat resistance of the pathogens on sunflower kernels was evaluated.

Methods: Sunflower kernels were oil roasted at 250 and 260°F for up to 5 min per the Almond Board of California's "Guidelines for Validation of Oil Roasting Processes". Sunflower kernels 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., *L. monocytogenes*, STEC, and *E. faecium* on sunflower kernels showed a log reduction of 2.35±0.58, 4.84±0.17, 2.93±0.22, and 2.59±0.38 at 280°F, respectively. A five-log reduction of *Salmonella* spp., *L. monocytogenes*, STEC, and *E. faecium* was achieved by oil roasting for 4 min or less at 250 and 260°F.

Significance: Reduction of pathogens during oil roasting of sunflower kernels is not consistent with oil roasting almonds. *E. faecium* was shown to be an appropriate surrogate for *Salmonella* spp. and STEC on sunflower kernels. *L. monocytogenes* showed significantly lower heat resistance than *Salmonella* spp., STEC, and *E. faecium* during oil roasting and heat-resistance testing.

P2-79 Survival of *Listeria monocytogenes* in Peanut Butter under Shelf-stable Conditions

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Introduction: Recent recalls due to *Listeria monocytogenes* in low-moisture, low-water activity foods have highlighted the need to understand the growth and survival kinetics in such products.

Purpose: The purpose of this study was to observe over time the survival of *L. monocytogenes* inoculated into various formulations of peanut butter.

Methods: Five peanut butter formulations were evaluated for the study. Peanut butter samples were individually inoculated with a six-strain cocktail of *L. monocytogenes* using a low-moisture vehicle (sand) to achieve a target inoculation level of ~10^5 CFU/g peanut butter Samples were stored at 22.2°C and enumerated for *L. monocytogenes* every 30 days from 0 to 570 days using U.S. Food and Drug Administration-approved microbiological methods. Samples showing results below the limit of detection were further enriched and analyzed for presence or absence of *L. monocytogenes* using the VIDAS LMO2 assay. All experiments were performed in triplicate. Analysis of variance was performed to understand the effect of storage conditions on the survival of *L. monocytogenes* in peanut butter.

Results: *Listeria monocytogenes* decreased (P<0.05) with increase in storage time, and the rates of inactivation varied based on the type of peanut butter formulation. *L. monocytogenes* was recovered through direct plating between 90 and 120 days of storage in all formulations. However, the assay, which includes an enrichment step, demonstrated that *L. monocytogenes* can persist up to 150 to 420 days. Complete die-off of *L. monocytogenes* was noted for all formulations prior to the end of the longest product shelf life of 570 days.

Significance: This study reported the survival of *L. monocytogenes* in five peanut butter formulations when the pathogen was introduced using a low-moisture vehicle of contamination (e.g., dust or debris from the manufacturing process). The study findings also underscore the importance of a manufacturing facility's hygienic design and employee hygienic practices in mitigating post-process contamination of peanut butter with *L. monocytogenes*.

P2-80 Evaluation of "Story of Your Dinner" Food Safety Campaign Video in 2016 and 2017

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Introduction: Foodborne illness is an important public health issue in the United States. The Partnership for Food Safety Education (PFSE) developed and launched a food safety campaign ("The Story of Your Dinner") in both the 2016 and 2017 holiday seasons (November to January) in the United States. A campaign video was developed to increase the awareness of and compliance with safe food handling practices among consumers.

Purpose: Evaluate the effectiveness of the campaign delivering food safety information and assess safe food handling knowledge and self-reported behavior of consumers.

Methods: Food safety educators on the PFSE mailing list were recruited to identify clientele to view the campaign video and respond to pre- and post-surveys, which collected demographic information and evaluated perceived risk, food safety knowledge, and food hygiene practices before and after the campaign. All surveys were entered into Microsoft Excel by a trained intern and reviewed by a separate researcher.

Results: A total of 161 participants (79% female, 45% 18-24 years old) in 2016 and 68 participants (92% female, 65% older than 55 years old) in 2017 completed the study. There was no significant difference between the two years and between the five participating states. After the campaign, participants' correct response to not wash raw meat or poultry under running water increased from 17 to 74% (2016) and 38 to 90% (2017). The correct response regarding the maximum time food should be out of refrigerator increased from 70 to 94% (2016) and 73% to 85% (2017), and correct response regarding the recommended temperature range for refrigerators increased from 56 to 86% (2016) and 65 to 81% (2017). Many reported that "not washing raw meat and poultry under running water" was the most memorable information. Further, many committed to using food thermometers and monitoring refrigerator temperature.

Significance: The campaign increased consumers' food safety knowledge and self-report practice compliance, especially when "surprising" information was included.

Poster

P2-81 An Examination of Microbiological Risks Associated with Almond Soaking and Drying

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Introduction: Soaking almonds before consumption has gained recent international popularity and widely varying instructions (almond to water ratios, soaking temperatures and times, drying practices) are available. The relative safety of these practices has not been adequately explored.

Purpose: To evaluate the behavior of foodborne pathogens during water soaking of almonds and during post-soaking drying.

Methods: Separate five-strain cocktails of rifampin-resistant *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* were inoculated onto untreated almonds that were then dried before soaking (direct inoculation), or added directly to the soaking water (indirect inoculation). The impact of almond to water ratios (w/w) of 1:1 and 1:3, incubation temperatures of 15, 18, and 23°C for up to 24 h, and post-soaking drying at 65°C for up to 14 h were evaluated. At each timepoint, pathogen concentrations were determined for triplicate almond samples (10 g or 10 almonds) by plating dilutions onto appropriate selective agar; moisture content and water activity (a_w) of the soaked almonds were determined. Each experiment was replicated at least two times; n = 6 (soaking) to n = 8 (drying).

Results: Populations of *Salmonella* did not significantly increase within the first 8 h of soaking at 23°C or within 24 h at 15°C. At a 1:1 almond to water ratio, increases of 1.13 and 4.44 log CFU/g of *Salmonella* were observed after 24 h of incubation at 18 and 23°C, respectively. No significant differences ($P < 0.05$) were observed between direct and indirect inoculation methods. At a 1:3 almond to water ratio, significant population increases (3.01, 3.66, and 3.62 log CFU/nut) were observed for *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes,* respectively, after 24 h of soaking at 23°C. Drying soaked almonds at 65°C for 14 h reduced moisture and a_w from 40% and 0.99, respectively to ~6% and 0.6, respectively. No significant reduction in *Salmonella* populations on soaked almonds were observed during drying.

Significance: Soaking almonds in water at ambient temperature supports the growth of foodborne pathogens. Choosing cooler soaking temperatures or shorter soaking times may lower risks of soaking almonds.

P2-82 Long-term Survival and Thermal Death Kinetics of Enterohemorrhagic *Escherichia coli* Serogroups O45, O121, and O145 in Wheat Flour

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Introduction: Wheat flour has recently been associated with several outbreaks related to *Salmonella* and enterohemorrhagic *Escherichia coli* (EHEC). Although *Salmonella* has been studied in flour to some extent, little is known about EHEC.

Purpose: The aims of this study were to assess the long-term survival of EHEC (O45, O121, and O145) and *Salmonella* in flour and determine their thermal death kinetics.

Methods: Wheat flour was inoculated (8 log CFU/g) with five-strain cocktails, stored at 23°C, and sampled for microbiological analysis during a long-term survival study. Pulsed field gel electrophoresis (PFGE) was performed on select colonies at days 84 and 168 of storage to identify surviving strains. Thermal treatments were conducted at 55, 60, 65, and 70°C. Viability count data were fit to a log-linear model and Weibull model, and thermal death kinetics were calculated.

Results: EHEC and *Salmonella* were quantifiable for up to 84 and 112 days, respectively, in inoculated flour. All samples were positive for more than six months with enrichment. Reduction curves were similar among serogroups and *Salmonella*, with the fastest reduction rate within the first week. *D*-values in long-term survival experiments were 13.5, 7.3, 8.3, and 7.7 days for *Salmonella,* EHEC O121, O45, and O145, respectively. Inactivation (δ) values were 9.7, 2.0, 5.5, and 9.3 days, respectively, with no statistical significance ($P \leq 0.05$). PFGE results revealed that the two O121 outbreak strains from the same outbreak were recovered more frequently. In thermal death kinetics studies, *D*-values, δ-values, and Z-values obtained were considerably higher than values for EHEC in foods with higher a_w, confirming that low a_w enhances thermal resistance of EHEC in flour. Overall, Weibull was a better fit to describe long-term survival and thermal inactivation of EHEC in flour.

Significance: This study reports findings that may contribute to designing interventions and strategies to control EHEC and *Salmonella* in flour.

P2-83 Survival of Shiga Toxin-producing *Escherichia coli* (STEC) O26, O111, and O121 in All-Purpose Flour

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Introduction: Foods and ingredients with low water activity have been the focus of increasing scrutiny, as multi-state outbreaks linked to contamination with both *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC) have been reported. Since 2016, three outbreaks of STEC associated with serotypes O26 and O121 in flour have occurred in North America.

Purpose: The purpose of this study was to evaluate the ability of STEC O26, O111, and O121 serotypes to survive in dry flour.

Methods: Representative serotypes from three STEC serogroups (O26, O111, and O121) were used to inoculate separate samples of sterilized all-purpose flour. Inoculated flour was stored in an environmental chamber at 25°C and relative humidity of 57% to simulate a dry storage environment. Flour was sampled on days 0, 2, 5, and 7 and weeks 2, 3, 4, 5, 6, and 7 to determine STEC populations by plating on tryptic soy agar. Samples were also enriched and plated on STEC CHROMagar to determine presence/absence. All experiments were independently replicated (n=9).

Results: At six weeks of storage, all three serotypes exhibited log reductions of 3.05, 2.46, and 4.10 log CFU/g for STEC O26, O11, and O121, respectively. All serotypes fell below the limit of detection (1.6 log CFU/g) at seven weeks. With enrichment, all three serotypes were found to be positive until 16 weeks. At 20 weeks, O26 was the only serotype that was positive through enrichment.

Significance: These data suggest that STEC can remain viable at low populations for extended periods of time when stored at room temperature in a low-water activity environment. More studies will be needed to further understand STEC's mechanism of survival in low-water activity foods and if specific isolates are better adapted for survival under these conditions.

P2-84 Identification of Novel Genes Mediating Survival of *Salmonella* under Low-moisture Conditions

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Introduction: Low-moisture foods (LMF) have been repeatedly linked to outbreaks of salmonellosis, but mechanisms mediating *Salmonella* survival on LMF remain poorly understood.

Purpose: To identify novel genes required for *Salmonella* survival on LMF.

Methods: Multi-gene deletion mutant panels of *Salmonella* Typhimurium 14028s were tested for survival on dry nitrocellulose (NC) as a model abiotic surface that allows multiplex screening (NC). Mutants were grown at 37°C for 12 h and spotted both on agar media and on sterile NC (8 by 8 cm). Agar plates were incubated at 37°C for 24 h, while inoculated NC was dried and stored in the dark at 22°C. After 10 and 20 days, inoculated NC was overlaid on agar media and incubated (37°C, 24 h). Spots with little or no growth on NC were identified, and putative mutants were retested for impaired survival on dry NC. Selected mutants were tested in triplicate for survival on in-shell pistachios, which were inoculated with a cell suspension (10^9 CFU/g), dried to their original water activity, and stored in the dark at 22°C. *Salmonella* populations after inoculation, after drying, and at 11 and 30 days were enumerated on non-selective and selective (XLD) media.

Results: Of 449 mutants, 10 were consistently impaired for survival on NC. Testing of three mutants on pistachios revealed that one (D1) showed significantly ($P<0.05$) greater reductions after drying in comparison to WT (reductions of 100-fold versus six-fold for D1 and WT, respectively). Subsequent reductions after 11 and 30 days were also significantly higher for D1 versus WT. Interestingly, the 18 genes deleted in D1, including *msyB*, *yceK*, *mdoHGC*, and the *csg* operon, were not among those previously found to be transcriptionally induced in *Salmonella* under desiccation and were not previously implicated in LMF survival.

Significance: Findings reveal novel genes required for *Salmonella*'s desiccation tolerance and LMF survival.

P2-85 Analysis of Desiccation Resistance of *Listeria monocytogenes* Strains

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Introduction: Listeria monocytogenes is a foodborne pathogen not historically associated with low-water activity foods. Due to recent outbreaks and recalls of low-water activity foods, there is a need for knowledge regarding the desiccation resistance of different strains of *L. monocytogenes*.

Purpose: The purpose of this study was to compare desiccation resistance of different *L. monocytogenes* strains on filters at 25°C for 28 d.

Methods: Five strains of *L. monocytogenes* were utilized for desiccation studies: 0827 (avocado isolate), 0352 (cream cheese isolate), 2153 (ricotta isolate), 0806 (hummus isolate), and ScottA. Each strain was individually cultured on brain heart infusion (BHI) agar at 37°C for 24 h and harvested using buffered peptone water (BPW). Each strain was inoculated separately onto duplicate 45-mm cellulose filters, resulting in 11 log CFU/filter. Filters were stored at 25°C with ambient relative humidity and enumeration conducted at 0, 1, 7, 14, 21, and 28 d. At each timepoint, filters were vortexed with BPW and serially diluted onto BHI for enumeration. Three independent trials were performed and data were statistically analyzed via analysis of variance ($P \leq 0.05$).

Results: All strains decreased significantly from their starting populations at 0 d. No significant differences occurred between the different *L. monocytogenes* strains through 14 d. At 21 d, 0827 and 0806 both had significantly higher populations (9.3±0.16 and 0.928±0.40 log CFU/filter) than 2153 (8.70±0.74 log CFU/filter). At 28 d, 0806 had a significantly higher population than 2153 (9.1±0.17 versus 8.30±0.77 log CFU/filter). Overall, a decrease of approximately 2 log CFU/filter occurred for all *L. monocytogenes* strains over the course of 28 d.

Significance: The results of this study can aid in identifying appropriate *L. monocytogenes* strains for use in desiccation studies.

P2-86 Effect of Water Activity and the Mixture of Sodium Lactate and Sodium Acetate on *Aspergillus flavus* Growth and Aflatoxin Production in Beef Jerky

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Introduction: Recently, Korea has started to produce various jerky products using different types of meats and seasonings. Spoilage due to mold growth in dried meat not only causes food safety problems, but also negative effects on the industry's reputation and future sales.

Purpose: The objective of this study was to investigate effect of water activity (aw) and the mixture of sodium lactate (SL) and sodium acetate (SA) on inhibition of *Aspergillus flavus* growth and aflatoxin production in beef jerky.

Methods: Sliced beef was marinated with 15% sugar and 10% soy sauce (control). Then, 0.75 or 1% of the 5:5 mixture of SL and SA (Opti.Form Powder Ace S50) was added into marinated sliced beef. The aw of marinated beef was adjusted to 0.70, 0.75, and 0.80 in the dehydration process using a food dehydrator. *A. flavus* (KCCM 11453) was inoculated onto the surface of beef jerky (5 g) and stored at 25°C for 4 weeks. Total aflatoxin was quantified using an AgraQuant Aflatoxin test kit.

Results: Growth of *A. flavus* in beef jerky containing the mixture of SL and SA was prevented at 25°C for 4 weeks, regardless of aw, indicating an antifungal effect from the mixture of SL and SA. At aw of 0.75 and 0.80, *A. flavus* in the control had 2 weeks of lag time and then increased up to 6.19 and 6.22 log CFU/g, respectively. Total aflatoxin was not detected in all samples at aw levels below 0.75. Total aflatoxin in the control was accumulated to 7.31 ppb at aw of 0.80, which decreased as the mixture of SL and SA increased.

Significance: Beef jerky showed risk of *A. flavus* and aflatoxin at aw levels of 0.80. The addition of the mixture of SL and SA and controlling aw at levels below 0.75 must be implemented for the safety of beef jerky.

P2-87 Validation of *Enterococcus faecium* NRRL B-2354 as a Surrogate for Thermal Inactivation of *Salmonella* in Date Paste

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Introduction: Dates are a common low-moisture ingredient in baked goods and desserts. *Salmonella* can be introduced into date paste during grinding, persist for months, and pose a food safety risk if not controlled by pathogen prevention and reduction processes. Therefore, methods to validate such processes need to be established.

Purpose: This study compared *Enterococcus faecium* as a surrogate for thermal inactivation of *Salmonella* in date paste and assessed reproducibility of the results across two laboratories (Michigan State University [MSU] and University of Georgia [UGA]).

Methods: A 600-g sample of pitted date pieces (~1 by 1 cm) was surface-inoculated with either *E. faecium* NRRL B-2354 or a five-strain *Salmonella* cocktail (Agona, Enteritidis PT30, Tennessee, Montevideo, and Mbandaka) on the outer skin, pre-conditioned to water activity (aw) of 0.65±0.025, and

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then ground into a paste by three passages through a meat grinder. Individual 1-g sub-samples were re-conditioned for three to four days and tested to ensure a homogeneous inoculum before use. Isothermal treatments were performed at 70, 75, and 80°C by heating 1-g samples (in triplicate, at two laboratories, with more than six durations per temperature) in aluminum test cells. Survivors were enumerated on designated non-selective/differential media after incubation.

Results: *E. faecium* exhibited higher thermal resistance (P<0.05) ($D_{75°C\text{-}Ef}$ of 3.8±0.4 min) than *Salmonella* cocktail ($D_{75°C\text{-}Sal}$ of 1.4±0.0 min) which represented by both MSU and UGA. MSU and UGA also demonstrated significant (P<0.05) impact of temperatures at 65,70 and 75°C for *Salmonella,* indicating repeatability of results between both laboratories (MSU: $D_{65°C}$ 12.7±0.7 min; $D_{70°C}$ 4.3±0.4 min; $D_{75°C}$ 1.5±0.1 min) and (UGA: $D_{65°C}$ 9.7±0.4 min; $D_{70°C}$ 4.4±0.2 min; $D_{75°C}$ 1.3±0.0 min).

Significance: Based on this cross-laboratory study, *E. faecium* NRRL B-2354 appears to be a valid surrogate for thermal inactivation of *Salmonella* in date paste.

P2-88 Effect of Talc on Thermal Resistance of *Enterococcus faecium* NRRL B-2354 in Almond Meal at a Water Activity of 0.45

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Introduction: Wet-inoculation methods produce high initial levels of *Salmonella* and have been used in *Salmonella* inactivation studies with low-moisture foods; however, dry carrier inoculation methods have been suggested to better represent conditions that lead to increased thermal resistance of *Salmonella*.

Purpose: This study aimed to determine: i) the effect of wet and dry inoculation on thermal resistance of *Enterococcus faecium* (a *Salmonella* surrogate) in almond meal at 80°C, and ii) the influence of talc powder as a carrier for dry inoculation.

Methods: Whole almonds were either immersed in an *E. faecium* suspension for wet inoculation (WI) or added to inoculated talc powder for dry inoculation (DI). After sieving the DI almonds to remove excess talc, almonds were pre-conditioned at a water activity (aw) of 0.45, ground into a meal, and re-conditioned to 0.45 aw. Thermal resistance of *E. faecium* in talc alone and WI almond meal with added talc (WT) was also determined. Isothermal treatments were performed by heating ~1 g of almond meal in aluminum test cells at 80°C (in triplicate with more than five heating times), and then immediately cooling, diluting, and plating samples on esculin tryptic soy agar with yeast extract (48 h at 35°C).

Results: WI yielded a higher initial population (7.6±0.2 log CFU/g) than DI (5.5±0.3 log CFU/g). *E. faecium* was more thermally resistant in DI ($D_{80°C}$=63.5±1.9 min) compared to WI almond meal ($D_{80°C}$=40.5±1.0 min, *P*<0.05), but the resistance in WT almond meal (46.9±0.9 min) was between and different (*P*<0.05) from both DI and WI.

Significance: Under-prediction of *Salmonella* inactivation in almond meal can be avoided by using *E. faecium* as a surrogate for *Salmonella*. However, efficacy of talc as an inoculum carrier for dry inoculation of low-moisture foods is complicated and needs further study.

P2-89 Influence of Water Activity on the Thermal Inactivation of *Salmonella enterica* in Low-moisture Pet Foods

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Introduction: Low-moisture products, including pet foods, have been associated with major recalls due to contamination by *Salmonella spp*. Increased heat resistance and prolonged survival of *Salmonella* at low-moisture conditions impede their effective inactivation in low-moisture foods. Water activity (aw) is one of the major factors influencing the thermal resistance of *Salmonella* in low-moisture foods. To achieve adequate reduction of *Salmonella* in low-moisture foods, elevated treatment temperatures or increased thermal treatment times are required when their aw values are decreased.

Purpose: This study aimed to analyze the effect of aw on the thermal resistance of *S. enterica* in pet food pellets.

Methods: Pet food pellets were inoculated with strain cocktail containing *S. enterica* ATCC 13311 and ATCC 43845 and equilibrated to aw 0.33, 0.53, and 0.73 values using controlled humidity chambers. Inoculated pellets were treated in closed aluminum cells at 65, 70, 75, 80, and 85°C. The reduction of cell counts of *Salmonella* populations on tryptic soy agar after thermal treatments were used to calculate their *D*-values and *z*-values.

Results: The *D*-values for *Salmonella* cocktail decreased with increase in aw of pet foods. For instance, at 0.33 aw, the calculated *D*-values at 75, 80, and 85°C were 11.7, 5.6, and 2.1 minutes, respectively, while the *D*-values were 7.9, 2.6, and 1.1 minutes, respectively, at 0.53 aw. We observed the lowest *D*-values of 6.1, 4.7, and 2.7 minutes at 0.73 aw after treatments at 65, 70, and 75°C. The *z*-values were determined as 13.6, 11.8, and 28.6°C at 0.33, 0.53, and 0.73 aw, respectively.

Significance: The quantitative analysis of thermal reduction of *Salmonella* with regards to aw will help us to develop effective thermal treatment protocols for adequate reduction of *Salmonella* in pet foods.

P2-90 Establishing the Microbial Profile of Retail "Raw" Almonds Purchased in the United States, 2013 to 2017

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Introduction: Almonds are an important crop to California's economy and make up 83% of the world's almond supply. Recalls due to *Salmonella* in retail almond products resulted in a new rule being established under the California almond marketing order in 2007 that requires almonds to be pasteurized before they are packaged and sold to consumers. These almonds are labeled "raw" or "natural" as it is thought that the changes in nutritional and sensory characteristics are negligible. Many studies have shown that pasteurization of almonds is effective in preventing foodborne illness. However, since the 2007 rule, no reported study has tested raw almonds purchased at the retail level for the presence of *Salmonella*.

Purpose: The purpose of this study is to validate the efficacy of mandated pasteurization of California raw almonds and to establish the microbial profile of raw almonds purchased at the retail level.

Methods: A total of 300 raw retail almond samples were collected from retail stores in California (*n* = 150), farmer markets or roadside stands in California (*n* = 16), or online (*n* = 134) over a 5-year period. Product labeling indicated that 29% of the samples were pasteurized, 10% were unpasteurized, and 61% of the almond samples did not indicate pasteurization status on the label. Samples were labeled as grown in California (27%), Europe (8%), USA

(6%), and 58% of the samples did not indicate origin. Samples were analyzed for the presence of *Salmonella*, total mesophilic aerobic bacteria, coliforms, generic *Escherichia coli*, and yeast and mold using standard methods.

Results: *Salmonella* was not detected in any of the samples. The total aerobic mesophilic bacteria averaged 2.66 $\log_{10}$ CFU/g, the yeast and mold averaged 2.01 $\log_{10}$ CFU/g, and coliforms averaged 0.23 $\log_{10}$ CFU/g. No generic *E. coli* was detected.

Significance: These data show that pasteurization of almonds is effective in protecting consumers from foodborne illness. Pasteurization is a valid food safety intervention method for nut processors and should be considered for other nuts recalled for presence of *Salmonella* (walnuts, macadamia nuts, pecans, cashews, pistachios, hazelnuts, and pine nuts).

P2-91 Effects of Temperature, Water Activity, and Physical Structure on Thermal Resistance of *Salmonella* Enteritidis PT30 on Multiple Almond, Date, and Wheat Products

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❖ ***Developing Scientist Competitor***

Introduction: Thermal resistance data for pathogens in low-moisture foods are typically reported for individual materials. In commercial processes, low-moisture products are often ground, milled, and/or included as ingredients in formulated products. However, little is known about the effects of fabrication and structure on pathogen thermal resistance.

Purpose: The objective was to quantify the effect of temperature, water activity, and physical structure on *Salmonella* thermal resistance on and in multiple almond, date, and wheat products.

Methods: Whole almonds, dates, and wheat kernels were inoculated with *Salmonella* Enteritidis PT30 (~108 CFU/g) and equilibrated (3 to 10 days) to water activities (aw) of 0.25, 0.45, or 0.65 before fabricating into almond meal, almond butter, wheat meal, wheat flour, and date paste. Inoculated and equilibrated almonds, wheat kernels, and date pieces were vacuum-packed in plastic bags, and fabricated products (~1 g) were loaded into aluminum test cells. Samples were subjected to isothermal water bath treatment (70 to 90°C), pulled at multiple intervals, immediately chilled, diluted in peptone water, and plated on modified (differential) trypticase soy agar to enumerate survivors. Primary (log-linear and Weibull) and secondary (Bigelow-type) models were applied to inactivation data (triplicate) to estimate parameters such as *D*-value, z_T, and z_{aw}.

Results: Overall, model fits yielded root-mean-square errors of 0.47 to 1.08 log CFU/g. The highest *Salmonella* thermal resistance was in almond meal ($D_{80°C,\ 0.25aw}$ = 75.2 min) and the lowest was in date paste ($D_{80°C,\ 0.65aw}$ = 0.7 min). There were no structural effects for wheat products (*P*>0.05), but *Salmonella* thermal resistance in fabricated almond and date products was higher (*P*<0.05) than on kernels and pieces. Overall, thermal resistance increased with decreasing aw (*P*<0.05).

Significance: The results show that water activity and structure of low-moisture products are critical factors that must be considered when validating pathogen controls for ingredients or formulated products.

P2-92 Survival of Various Microorganisms in Powdered Infant Formula

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Introduction: Microorganisms can survive in low-water activity foods such as powdered infant formula (PIF) for extended periods of time. Currently, the literature on microbial survival in PIF focuses on *Salmonella* and *Cronobacter sakazakii*, while data related to other microorganisms is limited. Recent recalls of low-water activity foods have involved *Listeria monocytogenes* and pathogenic *Escherichia coli*, exposing a need for additional research.

Purpose: To evaluate the survival of pathogenic and non-pathogenic microorganisms, including *Bacillus cereus*, *Staphylococcus aureus*, *C. sakazakii, E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium, and *Shigella,* in PIF.

Methods: Commercial PIF was inoculated with plate-harvested cultures using an ultrasonic atomizer and mixed for 30 min. Samples were stored in heat-sealed bags at 23±2°C and <25% relative humidity for the duration of the study. Four 11 g samples were enumerated in duplicate at 0, 3, 6, and 9 months using aerobic count Petrifilm incubated at 35°C for 48 h. Pathogens were confirmed using organism-specific selective agars.

Results: All pathogens except *B. cereus* exhibited a 1- to 2-log reduction during the duration of the study. *B. cereus*, *S. aureus*, *C. sakazakii, E. coli* O157:H7, *L. monocytogenes*, *Salmonella* Typhimurium, and *Shigella* had starting concentrations of 6.89±0.09, 7.38±0.01, 5.82±0.03, 6.77±0.06, 6.01±0.02, 6.81±0.04, and 6.78±0.05 log CFU/g and an ending concentration of 6.98±0.05, 6.05±0.11, 4.75±0.06, 5.19±0.16, 4.25±0.16, 6.07±0.04, and 5.90±0.07 log CFU/g, respectively. The non-pathogenic microorganisms showed similar survival trends.

Significance: Despite some die-off, significant population levels were observed for all pathogens analyzed in this study for months after inoculation. Previous studies demonstrate comparable results for *Salmonella* and *C. sakazakii*; however, this study emphasizes that other pathogens demonstrate long-term survivability in PIF and highlights the concern of post-processing contamination.

P2-93 Use of Residence Time Versus Screw Speed in the Response Surface Model for Microbial Inactivation during Single-screw Extrusion of Low-moisture Food

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❖ ***Developing Scientist Competitor***

Introduction: Many published validation studies on the factors that affect microbial inactivation during extrusion have primarily focused on screw speed instead of residence time. When scaling up the process to a different extruder, residence time serves as a useful tool for comparison.

Purpose: This study aims to: i) develop a response surface model for residence time of oat flour in a single-screw extruder as a function of moisture content, fat content, screw speed, and temperature, and ii) evaluate the use of residence time instead of screw speed on the accuracy of a response surface model for inactivation of *Salmonella* spp. and *Enterococcus faecium* NRRL B-2354 during extrusion of oat flour.

Methods: The oat flour was formulated to different moisture (MC, wet basis; 14 to 26%) and fat (FC; 5 to 15%) contents. A lab-scale single-screw extruder was used to extrude the prepared samples at different temperatures (T; 65 to 95°C) and screw speeds (SS; 75 to 225 rpm). The residence time (RT) was measured by adding 20 g red dye into the inlet of the extruder and measuring the time required for the color to appear at the die. A split-plot second-order central composite design was used where the central point was replicated six times. The RT data were then used to replace SS in a previously published response surface model for microbial inactivation.

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Results: Increase in SS, FC, and MC significantly (P<0.05) decreased the RT. However, the RT was not significantly affected by increasing T. The response surface equation for the residence time is: RT=140.09-3.16*FC-3.39*MC-0.66*SS+0.075*MC^2+0.143*FC^2+0.0015*SS^2(R^2=0.97). The use of RT instead of SS improved the R^2 value for response surface model from 0.83 to 0.85 for *Salmonella* and 0.84 to 0.88 for *E. faecium* inactivation.

Significance: A slight increase in accuracy due to use of RT instead of SS may not warrant the use of RT in the model due to complexity in determination of RT.

P2-94 Radio Frequency Pasteurization Process for Inactivation of *Salmonella* spp. and *Enterococcus faecium* NRRL B-2354 on Ground Black Pepper

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Introduction: Several *Salmonella* outbreaks linked to black pepper show a need for more effective inactivation processes, because current decontamination methods are limited by quality deterioration. Rapid volumetric heating by radiofrequency (RF) processing reduces the come-up time, which allows the design of high-temperature, short-time processing to inactivate *Salmonella* with minimal deterioration in product quality.

Purpose: The purpose of this study was to investigate RF heating for inactivation of *Salmonella* spp. in ground black pepper, evaluate *Enterococcus faecium* NRRL B-2354 as a surrogate for *Salmonella* spp., and assess quality deterioration during RF heating of ground black pepper.

Methods: The ground black pepper samples inoculated with either a *Salmonella* spp. cocktail or *E. faecium* were RF heated for 120 and 130 s and spread plated onto specific differential media for enumerating *Salmonella* spp. or *E. faecium.* Moisture content, color, piperine content, total phenolics, volatile compounds, and antioxidant activity were evaluated to assess quality of the pepper samples.

Results: RF heating the samples for 120 and 130 s gave a reduction of 3.98±0.17 and >5.98±0.13 log CFU/g for *Salmonella* spp. and 2.30±0.04 and 3.89±0.36 log CFU/g for *E. faecium*. At both heating times, a significantly (P<0.05) higher inactivation was obtained for *Salmonella* spp., which indicated that *E. faecium* was more thermally resistant than *Salmonella* spp. Color parameters (L*, a*, b*), piperine content, total phenolics, scavenging activity, and most of the volatile compounds in the samples RF-treated for 130 s were not significantly (P>0.05) different from the control samples.

Significance: The RF processing provided effective inactivation of *Salmonella* spp. with insignificant quality deterioration, and *E. faecium* was a suitable surrogate for *Salmonella* spp. during RF heating of ground black pepper.

P2-95 Survival of *Listeria monocytogenes* on Pistachios, Corn Flakes, and Chocolate Liquor at 4 and 23°C

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❖ ***Developing Scientist Competitor***

Introduction: Low-moisture foods (LMFs) such as dried fruits, cereals, and confections, are characterized by a water activity (a_w) below 0.85 and are emerging as novel vehicles for foodborne illness. Although the growth of bacterial pathogens is inhibited by low a_w, they have been shown to survive and persist for long periods of time in some LMFs. This presents a public health concern, especially when LMFs are consumed without undergoing any microbial inactivation steps.

Purpose: The main purpose of this study is to assess the survival of *Listeria monocytogenes* on artificially inoculated LMFs (dry-roasted shelled pistachios, chocolate liquor, and corn flakes).

Methods: Foods were inoculated with a four-strain cocktail of *L. monocytogenes* at an initial concentration of 8 log CFU/g by immersion (pistachios) or misting (chocolate liquor and corn flakes). They were then dried at 30°C and stored at both 23°C and 30 to 35% relative humidity (RH), and at 4°C and 30 to 35% RH. Bacterial enumerations were done on tryptic soy agar with 0.6% (weight/volume) yeast extract, with the exception of pistachios, for which Oxford agar was used due to interfering background microbiota. Analysis of significant population was determined by using a two-way repeated measures analysis of variance.

Results: During the initial drying/equilibrium period, populations of *L. monocytogenes* declined by 1.2 to 2.0 log CFU/g on pistachios and corn flakes. During the first two weeks of storage at 23 and 4°C, populations on pistachios remained relatively stable. *L. monocytogenes* populations on corn flakes also remained stable during the first month of storage at both temperatures. Monthly sampling of LMFs will continue for up to a year.

Significance: As the presence of any *L. monocytogenes* on ready-to-eat foods can lead to food recalls, research regarding foodborne pathogens on LMFs is a very important part of understanding the environmental mechanisms underlying the survival of pathogens. It also has great relevance for predictive modeling used in microbial health risk assessments.

P2-96 A Comparison between Two Methods for Determining Thermal Resistance of Microorganisms in Low-moisture Foods: TDT Disks and TDT Sandwiches

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❖ ***Developing Scientist Competitor***

Introduction: The thermal resistance of microorganisms is usually determined with a sealed vessel such as a thermal-death-time (TDT) disk immersed in a water or oil bath. However, it is difficult to control the come-up time (CUT) of samples in such a bath. A novel method called TDT sandwiches was developed to consistently control the CUT of inoculated food samples.

Purpose: To compare the measured thermal resistance of *Salmonella* spp. and *Enterococcus faecium* NRRL B-2354 in ground black pepper using TDT disks and TDT sandwiches.

Methods: Black peppercorn samples were spray-inoculated, equilibrated to 0.45 aw for 2 days, ground, then equilibrated again for a day to achieve stable initial populations of 6.44±0.24 and 7.51±0.12 log CFU/g of *Salmonella* spp. and *E. faecium*, respectively. Samples were packed into TDT disks or TDT sandwich pouches and heated to 65, 70, and 75°C for *Salmonella* spp. and 70, 75, and 80°C for *E. faecium,* with two replications at each time point. Samples were pulled out at selected time points, chilled in an ice bath, and plated on differential media.

Results: The D-value of *Salmonella* spp. at 65, 70, and 75°C for the TDT disk was 17.49, 10.87, and 4.09 min, respectively, and for the TDT sandwich was 44.47, 17.68, and 7.21 min, respectively. On the other hand, the D-value of *E. faecium* at 70, 75, and 80°C for the TDT disk was 27.54, 9.23, and 3.50 min, respectively, and for the TDT sandwich was 34.12, 12.01, and 4.18 min, respectively.

Significance: The consistently higher D-values in TDT sandwiches provide a conservative measurement of thermal resistance, which is appropriate for food safety validation. Additionally, the results encourages deeper investigation into possible factors such as leakage in TDT disks that could influence the results of traditional methods utilizing water or oil baths.

P2-97 Modeling Inactivation of *Salmonella* during Spray Drying

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❖ ***Developing Scientist Competitor***

Introduction: Pathogenic contamination of powdered foods and ingredients is a growing concern due to outbreaks and recalls associated with pathogens like *Salmonella* in low-moisture foods. Food manufacturers must consider how bacteria potentially survive and persist in various unit processes, such as spray drying, in order to prevent such outbreaks. Thus, modeling *Salmonella* inactivation in spray droplets will improve microbial safety modeling of the entire spray drying processes.

Purpose: This study aimed to model the inactivation of *Salmonella* during spray drying under controlled conditions in a convection oven.

Methods: A 100 ml suspension of 10% w/w soy protein isolate was inoculated with 6 ml tryptic soy broth containing *Salmonella enteritidis* PT 30 (10^9 CFU/ml). 40 µl of the suspension was pipetted onto a mesh disk (2.5 cm diameter, 160 µm mesh size, 80 µm thickness) to simulate droplets generated by spray dryer atomizers. Inoculated meshes (n=51) were placed in a pilot-scale convection oven for 5-25 s at 180, 190, and 200°C, <1% relative humidity, and ~2 m/s air velocity, then transferred into a bag containing chilled peptone water. Sample bags were ultrasonicated for 2 min to recover bacteria, plated on modified tryptic soy agar, and enumerated for survivors after incubation (48 h, 37°C).

Results: D-values for 180, 190, and 200°C were 4.6, 4.9, and 5.2 s, respectively, with RMSEs of 0.54 to 0.65 log CFU/ml. No significant difference in inactivation was found between air temperatures ($P \geq 0.05$). Recovered survivors were below detection limits after 25 s for all temperatures.

Significance: The methodology was able to estimate the instant and infinitesimal inactivation during the process of spray drying, which can be used to estimate the impact of other factors such as % solids, humidity and air velocity for secondary modeling.

P2-98 Comparison of the U.S. Food and Drug Administration's Bacteriological Analytical Manual and Metagenomic Shotgun Sequencing Methodologies in the Microbiological Isolation and Characterization of *E. coli* from Recalled Chapati "Atta" Flour

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Introduction: Flour has recently emerged as an important outbreak source for Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 and other STEC. In 2016 and 2017, wheat flour contaminated by O121:H19, O26:H11, and *E. coli* O121 was linked to three outbreaks resulting in 102 illnesses in the United States and Canada.

Purpose: Flour from a lot recalled in 2017 due to possible *E. coli* O121 contamination underwent testing using methods from the U.S. Food and Drug Administration's Bacteriological Analytical Manual (FDA BAM), shotgun sequencing, and whole genome sequencing (WGS).

Methods: Twenty subsamples from the chapati "atta" flour (20-lb bag) were placed in Whirl-Pak bags and 225 mL of broth (modified buffered peptone water with pyruvate) was added. Enrichment and subsequent plating onto three different agars proceeded per FDA BAM Chapter 4a. Enrichment aliquots underwent DNA extraction, and DNA was subjected to parallel screening by PCR and Bio-Plex suspension array screening per FDA BAM protocol and shotgun sequencing using the NextSeq 500 system for a metagenomics approach. Shotgun sequencing data analysis included microbial relative abundance, *E. coli* molecular serotyping, and virulence gene characterization. Typical-looking agar colonies from enrichments of interest were screened using the Bio-Plex and, upon a positive result, WGS.

Results: None of the 20 enriched flour subsamples were positive for *E. coli* O121 by any approach. However, we did identify *E. coli* O113 and O45 and virulence gene *eae* in enrichment subsamples #14, 20, and 8, respectively, all confirmed via PCR, Bio-Plex, and isolate WGS. Enrichment metagenomics data analysis confirmed *E. coli* virulence gene *eae* (#8), serotype O113:H2 (#14), and serotype O45:H19 (#20), even in the presence of other closely related *E. coli.*

Significance: The depth of resolution achieved in the analysis of shotgun sequencing data and its agreement with the FDA BAM method and whole genome sequencing demonstrates the potential of metagenomics as a tool to accurately detect STEC and other *E. coli* directly from enrichment in flour.

P2-99 Detection of Milk Proteins in Alkaline CIP Solutions Using High-resolution Mass Spectrometry

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Introduction: Re-use of clean-in-place (CIP) solutions can deliver environmental and economic benefits, but re-use between products containing different food allergens could potentially result in allergen cross-contact. The level of cross-contact risk associated with CIP re-use is unknown, largely due to the incompatibility of these solutions with existing allergen detection methods.

Purpose: The objective of this study was to develop a mass spectrometry method for the detection of milk proteins in alkaline CIP solutions.

Methods: Nonfat dry milk (NFDM) was analyzed by discovery proteomics. Specifically, proteins were extracted, reduced, alkylated, and digested with trypsin prior to analysis with a Q Exactive Plus Orbitrap mass spectrometer in data-dependent acquisition mode. Peptides were identified by database searching, and potential targets were selected based on several criteria. The performances of the selected peptides were evaluated in a targeted, parallel reaction monitoring method. NFDM was spiked into alkaline CIP solutions and heated to 85°C to mimic CIP conditions. Milk proteins were extracted from the alkaline solutions, and buffer exchanges of the extracts were performed using 3 KDa centrifugal filter units. Retained proteins were analyzed by discovery and targeted methods.

Results: Discovery proteomics identified 18 peptides from seven proteins as preliminary targets. A targeted method was used to analyze samples of 100 ppm NFDM in water or alkaline solutions. At room temperature, 16 of 18 peptides demonstrated similar recoveries. In heated samples (85°C, 1 h), dramatic reductions in signal (0.1 to 10% recovery) were observed under alkaline conditions, with only eight peptides detected. Additional analyses indicated heating in alkaline conditions resulted in substantial proteolysis. New, stable peptide targets (detectable after heating at 85°C for 3 h) were identified.

Significance: This method for milk protein detection in alkaline solutions will allow processors to determine the allergen risks associated with CIP chemical re-use and implement risk-based practices.

Poster

P2-100 Validation of a Rapid Immunochromatographic Method for Specific Detection of Coconut Protein in Clean-in-Place Water, Environmental Samples, and Food Matrices

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Introduction: Food allergies have increased over the years and currently affect about two percent of adults and five percent of young children, often requiring emergency room treatment, which impacts public health expenditures. Current food demand may require production facilities to manufacture both foods containing allergens and food that is expected to be allergen-free. Robust environmental monitoring and verification of allergen control measurements are essential in food safety programs. Allergic reactions to coconut protein have been reported, but are relatively rare, and the use of coconut-derived products is increasing.

Purpose: To validate an immunochromatographic method for the detection of coconut protein in food ingredients and environmental samples.

Methods: A reference material obtained from coconut milk containing 0.15% of protein was used to create a 40-ppm solution. For cross-reactivity testing, 15 coconut-free foods were analyzed in duplicate to test for interference; each of these foods was spiked with 0 and 2 ppm of coconut protein and analyzed. Performance of the method was also determined in clean-in-place (CIP) water and on 100 cm^2 of a stainless-steel surface, at 0 and 2ppm (*n*=3/sample). For lot-to-lot variability, four liquid samples were spiked with 2 ppm of coconut protein (*n*=6/sample) and non-spiked samples were used as a control (*n*=6/sample). All samples were extracted and analyzed by immunochromatography following 3M Coconut Protein Rapid kit manufacturer's instructions.

Results: No cross-reactivity or interference with any of the screened foods was determined, and all 2 ppm spiked samples, including food, CIP, and swabs were positive for coconut protein. All three kit lots had acceptable performance in liquid samples when spiked with 2 ppm.

Significance: The immunochromatographic method, provides a rapid result (~11 min) to determine the presence of coconut protein. This enables food processors to rapidly verify cleaning procedures as well as to test food ingredients and products for the presence of this allergen.

P2-101 Improved Sampling Methods for Detection of Food Allergens on Food Contact Surfaces

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Introduction: Food manufacturers rely on total protein or immunochemical tests to determine if allergens remain on processing equipment after cleaning. No uniform environmental sampling protocols for allergens currently exist.

Purpose: This project was designed to optimize the amount of allergens recovered from stainless steel surfaces during swabbing as measured with the bicinchoninic (BCA) protein assay.

Methods: This study evaluated the impact of swab material (rayon, polyester, foam), number of swabs (one, two, three), and phosphate buffered saline (PBS) swabbing buffer with and without detergent on recovery of allergens applied to surfaces with and without heat. Solutions of whole egg powder, peanut butter, and nonfat dry milk were pipetted directly onto swab heads or stainless steel plates. Plates were not dried, dried at ambient temperature, or dried with heat (65 or 80°C; 30 or 60 min) and then sampled for allergens using swabs wetted in the different swabbing buffers. PBS with 0.05% Tween 20 (PBST) was used to extract protein from swab heads, and was then analyzed for protein content with the BCA assay. Experiments were conducted in triplicate.

Results: In general, higher total protein recoveries were measured for all allergens pipetted directly onto swabs composed of rayon than other materials. Rayon swabs also recovered more protein than other swab types when allergens were sampled from stainless steel plates. Studies with milk using two rayon swabs resulted in protein recoveries of 65% (10% CV) on wet plates, 44% (14% CV) on ambiently dried plates, and 40% (8% CV) on plates heated at 80°C for 60 minutes. The highest milk protein recoveries were measured with PBST and PBS with 0.1% Tween 80 swabbing buffers, and the lowest were measured with PBS alone in single swab experiments.

Significance: Environmental sampling procedures were identified for improving allergen recovery from stainless steel food contact surfaces.

P2-102 Effectiveness of Push-through Cleaning Methods for Removing Milk Chocolate from a Stainless Steel Pipe and Butterfly Valve

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Introduction: Dark chocolate is considered a high-risk food for milk-allergic consumers when manufactured on processing lines that are also used to produce milk chocolate. Inadequate cleaning of shared chocolate processing lines can result in milk contamination of dark chocolate products.

Purpose: A study evaluated the effectiveness of push-through cleaning methods for removing milk chocolate from a contaminated stainless steel pipe and butterfly valve.

Methods: Melted milk chocolate (111 to 135 g, 85,000 ppm milk) was used to coat inner surfaces of a heated (40°C) standard (1.5-in) sanitary stainless steel pipe (30.5 cm in length) and attached butterfly valve. Milk-free dark chocolate (~27 kg, 40°C) was pumped (~140 g/sec) through the milk chocolate-contaminated pipe and valve after each of the following cleaning methods: i) no cleaning and, ii) use of a silicone pig (7.6 cm in length) to purge chocolate from the pipe. Dark chocolate samples (~300 g) collected at 7-sec intervals were homogenized and analyzed for milk concentrations by enzyme-linked immunosorbent assay (ELISA, Neogen Veratox for Total Milk). Experimental trials and ELISA analyses were conducted in triplicate. A recirculating cocoa butter (~27 kg, 40°C, 1 h) flush test was also explored.

Results: Dark chocolate push-through alone resulted in initial milk levels of ~3,200 (3.4% CV) to 6,000 (10% CV) ppm milk in samples obtained within the first few seconds of collection. After 13 to 15 kg of dark chocolate was pumped through the pipe and valve, milk levels were below the ELISA limit of quantitation (LOQ=2.5 ppm). Use of the pig dramatically reduced levels of milk in initial dark chocolate samples (110 [7.6% CV] to 180 [16%CV] ppm milk). However, ≥18 kg of dark chocolate was needed to obtain milk levels <LOQ. Recirculating cocoa butter decreased initial milk levels, but 11 (3% CV) ppm milk was detected after ~13 kg dark chocolate purge.

Significance: This study aids in development and assessment of push-through cleaning procedures for preventing allergen cross-contact.

Poster

P2-103 Investigation of Toxigenic Fungi and Mycotoxins in Baled Silage Produced in Korea

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Introduction: Silage can be spoiled by mycotoxins if forage crops were contaminated by toxigenic fungi before baling or during storage. Once silage is contaminated with mycotoxins, it is not easy to remove them, resulting in toxicosis in livestock and humans through the food chain.

Purpose: The purpose of this study was to investigate toxigenic mycobiota and mycotoxin contamination in silage produced in Korea.

Methods: A total of 219 samples of rice straw and Italian ryegrass produced from 2013 to 2017 were cut into pieces 1 by 1 cm, suspended in 0.1% peptone water, then spread onto malt extract agar to isolate and count fungal colonies. Fungal isolates were identified using PCR, by amplifying internal transcribed spacer, beta-tubulin, or translational elongation factor *1α* genes. To analyze aflatoxins (AFs, B1+B2+G1+G2), ochratoxin A (OTA), deoxynivalenol (DON), and zearalenone (ZEA), the samples were cleaned up using an immunoaffinity column, filtered through a 0.2-μm syringe filter, then injected into ultra-high performance liquid chromatography equipped with a fluorescence or UV detector.

Results: Penicillium paneum was the most prevalent species (26%, 57 of 219) found, ranging from 2.5×10^3 to 6.9×10^5 CFU/g. The next frequent species were *Fusarium fujikuroi* (4.1%, 9 of 219, 2.5×10^4 to 8.3×10^4 CFU/g) and *Aspergillus westerdijkiae* (4.1%, 9 of 219, 8.2×10^3 to 8.3×10^5 CFU/g). For mycotoxins, AFB_2 was detected most frequently in 49% of samples, ranging from 1.2 to 4.8 ppb (limit of quantification [LOQ]=0.1 ppb), followed by AFG_1 (37%, 81 of 219) ranging from 1.5 to 18.3 ppb (LOQ=1.5 ppb), and ZEA (20%, 44 of 219) ranging from 16.7 to 1,980.0 ppb (LOQ=16.7 ppb).

Significance: Several toxigenic fungi and mycotoxins were found in silages produced in Korea, but the mycotoxin levels were below the maximum limit. Constant monitoring of mycotoxin spoilage is needed.

P2-104 Rapid Detection of Added Sudan Dyes in Chilli Powder Using Magnetic Nanoparticle-based Extraction Techniques

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Introduction: The hotness and the intense red color enhance the flavor and appearance of chilli powder. Sudan dyes are synthetic coloring agents, and there is a risk that they are added to chilli powder illegally. These dyes are harmful to the human body and are thus banned in foods in many countries. In order to protect consumers, rapid and sensitive techniques should be developed to detect Sudan dyes.

Purpose: To develop a convenient, rapid, and reliable approach to detect illegal Sudan dyes in chilli powder.

Methods: Magnetic nanoparticles based on magnetic solid phase extraction (MPSE) were used to extract Sudan dyes from chilli powder samples. The adsorbent used was lab-prepared Fe_3O_4 magnetic nanoparticles coated with polystyrene (PST-MNPs), which were characterized using Fourier-transform infrared spectroscopy (FTIR), a vibrating sample magnetometer (VSM) and atomic force microscopy (AFM). The extraction process of Sudan dyes was also optimized by varying five parameters: type of desorption solvent, mass of PST-MNPs, desorption vortex time, volume of desorption solvent, and adsorption time.

Results: The presence of the peaks in the FTIR spectrum strongly indicated the presence of polystyrene and magnetite in the solid, suggesting that the coating of polystyrene on magnetite was successful and that the MNP was synthesized successfully. The saturation point of PST-MNP in the VSM curve was 0.221 emu, lower than that of uncoated Fe_3O_4 (0.380 emu). In addition, the recovery percentages (from 74.1 to 106%) and the relative standard deviation (RSD, <17.4%) were satisfactory for testing the spiked samples. According to the results, the proposed method is a convenient, rapid, and reliable approach for Sudan dye analysis in chilli powder and is promising to be applied for other food analysis as well.

Significance: The study proposed a promising approach for detecting illegal dye additives in chilli powder.

P2-105 Background Monitoring of Nonylphenol and Bisphenol a Levels in Foods around Taiwan and BPA Migrating Test from Packaging Materials of Coffee Products

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❖ ***Developing Scientist Competitor***

Introduction: Nonylphenol (NP) and bisphenol A (BPA) were classified as endocrine-disrupting chemicals and have raised public attention because of their potential adverse effects on human and environmental health and their wide occurrence in various environments. Monitoring of food contaminated with BPA and NP is an important issue to consumer risk assessments. However, data on the occurrence of NP and BPA in Taiwanese foodstuffs are sporadic and rarely addressed.

Purpose: The present study was aimed to investigate the background levels of NP and BPA in local foodstuffs. The migration of BPA was also conducted with different packaging materials of coffee products.

Methods: A total of 278 food samples from 13 categories were collected from the township with the highest food production according to the statistics published by the Korean Council of Agriculture and Fisheries Department. All food samples were extracted by acetonitrile, purified by Florisil or Oasis HLB cartridge, and then analyzed by high-performance liquid chromatography mass spectrometry. In addition, the BPA migration levels were compared in black and latte coffee packaged with different materials, as well as stored at different temperatures and times.

Results: Concentrations of NP and BPA in all food samples ranged from 0.97 to 98.4 μg/kg wet weight (ww) and ND to 14.0 μg/kg ww. Other sea foods such as shrimp contained the highest NP levels, followed by fish and canned food. The highest level of BPA was found in canned food, followed by oils and beverages. Our data also suggested that high-fat foods had lower NP concentration and higher BPA concentration in our investigated foodstuffs. There was a higher BPA concentration found in canned food, in which high-fat categories had higher BPA concentration. The data were consisted with other studies. For the BPA migration test from packaging materials of coffee products, the highest BPA level was found in high-caffeine coffee product when stored at 50°C.

Significance: The apparent higher levels of NP were detected in high-fat foods, with the exception of crustaceans. BPA migration increased with the level of caffeine and storage temperature of coffee products.

Poster

P2-106 Development of Monoclonal Antibody Specific to Thermal Stable-soluble Protein in Egg Whites as a Food Allergen

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Introduction: Food allergies are recognized as an important public health issue all over the world. Egg is one of the eight major allergens that must be listed on packaged foods sold in the United States and the most common allergenic food in infants and young children. The presence of food allergens in processed foods can cause fatal reactions in consumers. Therefore, food manufactures have established an appropriate cleaning process to remove allergens during processing operations and from equipment. Additionally, analytical methods have been developed for monitoring the cleaning effectiveness and the presence of food allergens in processed foods.

Purpose: The objectives of this study are to investigate the existence of thermal stable-soluble protein (TSSP) in egg white and to use TSSP as an immunogen for the development of monoclonal antibodies (MAbs).

Methods: Effective extraction conditions for TSSP were optimized using various buffers. Boiled and roasted peanuts were subjected to extract TSSP, and the TSSP extracted was used as an immunogen to develop MAbs specific to egg white protein. Hybridoma cells producing MAbs were developed by cell fusion and cloning. Enzyme-linked immunosorbent assay (ELISA) and western blot analyses were performed to characterize the MAbs.

Results: Results obtained from protein assay and sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis confirmed the presence of TSSP in peanuts. Seven hybridoma cell lines (MAbs; EWB 4A9-1, 4A9-2, 4A9-3, 4A9-4, 2E6-9, 3F8-11, and 2D10-12) were developed by cell fusion and cloning. The MAbs secreted from the hybridoma cells were confirmed to be specific to egg white TSSP and did not show cross-reaction with other food allergens in indirect ELISA and western blot analyses.

Significance: Since the seven MAbs are specific to TSSP of egg white, the MAbs can be used as a bioreceptor to develop immunoassay for the detection of egg white in processed foods.

P2-107 Concentrations of Perfluoroalkyl Substances, Phthalate Esters, Gallium, and Indium in Food

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Introduction: Perfluoroalkyl substances (PFASs) and phthalate esters (PAEs) are widely used in consumer and industrial products. Gallium and indium are common ingredients in the industries of semi-conductors and liquid crystal panels. Humans are exposed to these chemicals mainly through consuming contaminated food.

Purpose: This study measured the concentrations of ten PFASs (PFBA, PFPeA, PFHxA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFHxs, and PFOS), six PAEs (DEP, BBP, DEHP, DNOP, DINP, and DIDP), gallium, and indium in pork, pork liver, pork kidney, fish (tilapia and milkfish), clams, and oysters.

Methods: One-gram homogenized samples were processed using the QuEChERS method ("Quick, Easy, Cheap, Effective, Rugged, and Safe") and EMR-Lipid cleanup for analyzing the organics by ultra-performance liquid chromatography - tandem mass spectrometry with isotope-dilution techniques. Regarding metal analysis, 1-g samples were mixed with 5 ml nitric acid for microwave digestion; gallium and indium were determined with intact cell matrix-assisted laser desorption/ionization mass spectrometry.

Results: Food samples (*n*=3 of each item, 21 total) were purchased from local markets in Hsinchu City, Taiwan in June, 2017. Short-chain PFASs, such as PFBA, PFPeA, PFHxA, and PFOA were not observed in any sample. Geometric means (GM) of other PFASs in pork liver ranged 0.21 to 2.93 ng/g, with the highest being PFOS at 42.2 ng/g. DINP was detected in all foods (GM=9.4 to 261 ng/g) except for tilapia, and the two highest concentrations were 908 and 386 ng/g for pork kidney and oyster, respectively. DIDP was also found in most samples but was at lower concentrations (GM=4.2 to 10.8 ng/g). The concentrations of gallium in clams (GM=49.5 ng/g) and oysters (GM=18.3 ng/g) were much higher than other foods (GM=0.98 to 2.52 ng/g). Indium in seafood (GM=0.56 to 0.83 ng/g) was also higher than in pork, liver, and kidney (GM=0.37 to 0.47 ng/g).

Significance: Indium concentrations in shellfish can be a traced to contamination from the high-tech industry. Liver may accumulate much more PFASs and PAEs than muscle.

P2-108 Doses of Specific Peanut Allergens in Bamba

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Introduction: The "Learning Early About Peanut" (LEAP) study showed that peanut consumption in early life dramatically reduced the prevalence of peanut allergy among high-risk children. The preferred peanut snack used for the study was Bamba, a corn puff containing ~50% peanut.

Purpose: Our objectives were to compare Ara h 1, Ara h 2, Ara h 3, Ara h 6, and Ara h 8 levels in Bamba and to estimate the weekly doses of specific peanut allergens associated with oral tolerance.

Methods: Extracts of Bamba (100 mg/sample) from either the United Kingdom (UK, *n*=8) or United States (U.S., *n*=8) and various types of the German peanut snack ErdnußLocken (*n*=10), or 25 individual Bamba sticks, were analyzed by enzyme-linked immunosorbent assay for Ara h 1, Ara h 2, Ara h 3, Ara h 6, and Ara h 8 using natural or recombinant allergen standards. Allergen consumption in the LEAP study was estimated by calculating the amount of allergen per Bamba stick.

Results: The absolute amounts of peanut allergens in Bamba were remarkably consistent between the U.S. and UK. The German ErdnußLocken had three- to four-fold lower amounts of peanut allergen compared to Bamba. Individual Bamba sticks contained similar levels of specific peanut allergens. Median weekly doses of allergens were calculated based on consumption of 80 Bamba sticks (equivalent to 7.7 g of peanut protein).

Significance: Unlike other peanut food products, Bamba is a reproducible and consistent formulation of peanut allergens. The five peanut allergens are present in uniform amounts in Bamba, but vary greatly in level and composition in peanut foods. For the first time, the results provide target doses of specific peanut allergens associated with prevention of peanut allergy, which could apply to the induction of tolerance to other food allergens. Specific allergen content should be considered when using food formulations or specific foods for clinical trials of oral immunotherapy or prophylactic interventions.

Poster

P2-109 The Enzymatic Detoxification of Deoxynivalenol Via Epimerization

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Introduction: Deoxynivalenol (DON) is a mycotoxin produced by various fungal species which infect grains such as wheat, barley, and maize and has negative consequences on human health, having been shown to impair immune and gastrointestinal systems. High levels of DON are often found in milled wheat and corn products, such as breakfast cereals produced from brans.

Purpose: As DON is extremely heat stable and resistant to chemical degradation, biological detoxification is developed as a practical approach to mitigate DON contamination.

Methods: *Devosia mutans* 17-2-E-8, a bacterial isolate which transforms DON to the stereoisomer 3-*epi*-deoxynivalenol, has been studied for possible detoxification mechanism(s) and enzyme(s) involved.

Results: Our study has showed that DON epimerization proceeds through a two-step pathway; DON is transformed to 3-keto-DON and subsequently transformed to 3-*epi*-DON, a compound with at least 50-fold less toxicity than DON. The enzymes from the DON epimerization pathway are investigated to develop a system for reducing DON contamination in corn products. It is demonstrated that the enzymes in this system are stable at moderately high temperatures (50 to 60°C) and show great potential to be incorporated into the processing of cereals.

Significance: Further investigation of these enzymes and their ability to detoxify DON will help improve food safety by reducing the amount of toxic DON in foods.

P2-110 Development and Characterization of a Novel Monoclonal Antibody-based Sandwich Enzyme-linked Immunosorbent Assay for the Quantitative Detection of Lupin

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Introduction: Lupin (or lupine), a legume belonging to the same plant family as peanuts and soybeans, can serve as a rich source of protein and is increasingly used in bakery, confectionery, soup, snack, and gluten-free products. European Union (EU) directive 2007/68/EG and EU regulation No 1169/2011 identify lupin as one of Europe's 14 allergens to regulate, and mandatory declaration of lupin in Australia and New Zealand is scheduled to begin in 2018.

Purpose: The purpose of this study is: i) to develop a sensitive and specific monoclonal antibody-based sandwich enzyme-linked immunoassay (sELISA) for the detection of lupin, ii) to determine the cross-reactivity of this assay against a large panel of legumes, cereals, tree nuts, seeds, meats, and spices, and iii) to assess the assay's performance in detecting various strains of lupin in raw ingredients, processed foods, and quality-control materials.

Methods: The lupin sELISA assay limit of detection (LOD, 0.13 ppm) was calculated by testing standards made from lupin flour. Cross-reactivity was tested using a large panel of commercial samples from various vendors (*n*=116). Recovery from various food matrices and ingredients (*n*=31) and quality-control materials (*n*=4) was assessed.

Results: The LOD of the assay was calculated statistically to be 0.13 ppm and the range of quantification was determined to be 1 to 40 ppm lupin. The assay equally recognized the three major cultivars of lupin (*Lupinus albus*, *L. angustifolius*, and *L. luteus*), and no cross-reactivity was observed with other legumes (*n*=23). Recovery of lupin from a variety of food matrices and quality-control material was considered ideal (80 to 120%).

Significance: The MonoTrace Lupin ELISA represents a novel method for detecting a broad variety of lupin strains within raw food ingredients and processed foods. The assay is sensitive, specific for commercially important lupin species, and has a wide dynamic range.

P2-111 Development and Validation of a Quantitative Monoclonal Antibody-based Enzyme-linked Immunosorbent Assay for the Detection of Mustard in Differentially Processed Commercial Products

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Introduction: Mustard and cruciferous vegetables such as broccoli, cabbage, kale, collards, turnip, radish, and rapeseed are members of the *Brassicaceae* family. European Union (EU) regulation No 1169/2011 lists mustard as one of 14 allergens to regulate. The detection of mustard can be incomplete due to processing methods commonly used to prepare commercial products.

Purpose: The objectives of our study were to: i) develop a quantitative monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) for the detection of yellow, brown, and black mustard, and ii) validate the assay for sensitivity, recovery, and cross-reactivity (CR) against a large panel of cereals, tree nuts, legumes, seeds, meats, and spices.

Methods: Monoclonal antibodies (MAbs) were generated by BioFront Technologies and screened for reactivity to yellow, brown, and black mustard seed. A sandwich ELISA was developed using a pair of MAbs that efficiently detected denatured mustard protein. Internal validations were performed using external proficiency test and reference materials (*n*=6), as well as in-house and commercial samples (*n*=118) to determine key assay parameters such as sensitivity, recovery, and CR.

Results: The limit of detection for the assay was calculated to be 0.20 ppm, while the range of quantitation was determined to be 1 to 40 ppm mustard seed powder. Yellow, brown, and black mustard seed were equally recognized by the assay. Recovery from incurred and processed samples was satisfactory (80 to 120%) according to AOAC guidelines. The assay was shown to be detect other members of the *Brassicaceae* family, including rapeseed, cabbage seed, and radish.

Significance: The MAbs selected for the sandwich ELISA can capture and detect native or denatured mustard protein. The mustard ELISA represents a new method for detecting mustard contamination within raw food ingredients and, more importantly, processed foods such as condiments. The assay is sensitive, has a wide dynamic range, and is highly specific to mustard and other members of the *Brassicaceae* family which may cause allergic reactions.

P2-112 Withdrawn

P2-113 The Compliance Level of Pesticide Residues within the Canadian Marketplace

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Introduction: The Canadian Food Inspection Agency (CFIA) is committed to its mission to safeguard food, animals, and plants to enhance the health and well-being of Canada's people, environment and economy. The CFIA delivers inspection services to prevent and manage food safety risks and contribute to market access for Canadian agricultural products.

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Purpose: The National Chemical Residue Monitoring Program (NCRMP) is a valuable surveillance tool that helps the CFIA verify that food products are safe, and identify potential hazards in relation to chemical residues and contaminants. This allows the CFIA to take proactive measures to improve food safety and helps industry comply with the Canadian *Food and Drugs Act and Regulations*.

Methods: Yearly sample plans were created based on Codex Alimentarius principles that determine and prioritize potential chemical residue hazards within the Canadian Marketplace. The samples were collected by CFIA inspectors from federally registered establishments across Canada, and include both domestic and imported products. Each sample was tested at contracted third party laboratories across Canada which are ISO 17025 accredited. Results for pesticide residue levels were then assessed against Canadian standards and guidelines set by Health Canada.

Results: Between April 2015 and March 2016, a total of 12,325 samples were collected under the NCRMP from registered establishment across Canada. These included food commodities such as Dairy (481), Eggs (780), Fresh Fruits and Vegetables (4,867), Honey (239), Meat (5,630), and Processed Fruits and Vegetables (328). A total of 283 samples contained pesticide residues above the Canadian standards. The total compliance rate for pesticides residues for samples collected under the NCRMP during this period was 97.7%.

Significance: The data suggests that pesticide residues within the Canadian Marketplace have a high compliance rate with Canadian regulations, and that food is safe for consumers.

P2-114 The Political Economy of Antibiotics in Animal Feed

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Introduction: In 1969, a report from the United Kingdom documented that low levels of antibiotics in animal feed were creating antibiotic-resistant (AR) pathogens. In 2016, the U.S. Food and Drug Administration began to regulate antibiotics in animal feed. In an economic framework, AR is an unwanted side effect, or externality, associated with the use of antibiotics. The efficacy of antibiotics can be considered as a public good that must be managed with government involvement. This is because the costs of AR created by a single individual or farm is borne by society, and in the case of antibiotics, the global society.

Purpose: To evaluate the difference in costs between hog farms and chicken farms that did and did not use antibiotics in animal feed.

Methods: The United State Department of Agriculture's (USDA) Economic Research Service (ERS) used Agricultural Resource Management Survey data to evaluate the difference in costs between hog farms and chicken farms that did and did not use antibiotics in animal feed. Bowman and co-authors also examined the use of consumer labels on chicken and related chicken sales.

Results: ERS found that hog and chicken farms using antibiotics in animal feed did not have lower costs of production. In a benefit/cost analysis, the cost of withdrawing antibiotics is zero, but the public health protection benefit is conservatively estimated at $7 billion per year. The Centers for Disease Control and Prevention's (CDC) $7 billion estimate is conservative because it only includes medical expenses and longer treatment of AR illnesses and time lost from work. The benefits would be higher if CDC included long-term health outcomes for these AR illnesses and included the increased protection from AR "bugs" that are becoming more frequent globally. As for consumer labels, Tysons and Perdue initially had a USDA Food Safety Inspection Service-approved label of "Raised without antibiotics". Sanderson Farms took the two companies to court and won against Tysons in 2008. Perdue has new label for its chicken ("No antibiotics ever") and has seen sales grow by 10 to 20% annually, while conventional chicken sales are increasing by not more than 3% annually

Significance: There are different costs and benefits associated with the use and non-use of antibiotics in animal feed.

P2-115 Food Safety Enforcement and Regulation in Ghana: Current Situation and Future Outlook

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Introduction: Ghana is a developing country in West Africa. As in developed countries, all food products and commodities must obtain market authorization before being imported, locally manufactured, or sold/distributed in the country. The Food and Drugs Authority (FDA) is the organization mandated to ensure that this system works effectively, backed by the Public Health Act, Act 851 (2012).

Purpose: To describe the current role and responsibilities of the Ghana FDA and plans to address the challenges faced in food safety.

Methods: Inspections and audits of processing facilities and storage facilities for imported foods (key in tropical conditions conducive to bacterial) are conducted to ensure safety from farm to fork, with enforced codes based on good gygiene and manufacturing practices (GHP and GMP), along with food evaluation and registration for locally manufactured and imported products. A database of all registered food products is maintained so that unregistered products can be easily identified during post-market surveillance, which occasionally leads to the FDA supervising safe disposal to ensure non-conforming products do not enter the markets. Vetting and monitoring activities are carried out through radio, television, and print media to ensure food advertisement claims are substantiated and violators of the law are sanctioned.

Results: As the economy of Ghana grows, the FDA meets challenges such as lack of adequate equipment and insufficient number of staff. There are also overlapping roles and responsibilities with other agencies involved in food safety regulation that need to be addressed. To combat these problems, the FDA is considering procuring additional vehicles for officers to use, and a new National Food Safety Policy is in the draft stage and soon to be completed. A new challenge is excessive alcohol consumption, which is being addressed through press releases.

Significance: The Ghana FDA is continuing to progress to meet the food safety needs of the country.

P2-116 Lethality of *Salmonella* spp., *Escherichia coli*, and *Listeria monocytogenes* during Ketchup Processing

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Introduction: Recent recalls and outbreaks due to foodborne pathogens in thermally processed foods highlight the need for food industries to validate their thermal processes.

Purpose: Evaluation of the thermal inactivation kinetics of *Salmonella* spp., *Escherichia coli*, and *Listeria monocytogenes* during ketchup processing.

Methods: Two ketchup formulations (A and B) were inoculated with *Salmonella* spp. (five-strain cocktail), *E. coli* (O157:H7, and "Big 6" non-O157 Shiga toxin-producing *E. coli* [STEC] serotypes), and *L. monocytogenes* (five-strain cocktail) in separate trials. Samples were placed in a water bath set at 165±2°F (73.9°C) (set temperature represents conservative worse-case scenario) and allowed to come up to the minimum temperature (163°F) before starting the treatment. Samples were treated at temperature and pulled at time points of 2.5, 5, 7.5 and 10 min and were immediately placed in an ice bath to stop further heating. All the experiments constituted three replicates. Samples inoculated with *Salmonella* spp., *E. coli*, and *L. monocytogenes* were enumerated using microbiological methods approved by the U.S. Food and Drug Administration. Data was log-transformed and analysis of variance was performed to determine parameters that deliver a minimum of 5-log reduction in *Salmonella* spp., *E. coli*, and *L. monocytogenes*.

Results: Both formulas A and B achieved ≥6-log reduction ($P<0.05$) of *Salmonella*, *E. coli*, and *L. monocytogenes* after a treatment of 2.5 min. Formula A varied from Formula B with regards to titratable acidity (%), sodium chloride, pH value, and brix. However, this did not significantly ($P>0.05$) affect thermal inactivation kinetics of all evaluated pathogens.

Significance: The study findings provide scientific basis that the thermal process (175°F for 25 min) employed by Conagra Brands achieves an acceptable lethality of vegetative pathogens. Because this study indicates that production of a saleable product will result in achievement of adequate food safety parameters, this work supports the management of thermal processing as an operational pre-requisite program (not a critical control point) in a facility's food safety plan.

P2-117 Microbiological Growth Profile of *Staphylococcus aureus* and *Bacillus cereus* in High-moisture Foods during Routine Manufacturing Conditions

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Introduction: High-moisture foods with water activity (aw) greater than 0.91 and a pH value between 4.5 and 9.6 may permit growth of *Staphylococcus aureus* and *Bacillus cereus* and therefore may also allow for potentially heat-stable enterotoxins. Compliance with the U.S. Food and Drug Administration's Food Safety Modernization Act (FDA, FSMA) 21 CFR Part 117, Subpart B and Section 117.8 (c)(2) and (c)(3) requires performing a hazard analysis to determine if preventive controls are needed.

Purpose: The purpose of this study was to perform hazard analysis of high-moisture foods during routine production conditions from a food safety standpoint.

Methods: Two formulations were evaluated: i) pumpkin pie mix slurry, and ii) dairy-based slurry used in the production of table spreads. Samples were individually inoculated with multiple strains of *S. aureus* and *B. cereus* to achieve a target level of ~10^2 CFU/g. For the pumpkin pie mix slurries, samples were stored at 12.8, 18.3, and 23.9°C and sampled following 0 to 7 days of storage. For the dairy-based slurries, samples were stored at 20 and 35°C and sampled following 0 to 10 days of storage. Samples were enumerated for *S. aureus* and *B. cereus* using FDA-approved microbiological methods. The limit of food safety was defined as 10^5 CFU/g for both *S. aureus* and *B. cereus*. Analysis of variance ($\alpha=0.05$) was performed to understand the effect of storage temperature and time on the growth profile of *S. aureus* and *B. cereus*.

Results: Neither *S. aureus* nor *B. cereus* exceeded ($P<0.05$) the limit of food safety at the evaluated experimental conditions. For the pumpkin pie mix slurry, this may be attributed to "competitive inhibition" due to the presence of native microflora. For the dairy-based slurry, this may be attributed to the presence of preservatives potassium sorbate and sodium benzoate.

Significance: The study findings indicate no significant food safety risk associated with the current production practices. The data generated in this study provides scientific basis for the facility's food safety plan in compliance with FSMA guidelines.

P2-118 Comparison of Quantitative PCR and Crystal Diagnostic Immunoassay-based Method for Studying the Distribution of *Salmonella* and Shiga Toxin-producing *Escherichia coli* in the Air of Beef Abattoirs

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❖ ***Developing Scientist Competitor***

Introduction: Bioaerosols containing bacterial pathogens are a great concern in the meat industry, because air currents may transport these pathogens from high-movement areas to "clean" areas such as fabrication rooms and chillers. To detect pathogens, using reliable sampling and detection methods is paramount.

Purpose: Compare the effectiveness of quantitative PCR (qPCR) and CDx for detecting *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC) in air samples collected from abattoirs in Texas.

Methods: Air samples were collected from two small and two large plants in fall, spring, and summer seasons using a wall-wetted cyclone air sampler. The samples were divided equally into two parts; one part was used for real-time PCR testing, and the other part was enriched for 18 to 36 h and used for CDx testing. All samples thaht tested positive were confirmed by plating and characterization methods.

Results: Salmonella and STEC were found in 37.5 and 65.0%, respectively, of the 70 samples when using qPCR. When using CDx, these pathogens were found in 57.1 and 60.7% of the 70 samples ($P>0.05$). Air samples required longer enrichment time than is recommended for food samples. A recovery increase of 16 and 47% was obtained for *Salmonella* and STEC between 18-h and 36-h enrichment. The incidence of *Salmonella* and STEC was affected by the season, size of the plant, and process stage and was significantly higher during summer. The larger the plant size, the higher the frequency of both pathogens. Significantly higher frequency of positives was obtained at stunning and dehiding areas compared to fabrication rooms and chillers.

Significance: Detecting pathogens in bioaerosols at abattoirs can be enhanced by collecting large air samples. This study showed the potential for occasional transfer of pathogens from the air in highly busy areas to fabrication rooms and chillers. More evaluation of methods is needed for detecting pathogens in environmental samples.

P2-119 Modeling the Effect of Temperature on the Growth of *Staphylococcus aureus* in Fresh-cut Lettuce

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Introduction: Staphylococcus aureus is one of the major foodborne pathogens, causing a high number of illnesses linked to the consumption of raw lettuce in Taiwan. From harvest to consumption, raw lettuce is exposed to temperatures ranging from chilled storage to room temperature, which could reach as high as 40°C.

Purpose: The objective was to develop mathematical models to predict the growth of *S. aureus* in lettuce as affected by temperature throughout the supply chain in order to assess the associated risks to consumers in Taiwan.

Methods: S. aureus isolated from leafy salad sold in local markets was inoculated onto pre-cut lettuce to achieve an initial inoculation level of 4 to 5 log CFU/g. The inoculated lettuce was stored at 4, 18, 25, 30, and 40°C to observe the growth of *S. aureus*. The populations of *S. aureus* during storage

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were determined, and the growth curves were analyzed using United States Department of Agriculture Integrated Pathogen Modeling Program (IPMP) to develop predictive models. The models were validated with additional samples stored at 35°C.

Results: Among the several models evaluated, no lag phase model, Huang rate model, and polynomial model were more suitable for describing the bacterial growth and estimating the maximum specific growth rate (μ_{max}) and maximum growth population (Y_{max}) of *S. aureus* in lettuce, with A_f and B_f of μ_{max} very close to 1.0 and root-mean-square error of the growth models within a reliable range. The secondary model showed that the estimated minimum growth temperature (T_{min}) was approximately 7.9°C, which is in agreement with the reported T_{min} for *S. aureus* (8°C).

Significance: These mathematical models were validated and acceptable for predicting the growth of *S. aureus* in lettuce as affected by temperatures and could be used for assessing the potential risks of *S. aureus* in lettuce within the entire food supply chain.

P2-120 Statistical Process Control Systems for Assessing and Responding to Preharvest, Postharvest, and Processing Plant Pathogen Testing

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❖ ***Developing Scientist Competitor***

Introduction: In most food production pathogen testing scenarios, a simple prevalence calculation is insufficient to answer the underlying question: Is the production system in control vis-à-vis pathogen prevalence? It is therefore inadequate as a tool to inform decision-making. Biological systems are inherently prone to noise, and statistical solutions are necessary for tracking a signal within that noise. Statistical process control (SPC) systems flag changes in pathogen prevalence that represent true departures from a baseline, as opposed to random variation around it.

Purpose: To reduce the risk of foodborne pathogen transmission by marrying statistical control techniques to pathogen-related monitoring and response systems.

Methods: We develop a multi-tier SPC system consisting of primary and secondary SPC algorithms. The primary SPC system is built on previously published methods common to manufacturing processes and identifies short-term spikes, as well as slower long-term changes. The secondary SPC system utilizes a machine-learning (ML) approach that ingests historical data and is tuned to identify emerging problems in a firm-specific framework. This approach recognizes that live production and processing pathogen ecosystems vary by firm and product type and can change and evolve over time within a specific firm and product type.

Results: Pathogen test results are binned into groups (hourly/daily/weekly/monthly), based on sample size. The resulting groups are compared against a historically derived baseline and adjusted for the exact sample size to determine if the prevalence meets or exceeds the short-term statistical threshold. Bins are cumulatively compared against a long-term statistical threshold to detect gradual deviations resulting from persistent (but small) short-term fluctuations. Baselines are periodically recalculated to continue to monitor for outliers, even when the overall process mean shifts.

Significance: Statistical monitoring techniques enable firms to more efficiently allocate production resources related to pathogen monitoring and resulting actions, reducing the overall risk posed by pathogen transmission in a targeted manner.

P2-121 Consumers' Perception of Food Safety of Perishable Foods Sold at Northern West Virginia and Western Pennsylvania Farmers Markets

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❖ ***Developing Scientist Competitor***

Introduction: There is growing interest in purchasing fresh foods at local farmers markets, but there are microbial safety concerns regarding locally grown foods.

Purpose: This study was to determine consumers' awareness of, knowledge of, and attitude toward the safety of locally grown, fresh produce sold at local farmers markets in the eastern United States.

Methods: Surveys were conducted through face-to-face questionnaire interviews with consumers at two farmers markets in northern West Virginia (WV) and two farmers markets in western Pennsylvania (PA) from August to November of 2017. Questions included basic information of age, gender, and education level, frequency of purchasing, handling of perishable foods, and food microbial safety concerns. Data from approximately 190 completed surveys were analyzed using JMP software to compare the similarities and differences between locations. Descriptive statistics were calculated for each variable, and chi-square tests of independence were employed to examine bivariate relationships between categorical variables with statistical significance set at P=0.05.

Results: Results showed that 88.4% of participants always wash hands before preparing foods at home and 80% of them always wash produce, with no significant difference (P>0.05) across the four farmers markets. Only 28.57% of all participants from the four farmers markets were very concerned about microbial safety, with the highest (P<0.05) concern rate from participants in Canonsburg, PA (37%). Although 63.5% of the participants know the time to cause bacterial foodborne illness, only 46.52% of them refrigerate their purchased foods within 30 min, with the highest (P<0.05) rate at Canonsburg, PA (67.39%) and lowest (P<0.05) rate shown at Morgantown, WV (32.61%). The majority of participants (62.31%) believe that farmers and vendors are the most responsible for the safety of products sold at farmers markets.

Significance: A better understanding of consumers' perceptions allows stakeholders, including local and state government agencies, growers, and vendors, to make better-informed decisions regarding food safety policies and practices of locally grown food.

P2-122 The Prevalence of Shiga Toxin-producing *Escherichia coli* and *Salmonella* on Sheep Hides, Pre-eviscerated and Final Carcasses in Various Honduran Slaughter Facilities

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* cause foodborne illnesses on meat animal carcasses that are linked to fecal contamination and poor hygiene. Developing countries lack baseline research on these common foodborne pathogens. Minimal research is available for sheep slaughter and processing in both developed and developing countries.

Purpose: The objective of this study was to evaluate the prevalence of STEC 0157, non-O157, and *Salmonella* on Honduran sheep hides, as well as pre-eviscerated and final carcasses.

Methods: Sponge swabs from 58 sheep were collected from two Honduran slaughter facilities. Swabs were aseptically collected from 100-cm^2 sections of the brisket region on hide, pre-eviscerated, and final carcasses. Microbial detection of STEC was performed using the BAX system and confirmed through biochemical tests. Statistical analysis was determined for differences based on a P value of 0.05.

Results: ***Salmonella*** contamination significantly decreased from 21.74 to 10.50% on hides to pre-eviscerated carcasses, respectively (P=0.034). Moreover, there was no detectable *Salmonella* contamination observed on final carcasses. Similarly, STEC contamination decreased throughout the slaughter processing chain. Hide contamination was measurably increased from pre-evisceration to final carcass processing (P=0.034 and P<0.01, respectively). Statistically, no measurable difference was calculated between pre-eviscerated and final carcass STEC prevalence (P=0.167). Therefore, intervention methods currently used in harvesting facilities are reducing *Salmonella* and STEC prevalence throughout the processing chain; however, hygiene and sanitation measures must be implemented to eliminate overall pathogen contamination.

Significance: Collecting science-based evidence of contaminated products throughout sheep slaughter processing chains will create baseline regulatory guidelines specific to developing countries such as Honduras. Implementation of these recommendations for Honduran sheep slaughter facilities can effectively improve food safety, resulting in decreased pathogen prevalence and safer, more wholesome products.

P2-123 Effect of Bacteria on Bleach Inactivation of Human Norovirus Surrogates on Stainless Steel Surfaces

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Introduction: Human norovirus (hNoV) can be transmitted by food, leading to acute gastroenteritis. On food contact surfaces such as stainless steel (SS), bacteria and hNoV are possibly present at the same time. Moreover, hNoV can readily associate with bacteria, including *Bacillus cereus* and *Enterobacter cloacae*. However, the effect of bacteria-virus interactions on virus properties, including bleach inactivation, is unknown.

Purpose: This study investigated whether *B. cereus* and *E. cloacae* affect the resistance of hNoV surrogates murine norovirus (MNV), Tulane virus (TuV), and Aichi virus (AiV) against bleach inactivation on SS surfaces.

Methods: A mixture of 10^5 CFU/ml bacteria (*B. cereus* or *E. cloacae*) and 10^6 PFU/ml hNoV surrogates (MNV, TuV, or AiV) was prepared and placed on SS surface and air dried. Afterwards, 1,000 ppm bleach was applied for 5 min and stopped by neutralizer (minimum essential medium with 0.1% thiosulfate and 10% fetal bovine serum). An antibiotic (imipenem) was then added to inactivate any remaining bacteria. Viruses were recovered from the surface using a cell scraper and quantified by plaque assay.

Results: Virus-bacteria association had no statistically significant effect (P>0.05) on virus inactivation compared to the virus alone group. The highest reduction was observed for TuV, followed by MNV and AiV. Specifically, exposure to bleach resulted in an average of 3.86, 2.14, and 0.94 log PFU/ml reductions for TuV, MNV, and AiV without bacteria, respectively. The reductions were 2.57, 1.68, and 1.32 log PFU/ml with *B. cereus* present and 3.50, 1.88, and 0.61 log PFU/ml with *E. cloacae* present for TuV, MNV, and AiV, respectively.

Significance: The results allow for a better understanding of virus-bacteria interactions with respect to surface disinfection. Overall, the presence of bacteria showed no significant effect on hNoV surrogates under tested conditions.

P2-124 The South African Food System: Regulation and Control in the Context of the Recent *Listeria* Outbreak

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Introduction: The recent *Listeria monocytogenes* foodborne outbreak has shifted attention to the soundness of the South African food system. The country has been a major exporter of fresh fruit and has well-established links in the global food trade network. However, the South African food system has not adopted regulatory changes that effectively reflect the changing food safety trends in testing and assurance. A sharp increase in recent legal and illegal food imports have further contributed to the compromised integrity of the food system due to ineffective border control and inspection services.

Purpose: This paper presents an overview of the South African food system in the context of the recent *Listeria* outbreak and reflects on 10 years of food safety research.

Methods: A country profile study was done based on the World Health Organisation's assessment tools to determine the food landscape and regulatory profile of the country. Fresh produce and environmental samples (n=4,000) were collected over a period of eight years to assess the integrity of the food system. Fresh produce and processed or minimally processed foods were obtained from the formal and informal markets. Indicator organisms including coliforms, *Escherichia coli*, and *Enterobacteriaceae* were enumerated. Detection of *E. coli*, *Staphylococcus, Listeria*, and *Salmonella* species were conducted using selective media and enrichment. Presumptive isolates were confirmed using matrix-assisted laser desorption/ionization-time of flight and conventional PCR or quantitative PCR and droplet digital PCR.

Results: The regulatory framework reflects a compromised food system with gaps and overlaps, as well as inadequate inspection services and laboratory capacity. Counts of coliforms exceeded the specified limit (2.3 log CFU/g) and *E. coli* was detected in some samples. A low prevalence of pathogenic *Salmonella* and *Listeria* spp. were evident in the food system.

Significance: This study highlights the need for improved food safety regulations and control. A food safety regulatory body is urgently required, as well as improved laboratory capacity and inspection services.

P2-125 Detachment Kinetics of *Escherichia coli* O157:H7 and Non-living Surrogate from Surface of Spinach

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Introduction: Sanitization of fresh-cut produce at processing plants is a vital step in reducing pathogenic bacterial populations and improving shelf life. To validate the commercial fresh produce sanitation process, inoculation of raw materials with viable bacterial marker strains is desirable, but not practical. Recently, a class of abiotic surrogates of living bacteria, which are composed of specific short DNA sequences embedded in food grade matrices, termed SaniTracers, have been developed and tested for applications in sanitation process validation. SaniTracers can be quantified using reverse transcription quantitative PCR (RT-qPCR).

Purpose: The purpose of this study was to compare the detachment kinetics of potential bacterial surrogate SaniTracer to the detachment kinetics of *Escherichia coli* O157:H7 on spinach leaves.

Methods: Spinach leaves were co-inoculated with comparable quantities of *E. coli* O157:H7 and SaniTracer suspensions. Inoculated samples were dried at 4°C, then washed in water containing 0, 5, 15, and 50 ppm free chlorine at incremental time intervals over a 10-min period. After washing,

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SaniTracer and viable *E. coli* O157:H7 left on spinach were quantified by a specifically designed qPCR assay and plating on sorbitol MacConkey agar with cefixime and tellurite, respectively.

Results: There was a correlation between the detachment of SaniTracer and *E. coli* O157:H7 from the spinach leaves in various concentrations of chlorinated water. Both surrogate and *E. coli* O157:H7 followed a trend where remaining counts on spinach were inversely correlated to the increase in wash water chlorine concentration and wash time.

Significance: The similar rates of decontamination support the potential of this non-living bacterial surrogate to be used to validate produce wash systems for pathogen inactivation.

P2-126 Evaluation of Different Postharvest Cooling Processes on the Microbial Quality and Storage of Florida Peaches

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✦ ***Undergraduate Student Award Competitor***

Introduction: Florida peaches are currently picked and held overnight in a cold room (room cooling [RC]), and then packed and shipped on the next day. This research evaluated forced-air cooling (FAC) and hydrocooling (HC) as alternatives to RC.

Purpose: Determine and evaluate the efficacy of different postharvest cooling processes on microbial quality control of peaches.

Methods: Freshly harvested peaches from different orchards in Florida were collected in pallets, transported to a packinghouse, and cooled by RC, FAC, or HC with sanitized water (150 ppm free chlorine). Samples were collected from pallets (field), after cooling (pre-pack), and after packing (post-pack). Peaches from the post-pack line were incubated at 1.1°C for 21 days. Representative samples were withdrawn on days 1, 7, 14, and 21. Samples were collected in sterile plastic bags, which were transported in insulated coolers to the lab and immediately analyzed for aerobic plate counts (APC) and yeast and mold counts (Y&M). Three trials for RC and two trials each for FAC and HC were conducted, and results were analyzed for evaluating the efficacy of the cooling processes.

Results: The average APC from field samples was 5.3 log CFU/peach, which remained unchanged after RC or FAC but was reduced to 4.6 log CFU/peach after HC ($P<0.05$). The average Y&M counts from field samples (6.2 log CFU/peach) was reduced significantly ($P<0.0001$) to 4.0 log CFU/peach after HC. The handling process during packing increased ($P<0.05$) both APC and Y&M counts on HC peaches, while it showed no such effect on other peaches. At the end of the 21-day incubation period, there was no difference in APC or Y&M counts on peaches, irrespective of the cooling processes.

Significance: Hydrocooling significantly reduced the postharvest loads of APC and Y&M on peaches and showed promise in maintaining microbiological quality of the fruit during storage.

P2-127 Probiotic Potential of Phage-resistant *Lactobacillus plantarum* against Foodborne Pathogens

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Introduction: Lactic acid bacteria are commonly used as probiotics and for the fermentation of functional food products. The effect of lactic acid bacterial activity is extremely impacted when phage infection occurs. To overcome this limitation, isolation of a phage-resistant strain from a sensitive strain is an alternative approach.

Purpose: The purpose of this study is to isolate phage-resistant *Lactobacillus plantarum* mutant and investigate the probiotic potential of phage-resistant *L. plant*arum against foodborne pathogens.

Methods: Growth of phage sensitive and phage-resistant *L. plantarum* strains were determined by measuring the optical density and viable cell count at various time points. Antibacterial activity of a phage-resistant *L. plantarum* strain against *Salmonella enterica* serovars Typhimurium and Enteritidis, *Staphylococcus aureus*, enterohemorrhagic *Escherichia coli* (EHEC), and *Listeria monocytogenes* were investigated using the agar disk diffusion method. An inhibition assay of all the pathogens' association and invasion of human intestinal epithelial cells (INT-407 cells) was also performed.

Results: The phage-resistant *L. plantarum* mutant strain showed markedly higher growth (>1 log CFU/ml) than that of the phage sensitive *L. plantarum* strain. Cell-free supernatant of the phage-resistant *L. plantarum* mutant strain showed strong antibacterial activity against *Salmonella* Typhimurium (>12 mm diameter of clear zone), *Salmonella* Enteritidis (>13 mm diameter of clear zone), and *S. aureus* (>10 mm diameter of clear zone). Both phage-sensitive and phage-resistant *L. plantarum* strains were able to adhere to INT-407 cells, but only the phage-resistant *L. plantarum* mutant strain could reduce the invasion abilities of *Salmonella* Typhimurium (1.8 log CFU/ml), *Salmonella* Enteritidis (0.3 log CFU/ml), and *L. monocytogenes* (1.2 log CFU/ml) into INT-407 cells ($P<0.05$).

Significance: This study indicated that a phage-resistant *L. plantarum* mutant strain is capable of inhibiting major foodborne bacterial pathogens and could be a potential candidate for industrial applications in functional foods.

P2-128 Food Consumption Habits and Handling Practices among the Mexican Central Region Population and Their Association with Salmonellosis

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Introduction: Around 50 to 87% of foodborne illness outbreaks are associated with the consumption of food at home, and *Salmonella* one of the most commonly implicated pathogens. This fact highlights the importance of implementing good food handling practices. In 2016, 77,614 cases of salmonellosis were reported in Mexico; however, there is no information regarding food handling practices among consumers.

Purpose: The main goal of this study was to know food consumption habits and handling practices among the population of the central region of Mexico and their association with salmonellosis.

Methods: An online survey was conducted among the population of ten states in Mexico (Aguascalientes, Colima, Guanajuato, Hidalgo, Jalisco, Michoacán, Morelos, Querétaro, Estado de México, and Mexico City). The questionnaire was divided into eight sections: 1) general characteristics (sex, age, education level, suffering from gastrointestinal/chronic-degenerative diseases), 2 through 6) consumption habits of five food groups (meat, vegetables/fruits, meat products, dairy products, and fish/seafood), 7) handling practices, and 8) have suffered from salmonellosis. The association between some variables was obtained by Pearson's chi-square test.

Results: A total of 1,015 surveys were analyzed. Of the respondents, 17.4% of respondents said they have had salmonellosis at least once in their life; this was primarily associated with the consumption of meats and vegetables/fruits, which were the most consumed foods (91.1 and 99.1%, respectively). Age, education level, and suffering from gastrointestinal diseases also showed a significant relationship to respondents effected by salmonellosis

($P<0.05$). Regarding handling practices, one of the riskiest was the use of the same knife (16.7%) and cutting-board (12.9%) to cut more than one product, which could lead to cross-contamination in the kitchen.

Significance: Learning about food consumption habits and handling practices, their association with salmonellosis, and Mexican consumers' perception of this disease is the first step in determining a more realistic way to reduce the level of exposure to *Salmonella*.

P2-129 *Salmonella* and *Listeria* in Ready-to-Eat Products: Improving the Estimates of Positive Test Probabilities by Product Categories

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Introduction: In 2003, the United States Department of Agriculture Food Safety Inspection Service (FSIS) implemented a RTE sampling algorithm with product risk rankings (PRR) to assist FSIS inspection program personnel (IPP) in selecting the riskiest RTE products for sample collection.

Purpose: In October 2016, FSIS analyzed the algorithm used to select RTE products for sampling by FSIS inspection program personnel. One parameter in the algorithm is the probability of a positive test result by RTE category for each of two chosen pathogens (*Listeria monocytogenes* and *Salmonella*) as part of the agency's risk-based sampling protocols. While test results (positive or negative) are always recorded with high accuracy, the exact RTE category is often missing or inaccurately recorded. These data quality concerns make it difficult to calculate positive test probabilities accurately. Different ways of imputing missing RTE category data and correcting inaccurate RTE category data were evaluated.

Methods: After comparing several ways of entering missing RTE category data and correcting incorrect RTE category data, an algorithm was developed to compute positive test probabilities, compare data, and correct for deficiencies in the data.

Results: The accuracy of each method was assessed by comparing imputation results against a manually verified subset of the available data. After the application of each method, positive test probabilities were computed and compared. The data showed that percent positive varied only between RTE categories, with dried products having significantly less risk than diced or shredded, multi-component, and salad, spread, or pâté products.

Significance: This approach to correcting inaccurate or missing RTE category information was validated and adopted. Data quality could be monitored by annual verification of the RTE category for a small, random sample of observations.

P2-130 Comparison of Nine Surface Adenosine Triphosphate Test Devices at Different Environmental Control Temperatures and Their Consistency in Signal over Time

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Introduction: The use of adenosine triphosphate (ATP) hygiene monitoring systems is widespread within the food manufacturing industry and provides a quick, cost-effective, and practical method of food contact surface hygiene verification. However, there is a need to determine the usability of such devices at different operational temperatures in food manufacturing environments and establish the stability of signal readings over time.

Purpose: To compare and assess the effect of operational temperature and time delay on ATP signal kinetics following activation of different ATP test device hygiene monitoring systems.

Methods: The stability in signal reading of ATP test devices ($n=9$) was assessed under controlled environmental conditions at different temperatures (10, 20, and 35°C). ATP signals and relative light unit readings were recorded immediately and at determined intervals (20/30, 60, and 120 s) following activation to measure the consistency in the signals over time. Repeat tests ($n=10$) at each temperature were undertaken with each ATP system.

Results: The stability in the signal readings of ATP test devices varied according to operational temperature, and signal was shown to decay over time. The study determined that only one test device showed stability in signal reading (determined by <10% change in signal) over 120 s and across all temperature variables. The reliability and consistency of seven of the nine ATP test systems were highly effected by time and temperature; in some cases, signal decay of 100% was observed.

Significance: Findings suggest that operating factors in the food manufacturing environment such as operational temperature or delays in recording the reading can result in false confidence or misreporting of hygiene compliance for food businesses.

P2-131 Behavior of *Listeria monocytogenes* in Hummus

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Introduction: Listeria monocytogenes is a critical human pathogen and has been implicated in major outbreaks and recalls associated with various contaminated foods. Hummus from two companies was recalled due to *L. monocytogenes* contamination in 2016.

Purpose: To investigate the behavior of *L. monocytogenes* in hummus and the underlying mechanisms of the behavior.

Methods: Hummus from companies A and B was inoculated with strains of *L. monocytogenes* from clinical and environmental sources related to the Company A and Company B hummus recalls. Inoculated hummus was kept at room temperature (RT) and 4°C. *L. monocytogenes* was enumerated using direct plating. Metagenomic sequencing was performed to investigate whether background flora could affect the behavior of *L. monocytogenes* in hummus.

Results: There was no growth observed for all the strains in the hummus from both companies. Moreover, *L. monocytogenes* survived much longer when kept at 4°C than at RT. In Company B hummus, the clinical strains died off completely in 28 days at RT, but in 55 days at 4°C. In addition, in Company A hummus, the pathogen died off in 12 days at RT but had only a 0.3-log reduction at 4°C after 60 days when the enumeration was stopped. For environmental strains, *L. monocytogenes* survived for 12 to 14 days at RT, but for more than 100 days at 4°C for both types of hummus. Meanwhile, the pH values were reduced 8 to 10% in both brands of hummus when kept at RT, but there was no change at 4°C. *Lactococcus* and *Lactobacillus* were found very abundantly in both types of hummus, but further analysis is needed.

Significance: The survival of *L. monocytogenes* in hummus depends on storage temperatures and is product-specific. Our data also indicated that *Lactococcus* and *Lactobacillus* might contribute to the reduction of *L. monocytogenes,* but further evidence is required. This study will help understand the behavior of *L. monocytogenes* in hummus and contribute to the related risk assessments.

Poster

P2-132 Correlation between Enzyme Inactivation and Pathogen Lethality during Water and Steam Blanching of Vegetables

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Introduction: Water and steam blanching conditions can achieve thermal inactivation of pathogens associated with raw vegetables. Enzyme inactivation may be a reliable verification indicator of whether microbial lethality has been achieved during blanching.

Purpose: The objective of this study was to develop a correlation with thermal inactivation of pathogens under specific time and temperature conditions. The study follows previous work to establish conditions for thermal inactivation of *Listeria monocytogenes* and *Salmonella* during water and steam blanching of vegetables.

Methods: Carrots, peas, potatoes, spinach, and broccoli were treated with hot water at 85 and 87.8°C or steam at 85 and 96.7°C for up to 3.5 min. For hot water blanching, samples were immersed in a circulating water bath. For steam blanching, samples were placed over a water bath in such a way as to simulate steam blanching in a closed environment. A qualitative test method adapted from the United States Department of Agriculture Technical Procedures Manual was used to test for peroxidase activity.

Results: Samples blanched in hot water showed peroxidase in carrots, peas, and spinach was inactivated within 0.5 min at 85 and 87.8°C and in broccoli within 2 min. In potatoes, peroxidase was not inactivated at 85°C after 3.5 min, while it was partially inactivated at 87.8°C in 3.5 min. Under steam blanching, no peroxidase inactivation was observed in peas at 85°C for 3.5 min, while it was partial at 96.7°C in 3.5 min. Peroxidase in carrots and spinach was inactivated within 2 min at 85°C and 1 min at 96.7°C. In broccoli, peroxidase was not inactivated at 85°C after 3.5 min, while it was partially inactivated at 96.7°C in 3.5 min.

Significance: The research shows parallels between enzyme and pathogen inactivation resulting from blanching. Such a correlation suggests that enzyme inactivation is a reliable, real-time verification test to determine if blanching processes have achieved adequate thermal treatment.

P2-133 Impact of Air Movement on the Lethality of *Salmonella* and *Pediococcus acidilactici* during the Cooking Step of Beef Jerky Production

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Introduction: Multiple *Salmonella* outbreaks have been associated with beef jerky products. The United States Department of Agriculture Food Safety Inspection Service (FSIS) requires processors to achieve a 5-log reduction of *Salmonella* in beef jerky processing. Many processors rely on the time-temperature guidelines published in Appendix A of the FSIS compliance guidelines; however, there are many caveats to these combinations that reduce product development and processing creativity. Alternative heating conditions, including convection oven systems, need to be validated to support industry compliance and food safety efforts.

Purpose: The objectives were to: i) compare lethality of convection and conventional baking protocols to reduce *Salmonella* on beef jerky, and ii) evaluate Kerry SAGA 200 (*Pediococcus acidilactici*) as a potential surrogate for *Salmonella* for in-plant biological verification studies.

Methods: Raw beef strips were inoculated with a *Salmonella* cocktail or *P. acidilactici* (>7 log CFU/g). Strips were cooked at 140.5°C for 12 min in a Waring 500X Convection Oven using the conventional (fan off) or convection (fan on) bake settings. *Salmonella* and *P. acidilactici* survivors were enumerated on tryptic soy agar or Man, Rogosa, and Sharpe agar, respectively.

Results: Comparison between convection and conventional baking showed significant differences in the survivability of *P. acidilactici*. Convection baking at 140.5°C for 12 min resulted in a 0.85±0.32-log CFU/g reduction, whereas the same conditions using conventional baking (fan off) resulted in a 3.77±1.18-log CFU/g reduction. *Salmonella* survival was less impacted by air movement with average reductions of 1.96±0.45 log CFU/g and 2.43±1.22 log CFU/g for convection and conventional baking, respectively.

Significance: Air movement during baking significantly reduces lethality of *P. acidilactici* during beef jerky processing. Additional research is needed to predict inactivation of thermal treatments that utilize convection heating. This study also demonstrates the necessity of comparing pathogen reduction to potential surrogates using processing conditions that mimic the commercial process as closely as possible.

P2-134 Validation of Lactic Acid Spray Applied to Beef Shoulder Clod Subprimals as an Antimicrobial Intervention in a Beef Processing Environment

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Introduction: Lactic acid is listed in the United States Department of Agriculture Food Safety Inspection Service directive as a safe and suitable processing aid in the production of meat products. Lactic acid is commonly used on beef subprimals as an antimicrobial intervention. Although extensive data exist on the use of lactic acid on carcasses as an intervention, little information is available on chilled subprimals in actual processing situations and the reductions observed after vacuum packaging of the subprimal.

Purpose: This in-plant study was conducted to determine the antimicrobial efficacy of a lactic acid spray applied to beef shoulder clod subprimals in a commercial beef processing facility.

Methods: Throughout three separate production days, samples were randomly collected pre- and post-lactic acid spray treatment during three production times (beginning of shift, midday, and end of shift; *n*=180). Lactic acid spray parameters included a concentration between 2 and 5%, temperature between 110 and 130°F, greater than 15 psi, with a uniform spray. Post-treatment vacuum sealed clods, knives, gloves, and packaging were sanitized with bleach solution within one hour after packaging. Clods were sampled at 500 cm^2 areas on the fat side utilizing sterile, pre-hydrated sponges. All counts were determined and converted to log CFU/500 cm^2.

Results: Aerobic plate counts average 7.13 log CFU/500 cm^2 before intervention and decreased to 6.43 log CFU/500 cm^2. Total coliforms and *Escherichia coli* were reduced from 4.13 and 2.19 to 1.36 and 1.33 log CFU/500 cm^2 after treatment, respectively. *Enterobacteriaceae* counts were reduced by 1.15 log CFU/500 cm^2 after treatment. All microbial counts observed over the 500 cm^2 area after treatment application were biologically reduced.

Significance: Validating interventions such as lactic acid spray in actual processing situations will provide additional knowledge and understanding of how processing aids can be influenced by unforeseen variables in commercial facilities.

Poster

P2-135 Evaluation of *Listeria monocytogenes* Sub-lethal Injury under Different Stress Conditions Related to Food Processing

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❖ ***Developing Scientist Competitor***

Introduction: Food processing treatments may result in sub-lethal injury within a microbial population, which is a potential threat for food safety. Therefore, the evaluation of a food process requires information on its potential to induce injury in food pathogens such as *Listeria monocytogenes*.

Purpose: To compare how different processing treatments induce sub-lethal injury in two strains of *L. monocytogenes*, assess the mode/extent of injury, and investigate the effect of previous stress adaptation.

Methods: Viability and sub-lethal injury of *L. monocytogenes* strains EGDe and ScottA (serotypes 4b, 1/2a) was investigated after exposing 7 log CFU/ml cells to the following treatments: lactic acid (LA), pH 3.0/4.0, 7 h; heat stress, 55°C, 2 h; peracetic acid (PAA), 10 ppm, 5 days; quaternary ammonium compounds (QUAT), 5 and 10 ppm, 5 days, prepared in i) 1/4 strength Ringers solution (R) and ii) Microcosm Water (sterile double-dionized H_2O; MW) incubated at 4 and 20°C. Additionally, the effect of stress-adaptation (R-10ppm-4°C-24h) on cell injury was evaluated under exposure to QUAT (M-10ppm, M-20ppm, R-20ppm). TSAYE was used as growth medium and injury determination was performed with the maximum non-inhibitory concentration (MNIC) method (NaCL, 5% for *L. monocytogenes*). Populations' recovery capacity was evaluated on TSAYE+3 and 6% NaCl (weight/volume).

Results: The type of treatment affected not only cell viability, but also the extent of injury. At LA-R-20°C-7h-55°C-90min, both strains decreased by 1 log CFU/ml, but only heating resulted in ~99% cell injury. Exposure to QUAT-R-10ppm-20°C for 5 days did not reduce the population but induced ~99% injury only to ScottA, indicating a strain-dependent mode of injury. EGDe was not affected by exposure to PAA; ScottA was reduced by 1 log CFU/ml after 5 days in PAA-MW-20°C. The respective survivors on TSAYE+3 or 5% NaCl were 1 and 2 log CFU/ml lower compared to TSAYE survivors. Adaptation to QUAT-R-10ppm-4°C-24h enhanced the resistance of cells upon exposure to QUAT-R-10ppm-4°C but also increased the injured subpopulations within the survivors.

Significance: Understanding factors which influence the sub-lethal injury of *L. monocytogenes* might contribute to better selection of treatments applied during food processing.

P2-136 Growth of *Salmonella* during "Sprouting" of Nut, Seed, and Grain Products

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Introduction: There are a number of food entrepreneurs that are producing food products with "sprouted" ingredients. The marketing concept implies that initiating germination of seed products (nuts, seeds, grains) leads to improved health benefits. The "sprouting" process typically consists of soaking raw product in water overnight either at ambient temperature or under refrigeration prior to further processing (dehydration, blending, etc.).

Purpose: The purpose of this study was to quantify *Salmonella* growth during the "sprouting" of specific nuts, seeds, and grains.

Methods: Raw nuts (almond, cashew, hazelnut, peanut, pecan, walnut), seeds (flax, pumpkin, sunflower), and grains (buckwheat, millet, quinoa, rice) samples were inoculated with ~3 log CFU/g of a *Salmonella* cocktail and dried at 25°C for 24 h. Inoculated product samples (25 g) were mixed with 0.1% peptone water (25 ml) and incubated at 7 or 25°C for 24 h. *Salmonella* cell density was determined by enumerating on Hektoen enteric agar after incubation at 37°C for 24 h.

Results: Initial *Salmonella* concentrations on nuts, seeds, and grains averaged 2.76±0.83 log CFU/g. *Salmonella* was capable of growing in all products during "sprouting" at room temperature, reaching an average concentration of 7.58±0.87 log CFU/g after 24 h. Nuts tended to have a higher final concentration of *Salmonella* at 8.00±0.69 log CFU/g, while seed and grain samples averaged 7.06±0.78 log CFU/g. Refrigerated almond samples showed no growth.

Significance: Salmonella multiplies rapidly during the "sprouting" processes of various nuts, seeds, and grains at ambient temperature. The industry should be encouraged to conduct "sprouting" activities under refrigeration.

P2-137 Suitability of *Enterococcus faecium* atcc 8459 as a Surrogate for *Salmonella* during Dehydration of Infused Fruit Products

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❖ ***Developing Scientist Competitor***

Introduction: Dehydration is commonly used as a processing method to manufacture dried fruit products. Although these processes have been designed to optimize roduct quality, little is known about the efficacy of these processes in reducing foodborne pathogens. The food industry needs information on the efficacy of these processes, as well as the suitability of surrogate organisms for in-plant biological verification of complex processing conditions.

Purpose: The purpose of this study was to determine the suitability of *Enterococcus faecium* ATCC 8459 as a surrogate for *Salmonella* during the dehydration of infused fruit products.

Methods: Infused fruit (apples, blueberries, cherries, peaches) samples (25 g) were inoculated with ~8 log CFU/g of a *Salmonella* cocktail or *E. faecium* and held at 25°C for 24 h. Fruit products were dehydrated in a Harvest Saver cabinet dryer at 57°C for <20 h. Survivors were enumerated on tryptic soy agar after incubation at 37°C for 24 h.

Results: For infused apple samples, *Salmonella* concentrations initially dropped from 8.04±0.06 to 6.29±0.15 log CFU/g after 4 h and remained at similar cell density for the duration of the treatment (16 h). *Salmonella* was more sensitive to dehydration treatments on infused blueberries, cherries, and peaches, with survivors continuing to decline throughout the 16 to 20 h of treatment. The dehydration treatment (57°C, 16 h) achieved total *Salmonella* reductions of 2.52±0.42, 5.33±0.49, 5.58±0.38, and 4.75±0.81 log CFU/g in infused apple, blueberry, cherry, and peach products, respectively. Reductions of *Salmonella* and *E. faecium* were comparable throughout the dehydration of all fruit types tested.

Significance: This study demonstrates the suitability of *E. faecium* ATCC 8459 as a surrogate for *Salmonella* in the dehydration step of infused fruit products. Future studies will utilize *E. faecium* ATCC 8459 as a surrogate for biological verification studies of commercial-scale dehydration systems.

Poster

P2-138 Benefits of Using Cloud-based Temperature Data Loggers for Temperature-sensitive Food Storage and Transportation

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Introduction: Industry 4.0 is about digitizing the enterprise ecosystem with system interoperability, cloud-connectivity and the Internet-of-Things. So what does Industry 4.0 have to do with temperature data loggers and food safety? The focus here is on essential temperature logging functions in order to reduce manual measurement and report logs, mitigate foodborne illness in the cold chain, increase productivity, and yield greater efficiency by connecting cold storage warehouses and refrigerated transport to the cloud for complete supply chain visibility.

Purpose: To communicate to food safety stakeholders that the majority of temperature data logging products currently deployed are passive devices that require manual data retrieval. To communicate to food safety stakeholders that cloud-based temperature data loggers offer geo-location traceability, live reporting of temperature conditions, or push notifications for temperature alerts.

Methods: Provide live data samples collected from product shipments within the cold supply chain and detail the speed to detect the presence of a temperature abuse with passive and active modes.

Results: Three different case studies will be demonstrated detailing journey time and temperature range experienced during the journey.

Significance: Early detection of temperature abuse and removal of food products from the cold chain can mitigate foodborne illness, increase productivity, and yield greater efficiency.

P2-139 Fate of *Salmonella enterica* on Raw Chicken Breast Meat Marinated in Lemon Juice with Added Thyme Oil and Yucca Extract

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Introduction: Consumers are demanding more natural, minimally processed foods without synthetic additives. This consumer trend has fueled research on the use of plant essential oils such as thyme oil (TO) as antimicrobial food preservatives.

Purpose: This study aimed at evaluating the antimicrobial efficacy of thyme oil (TO) and yucca extract against *Salmonella enterica* on artificially inoculated raw chicken breast meat in a lemon-based marinade at 23°C.

Methods: Four lemon-based marinade solutions were prepared: lemon juice alone (control), lemon juice (LJ) with added 0.5% yucca extract (YEX), and LJ with YE and 0.5% and 0.75% thyme oil (LJ+YEX+TO). Raw chicken breasts were inoculated with a five-strain cocktail of *S. enterica* at 10^8/sample and submerged in marinade for 2, 4, 6, and 8 h at 23°C. Survivors were enumerated by surface plating diluted (10-fold) chicken homogenates on xylose lysine deoxycholate (XLD) agar and XLD plates overlaid with a thin layer of tryptic soy agar with yeast extract and counting colonies after incubation (35°C) for 48 h.

Results: S. enterica survived on chicken in the control marinade with a decrease in viability of ~2.0 to 2.2 log CFU/sample after 8 h. Lower numbers of the pathogen were observed in marinade with YEX; however, results were not statistically different from control (P>0.05). Both marinades with added YEX and TO (0.5% or 0.75%) significantly decreased the initial viable count of *S. enterica* at all times tested in the study (P<0.05). TO (0.75%) + YEX exhibited the strongest bactericidal effect with only 3.44 log CFU/sample remaining after 8 h of marination. Sub-lethal injury in *S. enterica* survivors increased with time and concentration of TO.

Significance: Thyme oil combined with yucca extract has good potential for use as a natural antimicrobial component in marinades to decrease the survival of *Salmonella* on raw poultry meat.

P2-140 Determination of Zilpaterol in Sheep Urine and Tissues Using Immunochromatographic Assays

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Introduction: Zilpaterol is a feed additive used to increase weight gain, improve feed efficiency, and increase carcass leanness in cattle. An on-site analytical method is needed to determine zilpaterol exposure in animals to assist producer and trade groups in avoiding unnecessary animal or carcass condemnation.

Purpose: To determine the lowest level of zilpaterol in a mixed feed that returns positive immunochromatographic screening (ICS) results in urine and edible tissues of exposed sheep.

Methods: Sheep were fed trace levels of zilpaterol HCl (0.001, 0.01, and 0.1 mg/kg of feed; L, M, H doses, respectively) for 12 consecutive days in four trials; animals were killed on withdrawal day (WD) 0 (L0, M0, H0) or 3 (H3). Urine samples were collected and analyzed by ICS on feeding days 0, 2, 4, 6, 8, 10, and 12 (L0, M0, H0) and on WD 1, 2, and 3 for H3 animals. Tissues were harvested at slaughter, ground, either diluted with water or extracted with ethyl acetate, and evaluated by ICS.

Results: Of 96 total control urine samples, three of the tests were false positives. Urine collected from sheep in the L group returned 25 to 75% positive ICS samples. After day 4, all M0, H0, and H3 urine tested positive during treatment. All urine from H3 sheep tested positive on WD 1, 50% on WD 2, and 25% on WD 3. All H0 kidney, liver, and lung tissue extracts tested positive, while 50% of muscle extracts were positive. Aqueous dilution of H0 tissues returned 75 and 50% positive for liver and kidney, respectively, and 0% for muscle and lung, less sensitive than extraction method.

Significance: Immunochromatographic assays can be performed on site to determine zilpaterol exposure and can provide "real time" (10 min) answers. Positive tissue results were returned only in H sheep, a zilpaterol exposure level approximately 1.3% of the level found in labeled feed.

P2-141 Multiple Fingerprinting Analysis for Investigating Quality Control of Cassiae Semen Polysaccharides

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Introduction: Cassiae semen, seeds of *Cassia obtusifoliol* L., has been widely used as a tea or traditional folk medicine in Asia for therapeutic purposes. The widespread occurrence of polysaccharides are also significant bioactive constituents of cassiae semen, which could display multi-pharmacological effects. However, quality control issues overshadow potential health benefits of cassiae semen due to analytic limitations.

Purpose: The aim of our study is to establish multi-dimensional fingerprints of cassiae semen polysaccharides using instrumental methods (Fourier-transform infrared spectroscopy [FT-IR], high performance liquid chromatography [HPLC], and gas chromatography [GC]) and multivariate statistical techniques. In addition, the multi-dimensional fingerprints of polysaccharides were also applied to distinguish the conformity or inferiority of cassiae semen polysaccharides harvested from different areas.

Methods: FT-IR spectroscopic and HPLC/GC fingerprints of non/partially/completely hydrolyzed polysaccharide extracts from ten batches of the authentic source were established and then applied to assess the quality of two foreign sources. Fingerprints of polysaccharide extracts were analyzed using chemometric methods, including Pearson correlation analysis, principal component analysis (PCA), and other similar methods to distinguish the foreign sources from the authentic sources.

Results: FT-IR spectroscopic fingerprints of non-hydrolyzed polysaccharides using correlation coefficients and PCA differentiated various sources of cassiae semen (similarity=0.771~0.880< 0.9). HPLC fingerprints of partially hydrolyzed polysaccharides with PCA distinguished the foreign sources. However, HPLC or GC fingerprints of completely hydrolyzed polysaccharides using the Similarity Evaluation System of Traditional Chinese Medicine could not completely distinguish the foreign sources from the authentic one. Additionally, HPLC could provide more complete information than GC while identifying the monosaccharide composition of polysaccharides.

Significance: Multiple chromatographic FT-IR/HPLC fingerprints of non/partially-hydrolyzed polysaccharides, respectively, using multiple chemometrics methods might be potentially applied in detecting and differentiating sources of cassiae semen, and may also be a useful tool in controlling the quality of its polysaccharide products.

P2-142 Inactivation Kinetics of *Bacillus cereus* Biofilms Grown on Leafy Greens with Slightly Acidic Electrolyzed Water Combined with Ultrasound and Mild Heat

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Introduction: *Bacillus cereus* aggregates in multicellular communities with extracellular polymeric substances on different surfaces, called biofilms. Biofilms are difficult to eradicate and are resistant to disinfectants. Thereby, *B. cereus* biofilms on leafy greens can act as a persistent source of contamination, which is of public health concern.

Purpose: This study aims to examine the inactivation kinetics of slightly acidic electrolyzed water (SAEW) with ultrasound and mild heat on *B. cereus* biofilms on leafy greens.

Methods: *B. cereus* (ATCC 14579 and ATCC 10987) biofilms were grown on spinach, beet, and lettuce leaves, as well as stainless steel and plastics. Biofilm formation was quantified using crystal violet (CV) assay and cell enumeration. Biofilms were treated with SAEW or NaOCl, ultrasound, and mild heat (60°C), and surviving populations were measured. Obtained inactivation kinetics data was determined by Weibull model.

Results: Based on the CV assay, both strains formed biofilms on leaves. However, there was no significant difference among the tested leaves for both strains. Inactivation kinetics of SAEW was largely dependent on available chlorine concentration level, dipping time, strain, and surfaces used. SAEW (40 ppm) reduced ATCC 10987 and ATCC 14579 biofilms on plastics to nondetection limit within 1 min and 30 s, respectively. However, biofilms on leaves showed higher resistance. SAEW with ultrasound and mild heat for 5 min showed an additional reduction for biofilms on leaves. In all conditions, biofilm reduction using SAEW was significantly higher than that of NaOCl (40 ppm). Calculated correlation coefficients and root mean sum of squared error values indicated that the Weibull model precisely predicted the inactivation kinetics of SAEW with ultrasound and mild heat on *B. cereus* biofilms.

Significance: These data suggest that SAEW with ultrasound and mild heat may allow us to create novel cleaning strategies against *B. cereus* biofilms on leafy greens.

P2-143 A Filtration-facilitated, Aptamer-based Detection of Salmonella Using Ultra-fast Surface-enhanced Raman Spectroscopic Mapping

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❖ ***Developing Scientist Competitor***

Introduction: The presence of *Salmonella* in natural fresh water and drinking water is a leading cause of intestinal illness all over the world and a possible transmission pathway of *Salmonella* typhoid fever. Therefore, the detection of *Salmonella* in water is of great importance to public health.

Purpose: The objective of this study is to develop a rapid screening method for detection of *Salmonella* in water involving surface-enhanced Raman spectroscopy (SERS), aptamers, and filtration. SERS offers a great alternative to traditional culturing methods and rapid methods, as it does not require pre-enrichment of samples.

Methods: The specific capturing and labeling of *Salmonella* is realized by an aptamer, a single stranded DNA sequence, which is more cost-effective and more easily handled than antibodies. The aptamer used in this study is modified by adding an additional chain of adenine, which has a distinctive peak at 733 cm^{-1} and is used as presence/absence of *Salmonella.* By integrating a filter system, bacteria cells are collected and concentrated onto a membrane, and then recognized after labeling with specifically designed aptamers based on the unique SERS spectrum of aptamers.

Results: The specificity of the method was validated by using other types of bacteria such as *Escherichia coli*. With an ultra-fast Raman microscope, which can generate 20 by 20 (point by point) mapping within a few minutes, the sensitivity of the method could go down to 10^2 CFU/ml in a short time.

Significance: This study demonstrates the great potential of this method for detecting *Salmonella* in environmental waters and other aqueous matrices such as rinse water from poultry and fresh produce.

P2-144 Evaluation of the Bio-Rad iQ-Check *Salmonella* II Assay in Select Foods: A Collaborative Study

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Introduction: Low-moisture foods typically do not support the growth of *Salmonella*, although studies have indicated that the organism can remain viable for long periods of time. While rare, *Salmonella* outbreaks associated with low-moisture products often impact large numbers of people. Over the last few decades, several low-moisture product outbreaks have occurred, involving chocolate, raw almonds, dry seasonings, pet food, and peanut butter. The Bio-Rad iQ-Check *Salmonella* II Kit is a test based on gene amplification and detection by the use of real-time PCR. Ready-to-use PCR reagents contain oligonucleotides specific for *Salmonella*, as well as DNA polymerase and nucleotides.

Purpose: The purpose of this AOAC Official Methods of Analysis Collaborative Study compared the candidate method to the U.S. Food and Drug Administration Bacteriological Analytical Manual method for milk chocolate and dry pet food using 375-g test portions.

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Methods: The candidate method was evaluated in a multi-laboratory collaborative study. A total of 14 technicians from 12 laboratories participted, representing government and industry throughout the United States and Canada. Each laboratory evaluated 12 replicates at three levels of inoculation: an un-inoculated control level (0 CFU/test portion), a low inoculum level (0.2 to 2 CFU/test portion), and a high inoculum level (2 to 5 CFU/test portion).

Results: Statistical analysis was conducted according to the probability of detection statistical model. No statistically significant difference was observed between the candidate and reference methods. The new method correctly identified whether a test portion was positive or negative more than 98% of the time.

Significance: The data from the study, within the statistical uncertainty, supports the certification of the candidate method as an AOAC Official Method of Analysis, First Action in select food matrices and environmental surfaces.

P2-145 Simultaneous Enrichment of *Salmonella* Typhimurium, *Escherichia coli* O157:H7, and *Listeria monocytogenes* in Cheese

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Introduction: Per the U.S. Food and Drug Administration's Bacteriological Analytical Manual (BAM), different pre-enrichment broths and biochemical tests are used for the detection of different pathogens. For foods where multiple pathogens are of concern such as cheeses, pathogens are tested for individually using different enrichment protocols. Previous research showed success in the simultaneous enrichment of *Salmonella* Typhimurium, *Escherichia coli* O157:H7, and *Listeria monocytogenes* in produce, seafood, and spices as detected by a multiplex quantitative PCR method (qPCR).

Purpose: The objective of this study was to further assess candidate enrichment broths for the simultaneous enrichment of *Salmonella* spp., enterohemorrhagic *E. coli*, and *L. monocytogenes* in cheese.

Methods: Salmonella Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* were co-inoculated in a 1:1:1 ratio into 25 g of cotija cheese blended with 225 g of a candidate enrichment broth. Enrichment broths were chosen based on results from previous research and included universal pre-enrichment broth (UPB), BMW, and buffered BMW. After 24 h incubation at 35°C, total DNA was extracted from these enrichment broths and multiplex qPCR was performed for pathogen detection.

Results: All three target pathogens were detected in cheese after enrichment in BMW and buffered BMW. Cycle threshold (Ct) values were 23.30±1.27, 22.17±1.58, and 36.14±1.35 in BMW and 21.16±1.52, 23.87±4.76, and 33.94±0.06 in buffered BMW for *Salmonella* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes*, respectively. Ct values for *Salmonella* and *Listeria* enrichment in buffered BMW were significantly lower than BMW ($P<0.05$). In UPB, *Salmonella* Typhimurium and *E. coli* O157:H7 were detected in cheese by qPCR after 24 h; however, *L. monocytogenes* was not detected.

Significance: A universal enrichment broth combined with multiplex qPCR detection will reduce media requirements and analysis time resulting in a high-throughput, streamlined approach that will increase the capacity of public health laboratories to rapidly detect multiple pathogens in a variety of food matrices.

P2-146 Rapid Enumeration of *Salmonella* Using Roka Atlas *Salmonella* SEN Detection Assay

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Introduction: Roka Atlas *Salmonella* SEN Detection Assay is a molecular method which uses transcription-mediated amplification to detect ribosomal RNA with enhanced specification and sensitivity. The chemiluminescence reaction in its final detection generates relative light units (RLU) to reflect the quantity of RNA amplicons in the reaction. These semi-quantitative signals have the potential to be utilized to enumerate *Salmonella* cells in test samples.

Purpose: The purpose of this study was to explore the possibility of using the *Salmonella* rRNA detection assay to rapidly enumerate *Salmonella* in pure cultures.

Methods: Three *Salmonella* serotypes (Montevideo, Typhimurium, and Weltevreden) were used in the study. Tryptic soy broth inoculated with *Salmonella* cells was incubated at 35°C for 24 h. The overnight cultures were serial diluted, and 400-µL samples at each dilution level were analyzed using the *Salmonella* detection assay with two to four repetitions. The dilutions were also plated on tryptic soy agar plates for traditional enumeration. A total of 357 data points were collected from different trials with multiple assay kits to evaluate data consistency.

Results: Scatter plots of RLU signals with matching viable cell numbers (log CFU/mL) showed that the signals correlated consistently with cell counts between 3 and 7 log CFU/mL. Below 3 log CFU/mL, some variation of RLU signals occurred and the signal plateaued at 6 to 7 log CFU/mL. Using concentration of cells below 6 log CFU/mL as response (y) and the average Signal/Cutoff data as potential predictor (x), polynomial regressions yielded well-fitted quadratic functions for enumerating *Salmonella* in pure culture: Montevideo, $y=0.09x^2-0.11x+1.10$ ($R^2=0.90$); Typhimurium, $y=0.07x^2-0.01x+1.23$ ($R^2=0.90$); Weltevreden, $y=0.08x^2-0.08x+0.84$ ($R^2=0.88$).

Significance: Relatively consistent correlations between cell counts and RLU signals indicated this *Salmonella* detection assay could be used for rapid enumeration of *Salmonella*. Enumeration results could be obtained in three hours with the automated Atlas system, which is much shorter than the one-day requirement for conventional plating methods.

P2-147 Evaluation of a Real-time PCR Method for Verification and Serogroup Identification of *Listeria monocytogenes* Isolates

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Introduction: Listeria monocytogenes is one of the leading causes of death due to foodborne illness, affecting primarily the elderly, pregnant woman, neonates, and the immunocompromised. Although *L. monocytogenes* can be classified into 13 serotypes, only four are typically linked with illness and food contamination. A PCR method was developed that detected these four serotypes, which also form broad phylogenetic groups. This quantitative PCR-based serogrouping method could help to quickly identify implicated foods during outbreak investigations, track existing trends of serotypes, and identify new trends, as in the case of 4bV strains recently linked to several outbreaks.

Purpose: To develop an accurate, rapid PCR-based method for species verification and serogrouping of *Lm.*

Methods: A qPCR method was developed, incorporating an amplification control, targets specific to *Listeria* spp. and *L. monocytogenes*, and four serogrouping targets. This method was compared with the U.S. Food and Drug Administration's Bacteriological Analytical Manual (BAM) and Denka Seikken serotyping methods, using a 156-strain panel to determine accuracy.

Results: Evaluation of the genus/species methods found high accuracy for the qPCR (>99%) compared to the BAM (97%) method. The BAM method failed to detect a few *L. monocytogenes* isolates due to variations in the biochemical assays. Analysis of serogrouping results found higher accuracy with qPCR (100%) versus sera-agglutination (95%) for strains where either both results agreed or independent serotyping information was available.

Additionally, the qPCR identified all 4bV strains. For some strains, independent serotyping was unavailable and whole genome sequencing was used to resolve these results.

Significance: This method provided a rapid and accurate verification of *L. monocytogenes* and determination of serogroup. Critically, the qPCR is ideal for rapid identification of serogroups, including 4bV, and can aid in outbreak investigation and identification of new serotypes and trends.

P2-148 Analysis of Biofilm Formation among *Staphylococcus aureus* Isolates Collected from a Firm Implicated in Multiple Staphylococcal Food Poisoning Outbreaks

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Introduction: The potential impact of biofilm-producing *Staphylococcus aureus* isolates in food processing facilities is apparent given their vast role in human disease. Increased resistance to antimicrobial agents and disinfectants is a characteristic of microorganisms proliferating within a biofilm. The intercellular adhesion (*ica*) operon is responsible for slime production. The *icaA* and *icaD* genes are known to play significant roles in biofilm formation.

Purpose: Seventy *S. aureus* isolates recovered from environment and food and collected during multiple staphylococcal food poisoning outbreak investigations associated with one firm were evaluated for *ica* genes and their phenotypic biofilm formation ability.

Methods: Preliminary testing on these *S. aureus* isolates established that all 70 were resistant to one or more antibiotic and 68 were positive for staphylococcal enterotoxins. The *icaA* and *icaD* genes were identified here by data generated using shotgun whole genome sequencing (WGS) of these isolates. Phenotype testing was evaluated twice in triplicate and accomplished utilizing three methods: tissue culture, tube, and Congo red agar spot inoculation. The phenotypic biofilm formation of each isolate was categorized as high-to-moderate and weak-to-no biofilm.

Results: WGS analysis identified *icaA* and *icaD* genes in all isolates. Phenotypic biofilm analysis using Congo red agar spot inoculation yielded 57% high-to-moderate and 43% weak-to-no biofilm formation. The tube method produced 54% high-to-moderate and 46% weak-to-no biofilm. Tissue culture results indicated 63% high-to-moderate and 37% weak-to-no biofilm formation.

Significance: The difficulty biofilms present within a food processing facility and the potential to inadvertently transfer bacteria from the environment to food products presents real challenges to any firm and may explain, in part, why indistinguishable *S. aureus* isolates were recovered from this firm during the initial investigation, post-cleanup, and almost one year later during another outbreak investigation.

P2-149 Development of Method Combined with Filtration and DNA Concentration for Rapid Detection of Foodborne Pathogens by Real-time PCR

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Introduction: Concentration of pathogens is essential for detection because foodborne pathogens exist on food at a very low level. In order to detect pathogens, it is necessary to perform enrichment to reach the detection limit.

Purpose: This study describes a quick and simple pretreatment method that combines bacterial concentration with DNA concentration for detection of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium that exist on lettuce and beef.

Methods: E.coli O157:H7, *L. monocytogenes*, and *Salmonella* Typhimurium were artificially inoculated into lettuce and beef. This pretreatment method is based on filtration, modified PrepMan method, and real-time PCR. Filtration was used to concentrate the pathogens. Modified PrepMan method was used to concentrate bacterial DNA by reducing DNA elution volume. Detection through real-time PCR was performed.

Results: The detection limits of *E. coli* O157:H7 and *L. monocytogenes* were improved from 10^3 CFU/g to 10^1 CFU/g, and from 10^2 CFU/g to 10^1 CFU/g in all foods. The detection limits of *Salmonella* Typhimurium were improved from 10^2 CFU/g to 10^0 CFU/g. Moreover, detection time (including filtration, DNA extraction, and real-time PCR) only took about two hours. Lettuce and beef samples weighed 25 g each.

Significance: These results indicate that the use of this method is expected to shorten the length of pre-enrichment time required to reach the detection limit so that rapid detection can be possible.

P2-150 Rapid Pre-concentration and Detection of *Salmonella* in Food Samples Using Magnetic Ionic Liquids and Recombinase Polymerase Amplification

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❖ ***Developing Scientist Competitor***

Introduction: The rapid detection of pathogenic microorganisms in food manufacturing processes is essential for prompt identification of potential sources of contamination in the food supply chain. Current methods used in industry are limited by time-consuming pre-enrichment steps, culture-based detection methods, and/or nucleic acid-based testing that requires cost-prohibitive equipment and technical expertise. Simple, streamlined sample preparation techniques may enable valuable reductions in both testing costs and analysis times.

Purpose: We have previously investigated the extraction and concentration of non-pathogenic bacteria from model systems using magnetic ionic liquid (MIL) solvents. Here, we combine MIL-based sample preparation with rapid isothermal amplification and detection of *Salmonella*-specific DNA using Recombinase Polymerase Amplification (RPA).

Methods: Salmonella *enterica* ser. Typhimurium ATCC 14028 was artificially inoculated in water and milk samples (1 ml) and extracted for 30 sec using one of three MILs, including: trihexyl(tetradecyl)phosphonium cobalt(II) hexafluoroacetylacetonate ($[P_{66614}^+][Co(hfacac)_3^-]$), trihexyl(tetradecyl)phosphonium dysprosium(III) hexafluoroacetylacetonate ($[P_{66614}^+][Dy(hfacac)_4^-]$), and trihexyl(tetradecyl)phosphonium nickel(II) hexafluoroacetylacetonate ($[P_{66614}^+][Ni(hfacac)_3^-]$). Viable cells were recovered from the MIL extraction phase with the addition of modified Luria-Bertani broth, and a 10 to 20 min isothermal RPA assay was used to amplify the *Salmonella invA* gene for detection using gel electrophoresis.

Results: The combined MIL and RPA approach enabled detection of *Salmonella* at levels as low as 10^3 CFU/ml. MIL-based sample preparation required less than 5 min to enrich sufficient cells for detection using RPA, which (including gel-based analysis) required approximately 45 min.

Significance: Our results suggest the utility of MILs for the rapid extraction and concentration of pathogenic microorganisms in food samples, providing a means for physical enrichment that is compatible with both culture-based and isothermal nucleic acid amplification detection methods. These experiments establish the foundation for further evaluation of new MIL structures that can improve the enrichment of viable microorganisms from complex food matrices.

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P2-151 Detection of *Salmonella* Typhimurium and *Listeria innocua* from Environmental Samples Collected from a Facility Processing Lyophilized Lactic Acid Bacteria

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Introduction: Environmental monitoring for pathogens in a facility in which dried lactic acid bacteria (LAB) are processed represents additional challenges to those present in other food processing environments. These dried preparations contain a large and concentrated load of LAB which often produce antimicrobial compounds that can impair the recovery and detection of targeted organisms.

Purpose: The objective of the study was to evaluate the ability of a loop-mediated isothermal amplification (LAMP) molecular system to detect *Salmonella* Typhimirum and *Listeria innocua* from environmental samples collected from a facility processing lyophilized LAB.

Methods: Fifty environmental samples (*n*=50) were collected from a facility dedicated to blending lyophilized LAB utilizing sponges containing 10 ml of buffered peptone water (BPW). For each target organism, 15 samples were inoculated with a low level of the organism (approximately 1 CFU/sample), five with a high level (approximately 15 CFU/sample), and five were uninoculated. For *Salmonella* spp., the samples were enriched in 90 ml of BPW supplemented with vancomycin at 41.5°C for 18 h. For *Listeria* spp., the samples were enriched in 90 ml of Demi-Fraser broth at 37°C for 24 h, followed by a secondary enrichment in Fraser broth at 37°C for 24 h. The enriched samples were analyzed using a LAMP-based molecular method and culture was confirmed. For culture confirmation of *Salmonella* spp., secondary enrichment in Rappaport-Vassiliadis broth was used.

Results: For low-inoculum samples, *Salmonella* Typhimurium and *L. innocua* were detected in 87 and 73% of the samples, respectively, by both molecular and culture confirmation methods. All samples inoculated at high levels were positives and uninoculated samples were negative. There was 100% agreement between the molecular and the culture confirmation for each of the samples.

Significance: The LAMP-based molecular method coupled with the enrichment protocols were able to reliably detect low levels of *Salmonella* Typhimurium and *L. innocua* in the presence of high levels of LAB.

P2-152 A Localized Surface Plasmon Resonance Sensor Coupled with Magnetic Nanobeads-based Immunoseparation for Rapid and Sensitive Detection of *Escherichia coli* O157:H7

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❖ ***Developing Scientist Competitor***

Introduction: *Escherichia coli* O157:H7 is one of the most commonly identified foodborne pathogens for humans and animals; it poses a threat to human health and causes substantial economic cost to society. The rapid and sensitive detection of foodborne pathogens is critical in order to ensure food safety.

Purpose: The objective of the present study was to develop a localized surface plasmon resonance (LSPR) sensor coupled with magnetic nanobeads-based immunoseparation (MNBs-IS) for rapid and sensitive detection of *E. coli* O157:H7 in foods.

Methods: Biotinylated anti-*E. coli* O157:H7 antibodies were immobilized on streptavidin pre-coated MNB (100 nm) surfaces to specifically capture and isolate target bacteria from a food matrix. The cleaned LSPR sensor chip was functionalized with 16-mercaptohexadecanoic acid and then activated with carbodiimide hydrochloride and *N*-hydroxysuccinimide to covalently bond with the anti-*E. coli* O157:H7 antibodies. After being blocked with polyethylene glycol, the sensor chip was ready for detection. In tests, 100 µl of the separated MNBs-*E. coli* complexes were pumped into the LSPR chip and measured using a LSPR analyzer.

Results: The results showed that the LSPR/MNB-IS sensor could shorten the detection time down to 4 min, with a flow rate of 20 µl/min. The detection range of *E. coli* O157:H7 was 10^2 to 10^7 CFU/ml, with a detection limit as low as 82 cells in a sample of 100 µl. The MNBs used in this study were served not only in sample pretreatment, but also in amplification of the detection signal. When *E. coli* pure culture with concentration below 10^5 CFU/ml was tested without MNBs, no binding signal was observed, which confirmed the signal amplification by MNBs. No interference was observed with non-target bacteria.

Significance: The developed LSPR/MNB-IS sensor is potentially a rapid, specific, and simple approach for detection of *E. coli* O157:H7 at low concentrations in foods.

P2-153 Evaluation of Three Enrichment Procedures for Improved Detection and Isolation of *Escherichia coli* O157:H7 in Artificially Contaminated Sprouts

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Introduction: E. coli O157:H7 has been implicated in foodborne disease outbreaks with sprouted seeds. However, detection and isolation by standard cultural methods can be difficult due to the high background microflora associated with this matrix. This study evaluated three procedures for rapid detection and isolation of *E. coli* O157:H7 from artificially contaminated sprout samples.

Purpose: Sprouts (alfalfa, broccoli and mung bean) were inoculated with *E. coli* O157:H7. At the low level (~1 cfu/g), 20 samples were inoculated, at the high level (~10 cfu/g), five samples were inoculated and 5 uninoculated controls were stored at 4°C for 72 h before being enriched for the pathogen. Three enrichment procedures were used: (1) BAM modified buffered peptone water + pyruvate (mBPWp) for 5 h at 37°C, followed by addition of acriflavine (A), cefsulodin (C), vancomycin (V) and further incubated at 42°C to further enhanced selectivity; (2) mBPWp with CV held at 42°C with shaking and (3) mBPWp with CV held at 42°C without shaking.

Methods: Enriched samples were streaked directly, diluted and plated or treated with Dynabeads® MAX *E. coli* O157 for immunomagnetic separation (IMS) then streaked onto TC-SMAC, R&F® *E. coli* O157 Chromogenic medium and Chromagar O157 for cultural recovery of the pathogen from the artificially contaminated samples. Real-time PCR detection of the *E. coli* O157:H7 was done on the ABI 7500 Fast platform, screening for *stx*1, *stx*2 and O157*wzy* gene targets.

Results: Primarily at the lowest levels of inoculation, test conditions (2) and (3) were more efficient at recovering the pathogen from alfalfa sprouts and mung bean sprouts than condition (1), while no difference was observed between the methods for broccoli sprouts.

Significance: This study demonstrates the importance of validation for various sample processing techniques in high background food matrices.

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P2-154 Comparative Growth of Alternate Environmental *Listeria* Strains in Selective Enrichments and Competitive Effect on Detection and Recovery of *Listeria monocytogenes*

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Introduction: Detection of *Listeria monocytogenes* typically includes selective enrichment steps due to the presence of low initial populations and background microbiota interference. Detection and recovery of *L. monocytogenes* can be limited by the choice of enrichment media. In produce handling environments, swabs are commonly found to contain mixed *Listeria* spp.

Purpose: To compare the growth kinetics of environmental *Listeria* strains in Actero Listeria enrichment broth (ALEM), Half-Fraser Broth (HFB) and *Listeria* enrichment broth (LEB) and the influence of these alternat *Listeria* spp. during co-culture with *L. monocytogenes*

Methods: Growth kinetics of 12 *L. monocytogenes* and 12 alternate *Listeria* isolates recovered from produce handling environments was determined using a microplate assay. Optical density values were converted to CFU/ml from standard curves. The growth rate (µ) and generation times (GT) were determined during the exponential growth phase. Three *L. monocytogenes* and alternate *Listeria* spp. were inoculated in pairs to the enrichment broths at final concentration of log 2 CFU/ml. After incubation, decimal series dilution aliquots were plated on CHROMagar *Listeria* and RAPID'*L mono* agars.

Results: Growth in HFB was slightly faster (GT=0.8 to 1.3) than ALEM (1.2 to 1.9 h) and LEB (1.3 to 2.4 h). *Listeria* strains showed a significantly shorter lag phase in ALEM (13±1 h) compared with HF (19±2 h) and LEB (19.5±1 h). Similar end-point populations were observed for HF-24 h (9.2 log CFU/ml), LEB-48 h (9.0 log CFU/ml), and ALEM-24 h (8.7 log CFU/ml). *Listeria* strains in HF were still in growing phase at the end of 24 h enrichment. In 18 of 27 isolate pairs, alternate *Listeria* spp. reached higher levels than *L. monocytogenes* from 0.2 to 1.7 log CFU/ml. The type of enrichment broth influenced the potential for interference from Listeria inter-species competition.

Significance: Environmental swabs harboring altnerate *Listeria* species can mask the presence of *L. monocytogenes.* This study contributes to improved *Listeria* and *L. monocytogenes* detection and recovery.

P2-155 Development and Evaluation of Sequence-based Typing Services for Epidemiological Tracking of *Vibrio parahaemolyticus*

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Introduction: *Vibrio parahaemolyticus* is a common inhabitant of coastal estuaries, and in warmer months can accumulate to relatively high levels in the shellfish that populate those waters. Human gastrointestinal infection typically follows ingestion of raw oysters and can lead to extended closures of implicated oyster beds with serious economic consequences.

Purpose: To reliably track down the source of human infection and to monitor the spread of virulent strains, user-friendly and affordable typing services that provide sufficient resolution for epidemiological analysis were developed.

Methods: Bioinformatic analyses of the GenBank Nucleotide database identified the polymorphic tandem repeat-containing loci VpMT1 and VpMT2 on chromosomes 1 and 2 as promising typing targets, resolving 19 of 20 and 20 of 21 strains, respectively. Further analysis of the larger RefSeq Genomes database (>300 strains) confirmed these results, yielding Simpson's diversity indexes of >0.99 (compared to 0.88 to 0.92 by length analysis of these same loci in previously reported MLVA studies).

Results: Phylogenetic analysis identified multiple clusters representing strains known or likely to be epidemiologically related, e.g., nine serotype O4:K12 strains isolated from the midwestern United States and Pacific Northwest patients in 2007. Correlation with multilocus sequence typing was strong, but resolution was higher, e.g., North American ST36 strains yielded 17 VpMT1 alleles. In the laboratory, VpMT1 and VpMT2 were robust, resolving 15 of 16 strains following amplification and sequencing directly from heat-killed colonies. Moreover, the ability to detect and type *V. parahaemolyticus* directly from oysters, without enrichment or colony isolation, was demonstrated. Specifically, three of seven oysters harvested in September and two of 24 harvested in October yielded VpMT-nested PCR products whose sequences were unique but closely matched GenBank entries from North American clinical or environmental isolates.

Significance: VpMT typing, facilitated by safe and convenient outsourcing, represents a promising new tool for *V. parahaemolyticus* epidemiology and surveillance.

P2-156 CbMT Sequence Typing for Identification and Tracking of Foodborne *Clostridium botulinum* Outbreaks

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Introduction: *Clostridium botulinum* sporadically contaminates various foods, where it may propagate anaerobically and produce a potent neurotoxin. To identify outbreaks and track down their sources, rapid epidemiological analysis is required, which in turn reqires strain typing. Multiple methods have been developed for *C. botulinum* typing, including multilocus variable number of tandem repeats analysis (MLVA) and, most recently, whole genome sequencing. To varying extents, these methods have limitations related to strain resolution, turnaround time, data portability, technical complexity, and cost. Furthermore, these methods typically depend on pure cultures and are hence incompatible with crude samples such as foods and stool; this is problematic, as clinical laboratories increasingly rely on culture-independent diagnostic tests.

Purpose: A promising alternative is polymorphic locus sequence typing (PLST), which analyzes through conventional, inexpensive, and technically robust PCR and Sanger sequencing the one or two most phylogenetically informative loci within the genome of a bacterial species.

Methods: To extend PLST to *C. botulinum*, genome sequences of representative strains were bioinformatically analyzed for tandem repeat regions that combined extensive polymorphism (insertions/deletions and single nucleotide variants) with presence in all or nearly all strains.

Results: The most promising loci, CbMT1 and CbMT2, include TCTATAC and AGTTCT repeats within intergenic and membrane protein-coding regions, respectively. Both repeats were previously reported to be the most informative loci in MLVA studies, yielding diversity indexes of 0.95 and 0.92 based on length alone. CbMT1 and CbMT2 loci from the approximately 120 *C. botulinum* strains represented in GenBank databases were downloaded and phylogenetically analyzed. This resolved 69 and 87 distinct alleles, respectively, with several clusters representing strains known or likely to be epidemiologically related. Diversity indexes, following removal of epidemiological replicates, were 0.97 and >0.99.

Significance: CbMT typing, facilitated by safe and convenient outsourcing, represents a promising new tool for *C. botulinum* epidemiology.

Poster

P2-157 Sequence-based Typing for Tracking Foodborne Shiga Toxin-producing *Escherichia coli*

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Introduction: Detection of Shiga-toxigenic *Escherichia coli* (STEC), particularly O157 and the six additional serotypes that are considered adulterants, is a major component of food safety efforts. However, detection alone cannot reliably track down the source of the contamination; for this, strain typing is required. Typing methods currently used by regulatory agencies, including pulsed-field gel electrophoresis and whole genome sequencing, are costly, technically complex, and require pure cultures, and hence are impractical for the routine typing needs of food processors.

Purpose: Polymorphic locus sequence typing (PLST) is an affordable and user-friendly alternative based on PCR and sequencing of the one or two most phylogenetically informative loci within a species genome. PLST application to *Listeria* and *Salmonella* have recently been described; here, PLST is extended to STEC and compatability with diverse samples demonstrated.

Methods: Tandem repeats, which exhibit relatively high rates of polymorphism, were bioinformatically identified in the genome sequences of representative STEC strains. Repeats plus flanking sequences were screened by BLASTN analyses to GenBank genome databases (including >5,000 *E. coli* strains).

Results: Two promising candidates were identified: EcMT1 for pan-*E. coli* typing and EcMT2 optimized for O157 strains. Literature review revealed that both encompass tandem repeats with high diversity indexes (0.90 and 0.95, respectively, based on length alone) within established protocols for multilocus variable number of tandem repeats analysis. Diversity indexes based on EcMT1 and EcMT2 sequence analysis were higher (0.96 and 0.99, respectively), reflecting polymorphism in the form of both insertion/deletion and single nucleotide variants. In the laboratory, EcMT typing was robust, yielding high-quality sequences following amplification from crude samples including heat-lysed colonies, ground beef enrichments, unenriched pork sausage, and total stool DNA.

Significance: Application to tracking was illustrated by EcMT clustering of bovine, spinach/lettuce/beef, and clinical isolates from three unrelated 2006 STEC outbreaks.

P2-158 Toward an Advanced Analytical Approach for Detection of Enterohemorrhagic *Escherichia coli* in Food Using New Genetic Markers

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Introduction: Standard molecular methods for detecting pathogenic Shiga toxin-producing *Escherichia coli* (STEC) in food relies on screening of *stx* and *eae* genes, followed by detection of the Top 5/7 enterohemorrhagic *E. coli* (EHEC) serogroup associated genes. The major limitation is that co-amplification of these genes does not signal association of these genes in the same cell. Thus, this approach generates a significant number of presumptive positives for EHEC which cannot be confirmed. Optimization of the current EHEC screening method is needed to avoid this limitation that causes substantial loss for the food industry.

Purpose: The present study aimed at proposing and validating a new EHEC screening approach based on co-detection of *stx* & *eae* genes and three new single DNA EHEC markers (*espK, espV*, *CRISPR_O26E*) to identify all EHEC strains and reduce the number of presumptive positive samples subjected to a serogroup screening.

Methods: A total of 320 beef, 22 raw milk, and 74 raw milk cheese natural broth enrichment samples were analyzed. DNA extraction was done with the GENE-UP Lysis kit. PCR screening of *stx* and *eae* genes was performed with the GENE-UP STEC-*stx*&*eae* kit, and detection of *espK*, *espV* and *CRISPR_O26E* markers was realized with the GENE-UP New Marker EHEC RUO kit. A confirmation step on dairy samples was performed by direct spread of enrichment broth onto ChromID-CT media and PCR screening on bacterial colonies.

Results: The co-detection of *stx* & *eae* and at least one of the three new EHEC markers reduced the number of presumptive positive beef samples by 60%, raw milk samples by 20%, and raw milk cheese samples by 75%. A total of five EHEC and five attaching and effacing *E. coli* (AEEC) were isolated from the 36 dairy products that tested positive for both *stx, eae*, and new EHEC markers. No STEC or AEEC were isolated from the *stx* and *eae* positive samples that tested negative for the new EHEC marker.

Significance: This new approach allows a significant reduction in the number of presumptive positive intended to be screened for serogroup determination.

P2-159 Withdrawn

P2-160 Performance Evaluation of Lyophilized *Listeria monocytogenes* and *Salmonella* spp. Green Fluorescent Protein Variant Strains for Industrial Quality Control Applications

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Introduction: The implementation of U.S. Food and Drug Administrationn's Food Safety Modernization Act (FSMA) has increased the focus on prevention-based food safety systems across the food industry. This has in turn increased the significance of all quality control (QC) processes, especially those related to microbial testing, and their impact on maintaining quality standards and meeting regulatory requirements. Microbes with known phenotypic properties are routinely used to monitor the quality of the testing processes; however, genetic modifications due to repeated culturing often alter the phenotypic integrity of these microbes. Moreover, the QC organisms utilize the same phenotypes as the target pathogens, making it difficult to test a QC microbe alongside an unknown sample or to parse a contamination event. Here we describe performance evaluation of lyophilized green fluorescent protein (GFP)-variants of *Listeria monocytogenes* and *Salmonella* spp. (Bioball Luminate [BL]) with known 30 CFU/unit organisms.

Purpose: The objective of this study was to assess any interference of GFP on the widely used VIDAS (VD) immuno-based system or the GENE-UP (GU) based PCR system, while also confirming all results with the culture methods.

Methods: In total, 180 *L. monocytogenes* samples were tested in two (LPT, LMX) media, where LPT was tested at 30°C (VD; *n*=60) and 35°C (GU; *n*=60), and LMX was tested at 37°C (VD; *n*=60). For *Salmonella*, 120 samples were tested on VD (buffered peptone water +supplement @42°C; *n*=60) and GU (BPW @42°C; *n*=60). All the presumptive results were confirmed by culture methods, i.e., ISO/FDIS 11290-1: *Listeria* and ISO 6579-1: *Salmonella*.

Results: Data showed no interference of GFP on the functioning of either system, and the culture methods confirmed all the presumptive results from VD and GU.

Significance: These data support: 1) the reproducible performance of BL providing consistent results regardless of the detection system used, and 2) a viable QC organism alternative that offers the advantage of an easily discernible phenotype.

P2-161 Performance Evaluation of a Fluorescence Resonance Energy Transfer-based Real-time PCR Assay for the Detection of *Salmonella* spp. in Pecans

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Introduction: Even though recent surveys by the U.S. Food and Drug Administration (FDA) and others estimate the overall prevalence for *Salmonella* in tree nuts (including pecans) to be low (0.55 to 4.20%), the need for better mitigation strategies is driven by the recent implementation of the Food Safety Modernization Act (FSMA). *Salmonella* detection systems are a crucial component of this larger prevention-based strategy and with the increasingly complex food distribution logistics, the significance of accuracy and faster time to results is increasingly important. Largely, PCR has filled this need over the traditional culture methods; however, most commercial PCR systems remain inherently burdened with cumbersome sample preparation, and complex instrumentation and interpretation tools.

Purpose: The objective of this study was to evaluate the performance of fluorescence resonance energy transfer (FRET)-based real-time *Salmonella* PCR assay (GENE-UP [SLM]) for the detection of *Salmonella* spp. in pecans per AOAC validation guidelines.

Methods: The evaluation was performed on pecans (25 g) using the following study design: 30 unpaired samples for each matrix, five replicates were inoculated at a high inoculation level, 20 at a low inoculation level and evaluated along with five uninoculated control replicates. After sample enrichment in BPW broth, unpaired test portions were evaluated by both the SLM and the reference (FDA Bacteriological Analytical Manual [BAM], Ch. 5). All presumptive SLM results were confirmed with reference and the SLM alternative confirmation procedure.

Results: Statistically equivalent performance (95% confidence interval) were observed upon {dPOD}, where the SLM demonstrated no significant differences from the reference method. [$dPOD_{cp}$: 0.0; LCL:-0.43; UCL: 0.43] for high and uninoculated, and [$dPOD_{cp}$: -0.10; LCL: -0.34; UCL: 0.15] for low samples.

Significance: These data demonstrate a highly sensitive detection of *Salmonella* spp. using the SLM assay that is comparable to FDA-BAM, Ch. 5. The convenient sample preparation along with a user-friendly instrumentation and software interface makes SLM a viable alternative for *Salmonella* detection in pecans.

P2-162 Comparison of Serological Method with Two Molecular Methods in Serotyping *Salmonella* Strains

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Introduction: Traditionally *Salmonella* is serotyped by agglutination, but commercially available Luminex xMAP *Salmonella* serotyping assay and Check&Trace *Salmonella* Kit by Check-Points have been used in recent years. The Luminex assay identifies seven O Groups, 35 H antigens, and three additional targets (*fljB, sdf, Vi*), and in combination, can detect about 100 most commonly seen serotypes. The Check-Points assay is a microarray-based serotyping method that targets on multiple single-nucleotide polymorphism loci across the genomes and can identify over 300 serotypes.

Purpose: Comparing agglutination testing, Luminex xMAP *Salmonella* serotyping assay, and Check&Trace *Salmonella* kit by serotyping 200 *Salmonella* isolates.

Methods: Agglutination was done in the National Veterinary Services Laboratories (NVSL) and/or a commercial laboratory; Luminex xMAP and Check&Trace serotyping were done in our lab following manufacturers' instructions.

Results: A total of 27 serotypes were identified from the 200 *Salmonella* strains; the number of isolate per serotype ranged from one to 32. The traditional serological method detected 199 samples that matched either Luminex xMAP and/or Check&Trace results. Assuming the serotyping result is correct if it is detected by at least two of the three methods, the correct rate for agglutination is 99.5%. Luminex xMAP detected 190 samples that matched the traditional and/or Check&Trace serotyping results with a correct rate of 98%. Check&Trace detected 194 isolates that matched with traditional and/or Luminex xMAP genotyping data, fewer than the other two methods, resulting a correct rate of 97.0%. However, the remaining six have generated unique microarray patterns and have been assigned to specific serotypes. Once the database is updated, the correct rate would be 100%.

Significance: Traditional *Salmonella* serotyping is generally time-consuming and labor-intensive. Both Luminex and Check-Points assays are nucleic acid-based high-throughput methods. Our results demonstrated that molecular serotyping with Check&Trace or Luminex assays are accurate, rapid, and high-throughput alternatives to the traditional serological method for *Salmonella* serotyping.

P2-163 Validation of the 3M Molecular Detection Assay for the Detection of *Salmonella* Version 2 in a Variety of Foods against Traditional Methods

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Introduction: Salmonella is frequently associated with food and is a significant food safety concern. The 3M Molecular Detection Assay *Salmonella* version 2 (MDA2) is used with the 3M Molecular Detection System for the rapid and specific detection of *Salmonella* in enriched food and environmental samples. Results can be obtained after as little as 18 h of incubation. The 3M Molecular Detection Assays employ isothermal amplification of nucleic acid sequences to achieve specificity, efficiency, and rapidity, utilizing bioluminescence to detect the amplification.

Purpose: The objective of this study was to evaluate the performance of MDA2 against the Compendium of Analytical Methods MFHPB-20 in a variety of food matrices for the inclusion in the Compendium of Analytical Methods as a Laboratory Procedure (MFLP).

Methods: MDA2 and the comparative reference method (MFHPB-20) were analyzed by testing five food categories (raw poultry, dairy, RTE meat and poultry, fruit and vegetable products, and eggs and derivatives). Three separate inoculum levels were used: 20 samples at a "low inoculum level" to give fractional recovery (1 to 5 CFU/25g), 20 samples at a "high inoculum level" at approximately 1 log CFU/25g higher, and five negative controls. Both limit of detection (LOD) testing and compositing were incorporated into the study design.

Results: Statistical analysis was conducted using the probability of detection statistical model and exceeded the criteria outlined in "The procedure for the development and management of food microbiological methods" (Health Canada, Microbiological Methods Committee). LOD results showed a range of 0.294 to 2.5 MPN/25g.

Significance: MDA2 showed excellent performance and exceeded the requirements of the MMC. This new method offers the capability of detecting *Salmonella* species in foods after only 18 to 24 hours of incubation, thereby reducing presumptive reporting times.

Poster

P2-164 Amplified Luminescent Proximity Homogenous Assay-linked Immunosorbent Assay for the Detection of Shiga Toxin 2 in Foods Containing Shiga Toxin-producing *Escherichia coli*

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Introduction: Food poisoning due to Shiga toxin-producing *Escherichia coli* (STEC) is a consistent cause for concern because of its association with hemorrhagic colitis and hemolytic uremic syndrome. Thus, detection and elimination of STEC in food products is crucial for reducing the incidence of foodborne illness.

Purpose: Amplified luminescent proximity homogenous assay-linked immunosorbent assay (AlphaLISA) is an emerging technology that employs an efficient, homogeneous, bead-based immunoassay. Here, a novel AlphaLISA was developed and optimized for the detection of Shiga toxin 2 (Stx2).

Methods: Monoclonal antibodies for each of the two major subunits of Stx2 were employed in this assay. Donor beads coated with streptavidin bound one set of biotinylated antibodies, while the other set of antibodies were directly conjugated to acceptor beads. When the antigen is present, it becomes bound by both antibody sets, placing the corresponding beads in close proximity. Upon excitation by an Alpha laser, the donor bead emits singlet oxygen molecules that excite a photosensor on the acceptor beads, creating a detectable signal.

Results: Efficacy and sensitivity trials showed the AlphaLISA could detect ≥0.5 ng/ml of purified Stx2, which was comparable to the industry standard enzyme-linked immunosorbent assay (ELISA) tests for Stx2 detection. Further evaluation with STEC-inoculated romaine lettuce and ground beef samples demonstrated that both the AlphaLISA and the ELISA could discern uninoculated samples from 1x and 10x diluted samples containing 10 CFU/ml of STEC enriched for 16 h in modified tryptic soy broth containing mitomycin C.

Significance: There are many benefits to the newly developed AlphaLISA, including a shorter and simpler protocol that requires less manual manipulation and no wash steps compared to the ELISA. Overall, this AlphaLISA allows for the rapid detection of Stx2 in food matrices using a highly sensitive, robust, and automatable process that is amendable to high-throughput screening.

P2-165 A Fit-for-Purpose Evaluation of VIDAS LPT and LIS Immunoassays Compared to U.S. Food and Drug Administration Bacteriological Analytical Manual Cultural Methods for Growth and Detection of *Listeria monocytogenes* in Fermentation Starter Culture Products

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Introduction: Fermentation starter culture products contain high levels of live bacteria from single or multiple species. It was not known if the high background flora would present difficulties in the growth and recovery of *Listeria*. Therefore a review of *Listeria* detection from 11 starter cultures (liquid and dried) was performed.

Purpose: The purpose of this study was to challenge 11 starter cultures with *Listeria* levels low enough to challenge the assay, but also high enough to ensure growth if matrices were not inhibitory.

Methods: Samples of 25 g each were diluted 1:10 in enrichments specific to the detection kit or the standard cultural method (U.S. Food and Drug Administration Bacteriological Analytical Manual). Enrichments were inoculated in triplicate with *Listeria monocytogenes* (30 CFU, BioBall, *n*=33) and incubated. The data set included three uninoculated replicates of each product type (*n*=33). Inoculated and uninoculated enrichments were screened by immunoassays. All immunoassay and reference sample enrichments were streaked to selective/differential media and examined for the presence of typical colonies.

Results: The LIS immunoassay recovered *Listeria* from all nine of the liquid fermentation products, but failed for two dried starter culture products. The LPT immunoassay and reference method also demonstrated no positive *Listeria* growth in the dried products. A 1:20 dilution of these products recovered *Listeria* by all three methods. The LPT enrichment displayed difficulty in recovering *Listeria* from a single liquid product, while the reference method demonstrated difficulties from a different liquid product with an overall agreement of 96.96% with the LIS immunoassay and 92.42% with the LPT immunoassay.

Significance: Determining the proper conditions for growth and recovery are important with new matrices. Starter cultures with high levels of competing microflora can be challenging for cultural or rapid methods. The data from this study demonstrate the efficacy of automated immunoassays compared to cultural methods, but also illustrate the need to verify and optimize methods for individual sample types.

P2-166 Evaluation of Several bioMérieux VIDAS Assays and U.S. Food and Drug Administration Bacteriological Analytical Manual Cultural Methods for the Detection of *Salmonella* Typhimurium in Fermentation Starter Culture Products

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Introduction: According to U.S. Food and Drug Administration (FDA) and AOAC guidelines, a validation study is required for food matrices not previously validated *and* not included in the food category in the original validation study. Preliminary screening of groups of matrices using several rapid methods would detect obvious recovery issues of target pathogens due to matrix effects and help determine the most appropriate method for a subsequent matrix validation study.

Purpose: The purpose of this study was to design a preliminary "fit-for-purpose" method assessment and trial the protocol using a group of challenging food matrices and several methods. Eleven fermentation starter culture products with high levels of mixed and individual cultures were inoculated with *Salmonella* Typhimurium and were screened using the VIDAS SLM, SLM Easy, and *Salmonella* UP immunoassays or a standard cultural method.

Methods: Samples of each fermentation culture product (25 g, liquid and lyophilized) were diluted 1:10 in sample enrichments specified by each detection kit or the standard cultural method (FDA Bacteriological Analytical Manual [BAM]). Enrichments were inoculated in triplicate with *Salmonella* Typhimurium at 30 CFU (BioBall) and incubated (*n*=33). The data set included three uninoculated replicates of each product type (*n*=33). Inoculated and uninoculated enrichments were screened by the immunoassays. All immunoassay and reference sample enrichments were streaked to selective/differential isolation media and plates were examined for the presence of typical colonies.

Results: The SLM immunoassay performed the best with all 11 starter cultures and was comparable to the FDA BAM reference method in its recovery of the target organism. The other immunoassays varied in their detection of specific fermentation cultures and cultures in lyophilized form.

Significance: A preliminary method assessment is a useful and necessary tool to rapidly identify matrix interference and appropriate assays for subsequent matrix validation studies.

P2-167 Comparison of Shiga Toxin-producing *Escherichia coli* Detection Systems

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Introduction: Testing of beef products for the presence of Shiga toxin-producing *Escherichia coli* (STEC) occurs in a number of international markets. Exporters wishing to send beef products to North America must ensure their products are free of detectable strains of seven STEC serogroups (O157 and Big 6). The number of STEC test systems available has increased steadily over the past five years.

Purpose: Evaluate the performance of STEC test systems not presently routinely used in Australia.

Methods: One hundred manufacturing beef enrichment broths that were identified as potential positive (PP) for a Big 6 serogroup and had been tested using a government-approved STEC confirmation process were included in the study. Each broth was subsequently tested by a total of eight STEC screening tests and one STEC confirmatory test. In addition, quantitative PCR was used to determine the concentrations of *stx* and O-antigen genes present in each broth.

Results: The number of broths yielding positive results in each screening test ranged from 39 to 85. Screening tests that used additional gene targets or that included immunomagnetic separation prior to PCR were less likely to give a positive result (39 to 56 positives) in comparison to systems that tested for *stx*, *eae*, and O antigens only (64 to 85 positives). Twelve of the 100 samples were culture confirmed as positive for Big 6 STEC, and the majority of test systems detected 11 (91.7%) of 12 confirmed samples. In contrast, the STEC confirmatory test concluded that 16 samples were positive for Big 6 STEC.

Significance: In Australia, the number of PP that culture confirm is low. In this study, STEC test systems that utilised additional gene targets generated reductions in PP call rates without impacting the ability to identify broths that ultimately confirm for STEC.

P2-168 Evaluation of Pall GeneDisc STEC Top 7 Test System for Detecting Shiga Toxin-producing *Escherichia coli*

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Introduction: Testing of beef products for the presence of Shiga toxin-producing *Escherichia coli* (STEC) typically relies on detecting genes that encode Shiga toxin (*stx*), intimin (*eae*), and specific O antigens. STEC test systems that incorporate additional gene targets or utilise a STEC concentration procedure may assist in decreasing the number of potential positives (PPs) that require culture confirmation.

Purpose: Evaluate the performance of the Pall GeneDisc STEC Top 7 on Australian manufacturing beef enrichment broths.

Methods: A total of 101 manufacturing beef enrichment broths previously identified as PP for a Top 7 serogroup were included in the study. Twenty-one of the broths were confirmed positive for O157 (11) or O26 (10). Each broth was subsequently tested using the Pall STEC Top 7 system, which involved initial PCR analysis of the samples followed by immunocapture and secondary PCR analysis if the initial PCR analysis indicated the sample was a PP. For comparison, all samples were screened for Top 7 STEC using the United States Department of Agriculture Food Safety Inspection Service (FSIS) primers and probes.

Results: The number of broths yielding positive results following initial screening with the Pall STEC Top 7 test system was 53. Secondary PCR screening of the samples following immunocapture reduced the number of positives from 53 to 46. The group of 46 samples included all 21 samples that had previously been confirmed as positive for O157 or O26. In comparison, screening of the 101 samples using the FSIS primers and probes gave 79 positive results and also identified all 21 confirmed samples.

Significance: In Australia, the ratio of confirmed STEC-positive samples to PPs remains low. In this study, the Pall GeneDisc STEC Top 7 test system utilized additional gene targets and a STEC concentration step to reduce the number of PPs requiring culture confirmation by >40%. Such reductions could provide economic benefits to Australian beef processors.

P2-169 Metagenomic Assessment of Manufacturing Beef Enrichment Broths

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Introduction: Culture confirmation of non-O157 Shiga toxin-producing *Escherichia coli* (STEC) is a laborious process that is often unable to isolate the organism that the screening test indicates may be present. Reasons for low isolation rates are plentiful; however, they may relate to relatively low concentrations of STEC being present in high concentrations of diverse bacterial populations following enrichment.

Purpose: Determine if manufacturing beef enrichment broths that confirm have lower bacterial diversity and increased virulence gene content than those that do not confirm.

Methods: Twenty enrichment broths of manufacturing beef comprising nine confirmed positives and 11 potential positives (O-antigen, *stx*- and *eae*-positive) were included in the study. Metagenomic analysis of the broths was used to determine the relative abundance, taxonomic composition, and *E. coli* virulence and O-antigen gene prevalence of each sample. Principal components analysis (PCA) analysis was used to compare taxa present at genus and species level, as well as virulence gene content. In addition, quantitative PCR was used to determine the concentrations of *stx* and O-antigen genes present in each broth.

Results: Manufacturing beef enrichment broths were dominated by the presence of *Clostridium perfringens* and *E. coli*, with a mean abundance of 36.3 and 19.7% across all samples. Mean *E. coli* abundance was lower in confirmed samples (10.94%) than samples that did not confirm (26.87%). Confirmed samples did not possess lower bacterial diversity and increased virulence gene content when compared to broths that did not confirm. Furthermore, in confirmed samples the target serogroup was often present at ratios lower than 1 in 1,000 *E. coli*.

Significance: Manufacturing beef enrichment broths are often dominated by non-*E. coli* organisms, with STEC likely to be present in concentrations of <1.0%. Further evaluation of STEC media and time/temperature enrichment conditions is required to achieve optimum isolation efficiency.

P2-170 Modification of Thread-based Microfluidic Device with Polysiloxanes for the Development of an Innovative Immunoassay to Detect *Salmonella* in Foods

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Developing Scientist Competitor

Introduction: The relatively poor sensitivity of thread-based microfluidic devices has limited their wide application. Polysiloxanes are inorganic polymers that have the ability to resist breaching by liquids and surfactants with low surface energy. Considering the potential of tuning its hydrophobicity, incorporating it into a thread-based device can potentially achieve desirable fluidic control and optimum device performance at a minimal cost.

Purpose: We aimed to demonstrate a new approach of tuning the hydrophobicity of polysiloxanes to manipulate the fluidic flow in the pores of cotton thread-based microfluidic devices.

Methods: A mixture of methanol and isopropanol was used as a diluent for siloxane precursor, which was included into the thread to enable rapid curing of polysiloxanes for fluidic control and enhanced detection sensitivity. *Salmonella enterica* Enteritidis was used as a model bacterium. The phenomenon of fluidic delay was evaluated by mathematical simulation.

Results: Twelve-fold diluted polysiloxanes enabled desirable fluidic delay and optimum interaction between the targeted antigen and detection antibody gold nanoparticles (dAb-AuNPs) in the thread-based immunoassay, generating more antigen dAb-AuNP complexes that bound to the capture antibody at the test zone and achieved signal enhancement (~10-fold compared to the unmodified device). This polysiloxane-modified device could detect *Salmonella* Enteritidis in phosphate-buffered saline, spiked whole milk, juice, and lettuce with the detection limit of 500, 1,000, 1,000, and 5,000 CFU/mL, respectively, without enrichment, which was comparable to or even more sensitive than the existing immunoassays.

Significance: This work expands the application of polysiloxanes in microfluidic devices for the detection of pathogenic bacteria in foods.

P2-171 In Vivo Screening Platform for Shiga Toxin-producing *Escherichia coli* Using *Caenorhabditis elegans* Model

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Undergraduate Student Award Competitor

Introduction: Enteropathogenic *Escherichia coli* (EPEC) cause life-threatening infections in humans as a consequence of the production of Shiga-like toxins. Shiga toxin-producing *E. coli* (STEC) like O157:H7 strains and non-O157 strains, containing four serogroup strains that induce severe diarrhoea and haemorrhagic colitis (HC), and can also lead to haemolytic uremic syndrome (HUS). Therefore, it is necessary that the toxic mechanism of these bacteria is investigated further.

Purpose: This study aimed to determine the virulence of STEC using the nematode *Caenorhabditis elegans* as a model system.

Methods: The toxicity of STEC was confirmed by measuring the reproductive power and lifespan of the *C. elegans* exposed to STEC strains. After identifying the virulence of STEC strains, two hypotheses were made about the toxicity factor: 1) STEC strains would form colonies in the intestinal tract of *C. elegans* host, creating toxic infections, and 2) the toxicity factor would be heat labile. Colonization and heat-treated bacteria killing assay were conducted.

Results: Statistically, a significant difference in the survival of *C. elegans* was demonstrated, causing fast death at eight to 10 days but OP50 on day 15. STEC strains severely reduced the reproduction of worms. The intestinal tract of the adult stage nematodes harboring OP50 was found to be 3.5, but STEC strains E15, 18, and 22 resulted in 4.1, 4.2, and 4.7 log CFU/ml per nematode, respectively. The heat-treated STEC strains revealed that they significantly increased the longevity of worms compared to non-heated STEC strains. In addition, PCR-based genomic profiling of Shiga toxin genes *stx1* and *stx2* among the selected STEC strains demonstrated that these toxins may be unrelated to the virulence of STEC strains.

Significance: These data suggest that STEC strains cause the toxicity infection and form colonies in the intestine of the nematode *C. elegans*. In addition, the Shiga toxin produced by STEC strains was heat labile.

P2-172 Development of a Liquid Crystal-based Immunoassay for *Campylobacter* spp.

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Introduction: The foodborne pathogen *Campylobacter* is a significant contributor to human gastrointestinal infections. Rapid and accurate detection assays for *Campylobacter* spp. are urgently needed. Liquid crystal-based immunoassay (LCIA) is a combination of immunomagnetic separation and a liquid crystal-based biosensor to detect foodborne pathogens. With the ability to detect the physical presence of microsphere-cell aggregations, the LCIA rapidly detects pathogens without a secondary antibody, which reduces the assay time compared to standard enzyme-linked immunosorbent assay (ELISA).

Purpose: The objective of this study was to develop an innovative rapid detection assay for *Campylobacter* spp. and evaluate its detection limit.

Methods: A LCIA designed against *Campylobacter* species was used to test cell cultures including *C. jejuni*, *C. lari*, and *C. coli;* other common foodborne pathogens including *Escherichia coli*, *Salmonella*, and *Listeria* were tested for exclusivity. A commercial positive control was used to evaluate the sensitivity of the assay.

Results: The LCIA detected 100% (12 of 12) of the tested *Campylobacter* spp. The assay excluded 85% (17 of 20) of the strains tested. The sensitivity of the assay was 6.8 log CFU/ml. The total assay time, including incubation, was less than 42 h. Ongoing tests will improve sensitivity with optimized capture antibody and incubation conditions.

Significance: This study demonstrates the use of this LCIA for *Campylobacter* spp. detection. The shorter assay time to result, compared to culture assays and standard ELISA, provides a quicker intervention and improved consumer safety.

P2-173 Real-time Detection of *Escherichia coli* O157:H7 and *Salmonella* in Raw Milk Using the BAX System

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Introduction: The trend of consuming more natural foods such as raw milk has been on the rise due to its perceived health benefits and enhanced nutritional qualities. However, natural foods are not necessarily safer. Raw milk is unpasteurized and more likely to harbour pathogenic bacteria compared to pasteurized milk. In fact, consumers who ingest unpasteurized dairy products are 840 times more likely to experience foodborne illness.

Purpose: Control measures to ensure the safety of raw milk generally involve quality and hygiene testing. These tests do not provide information on whether a pathogen could be present. With at least three raw milk recalls last year, this study was designed to evaluate the performance of a real-time PCR method to detect two important foodborne pathogens: *Escherichia coli* O157:H7 and *Salmonella*.

Methods: Forty 25-ml samples of raw milk were fractionally inoculated with either *E. coli* O157:H7 (*n*=20) or *Salmonella* Dublin (*n*=20) and held at 4°C for 48 h. *E. coli* O157:H7 samples were homogenized with 225 ml of pre-warmed (42°C) modified tryptic soy broth + 20 mg/L novobiocin, and *Salmonella* samples were homogenized with 225 ml of pre-warmed (42°C) buffered peptone water + 20 mg/L novobiocin. All samples were incubated at 42°C for 18 to 22 h and tested using real-time PCR both directly from the primary enrichment and after a brain heart infusion regrowth. Results were confirmed using the appropriate reference culture method.

Results: Samples tested with the real-time PCR assays for *E. coli* O157:H7 and Shiga toxin-producing *E. coli* screening detected seven positives, with 100% agreement to the ISO reference method. Samples tested with the real-time PCR assay for *Salmonella* detected 10 positives, with 100% agreement to the Health Canada reference culture method.

Significance: The results of this study demonstrate that the BAX System real-time PCR assays can accurately detect *E. coli* O157:H7 and *Salmonella* in 25 ml of raw milk with no significant statistical difference compared to the reference culture methods.

P2-174 Withdrawn

P2-175 Evaluation of the TRANSIA PLATE Staphylococcal Enterotoxins Kit for the Detection of Staphylococcal Enterotoxins in Selected Foods

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Introduction: Food poisoning with staphylococcal enterotoxins (SE) is a major health concern. Outbreaks of SE in a variety of foods have been reported. There is a need for a fully validated detection kit with good sensitivity.

Purpose: To validate TRANSIA PLATE Staphylococcal Enterotoxin (TPSE) according to AOAC International guidelines for validating qualitative binary chemistry and microbiological methods. Inclusivity/exclusivity, kit stability, and kit robustness were also tested.

Methods: Foods that have been implicated in SE poisoning were artificially contaminated with low levels of SE (SEA, SEB, SEC_1, SEC_2, SEC_3, SED, and SEE). These included chocolate eclairs, deli ham, canned mushrooms, raw milk cheese, and liquid infant formula. These samples were subjected to extraction to recover the SE. Contaminations were done at concentrations that yielded OD values near the threshold to achieve a fractional recovery (probability of detection [POD] between 0 and 1). Bacteria for inclusivity/exclusivity were grown in brain heart infusion broth and centrifuged, and the supernatant was sterile filtered and tested in TPSE. The robustness and stability of the kit were also tested.

Results: There was 100% detection of the SE at 0.1 to 0.3 ng/g or ml of food. Inclusivity showed that all 54 *Staphylococcus aureus* strains that produce SE were positive and all 30 non-SE-producing bacteria were negative. Kits that were 12 and 14 months old performed as well as a recently made kit.

Significance: Based on the results of the POD, inclusivity and exclusivity, and stability and robustness studies, TPSE has good sensitivity. This data was also used for the certification of TPSE as an AOAC Performance Tested Method.

P2-176 Robustness Study of Assurance GDS Assays on the Rotor-Gene Q Platform

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Introduction: Food pathogen detection systems are an integral part of today's food safety systems. It is critical that they provide accurate and precise results. Quality systems within the international regulatory environment mandate an acceptable level of accuracy and uncertainty in the assays used in the food industry in order to maintain a safe, high-quality food supply. This poster presents results on a couple of key instrument factors related to the robust performance of Assurance GDS assays (AGDSA).

Purpose: To establish a range of conditions within which the AGDSA, in conjunction with the Rotor-Gene Q rotary thermocycler, will perform to stated kit specifications.

Methods: Three different Rotor-Gene Q instruments were used for this study. Each was calibrated using the manufacturer's recommended Optical Temperature Verification system. A set of control runs was performed on each instrument with the kit in question following the exact details of the directions for use and proper instrument settings. Eight positive controls and eight negative controls were run to compare various deviations from the instrument parameters to compare performance under a variety of conditions. Those parameters include instrument cycling time and cycling temperature.

Results: Quality control specifications were met across the following ranges for the parameters tested: instrument cycling times (denature time=1 to 5 sec; anneal time=10 to 30 sec; extend time=10 to 30 sec), instrument cycling temperatures (denature temperature=87 to 97°C; anneal temperature=49 to 63°C; extend temperature=69 to 83°C), for the five AGDSA kits studied (*Salmonella* Tq, *Escherichia coli* O157:H7 Tq, *Listeria* spp. Tq, *Listeria monocytogenes* Tq, and *Cronobacter* Tq).

Significance: This study clearly demonstrates that the AGDSA function well and can be considered robust in regards to thermocycler cycling parameters.

P2-177 Detection of *Escherichia coli* O157:H7 and *Salmonella enterica* serotype Typhimurium Based on Cell Elongation Induced β-Lactam Antibiotics

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Introduction: Pathogenic bacteria such as Shiga toxin-producing *Escherichia coli* and *Salmonella* can cause severe foodborne diseases. Rapid and sensitive detection of these foodborne pathogens are therefore critical and can aid in reducing associated mortality rates.

Purpose: Rapid detection of *E. coli* O157:H7 and *Salmonella enterica* serotype Typhimurium in water and milk.

Methods: A novel method based on cell elongation induced by β-lactam antibiotics for direct microscope counting of bacteria was established. Combined with sample preparation steps of membrane filtration and magnetic separation, detection of pathogens could be realized by direct optical microscope counting of the elongated bacteria.

Results: The limit of detection of *E. coli* O157:H7 and *Salmonella* Typhimurium could reach 5 CFU/ml. Recoveries were 83.33±4.93% to 120.00±2.65% and 80.00±2.83% to 120.00±2.45% for water and milk samples spiked with 5 to approximately 100 CFU/ml bacteria, respectively. Results agreed with traditional plate counting method.

Significance: The proposed method could be successfully applied in the detection of *E. coli* and *Salmonella* Typhimurium in real samples (water and milk). This method is sensitive, cost-effective, rapid (<2 h) and shows a great potential for the detection of pathogens in various environmental and food samples.

P2-178 Comparative Evaluation of the Detection of *Salmonella* spp., *Salmonella* Typhimurium, and *Salmonella* Enteritidis in Different Poultry Matrices from a Slaughterhouse in Brazil

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Introduction: Salmonella spp. are the most frequent microorganism found in outbreaks of foodborne diseases; among its 2,500 serovars, *Salmonella* Typhimurium and *Salmonella* Enteritidis are highlighted in many food safety programs, especially in the poultry industry. Due to the need to confirm the absence of these serovars in poultry products, quality assurance laboratories face a daily challenge to obtain alternative, faster, and more specific methodologies. In this sense, the use of the iQ-Check real-time PCR system (iQC) ensures a reliable, less laborious, and less time-consuming method, with confirmatory negative results in 22 to 26 h.

Purpose: This study aimed to detect *Salmonella* Typhimurium and *Salmonella* Enteritidis in different poultry matrices from a slaughter house in Brazil.

Methods: Isolated colonies of *Salmonella* serovars (Typhimurium, Enteritidis, Heidelberg, and Minnesota) and *Citrobacter freundii* were tested with iQC kits and used as artificial contaminants. Nine aliquots of each *Salmonella* serovar confirmed-negatives-matrix (chicken cuts, mechanically separated meat, and seasoned turkey [*n*=27]) were prepared and inoculated with a mixture of contaminants post-non-selective enrichment: *Salmonella* Minnesota 10^6 + *C. freundii* 10^6; *Salmonella* Typhimurium 10^4 + *Salmonella* Heidelberg 10^6; *Salmonella* Enteritidis 10^4 + *Salmonella* Heidelberg 10^6. They were then tested in triplicate with the alternative method in comparison to the ISO6579 method; two negative samples from each matrix were used as controls. Samples were also tested with a commercially available loop-mediated isothermal amplification isothermal PCR test.

Results: The results from the isolated strains and the artificially contaminated samples tested with the alternative method were 100% in agreement with the reference method ISO 6579. The sensitivity, specificity, and efficacy were 100% compared to the reference method and dPOD analysis showed that there was no statistical difference between the test method and the reference method ISO 6579.

Significance: The iQ-Check real-time PCR system is a reliable, specific, and sensitive alternative method with the release of negative reports in 22 to 26 h, which ensures process cost savings in the food industry. The study also showed the importance of having an internal amplification control to monitor for inhibition in the sample.

P2-179 Rapid Detection of *Salmonella* Using Real-time PCR Assay in Meat, Poultry, and Whole Liquid Egg Enriched with an Improved Culture Broth

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Introduction: Although rapid testing of *Salmonella* in foods has advanced over the past two decades, the development and implementation of rapid, user-friendly, and accurate methods remains an important task for the food industry.

Purpose: This study aimed to improve Actero Salmonella Enrichment Media and optimize enrichment conditions for the rapid detection of *Salmonella* in foods using a reverse transcription PCR method.

Methods: Growth kinetics studies were carried out *in vitro* with intact and heat-injured *Salmonella* that were cultured for 18 h at 35°C in the proprietary broth V1.2 in comparison with buffered peptone water and universal pre-enrichment broth. Overall, 150 samples including raw ground beef (375 g), chicken carcass rinsate (30 ml), and whole liquid egg (100 g) were artificially contaminated with *Salmonella* and stabilized at 4 to 8°C for 48 to 72 h prior to testing. The samples were enriched with 30 to 1125 mL of the proprietary broth for 16 to 20 h at 35 to 39°C and then analyzed using Hygiena's BAX System Real-Time PCR Assay for *Salmonella*, as well as by direct plating.

Results: The proprietary broth showed a strong ability to provide an ideal growing environment for intact and sublethally heat-injured *Salmonella*, resulting in a significant reduction of lag phase duration and an increase in the growth rate of bacteria. The probability of detection model confirmed equivalent or better performance of the alternative method as compared to the United States Department of Agriculture Food Safety Inspection Service Microbiology Laboratory Guidebook 4.09 method. No false negative or positive outcomes were detected among 90 food samples analyzed by the alternative method.

Significance: The incorporation of specific selective growth substrates and stimulating co-factors, previously used as liquid supplements into a powdered formulation of Actero Salmonella, resulted in significant improvement of performance. This made the broth more user-friendly for application with different rapid assays.

Poster

P2-180 Improving the Recovery of *Shigella,* and Potentially Other Foodborne Pathogenic *Enterobacteriaceae,* in Presence of Commensal *Escherichia coli*

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Introduction: Shigella bacteria cause bacillary dysentery and are primarily transmitted between people or by ingesting contaminated food or water. Current detection methods involve anaerobic enrichment in a low-carbohydrate media at 42°C in presence of novobiocin. However, we recently observed that resident *Escherichia coli* were strong competitors for the recovery of *Shigella* under such conditions.

Purpose: This work aims to optimize the broth and conditions for the recovery of *Shigella flexneri* from high-level background food types in the presence of commensal *E. coli* and to compare this optimized broth for the recovery of other strains of *Shigella*, pathogenic strains of *E. coli*, and *Salmonella* using the broth and conditions described in their respective methods in the U.S. Food and Drug Administration's Bacteriological Analytical Manual as references.

Methods: We prepared multiples aliquots of concentrated rinsates from the surface of green onion, spinach, and cilantro to study the effect of the tested parameters in the presence of the same competitive flora in five replicate samples containing 1 to 7 *S. flexneri* cells expressing green fluorescent protein prior to enrichment. Except for cilantro samples, which naturally contained *E. coli*, samples were also spiked with 10 to 50 cells of *E. coli* K12 when needed. Numbers of fluorescent and total bacteria were obtained from serial dilutions onto non-selective tryptic soy agar to calculate the number of doublings of both the pathogen and the microbial population over the 22-h incubation and the proportion of *Shigella* post-enrichment.

Results: A tryptone broth containing casamino-acids, glutathione, vancomycin, novobiocin, and agar maximized the recovery of *S. flexneri* from high–level background food types containing *E. coli* after incubation in a generic incubator at 42°C. This optimized broth is currently tested for other strains of *Shigella*, pathogenic *E. coli*, and *Salmonella*.

Significance: The work emphasizes the importance of adding *E. coli* to spike and recovery experiments used to assess the performance of methods for the recovery of *Shigella* (and potentially pathogenic *E. coli* and *Salmonella*) from foods.

P2-181 Evaluation and Comparison of Rapid Methods for the Detection of *Salmonella* in Sprouted Chia Powder Using Different Preenrichment Media

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Introduction: Chia seeds (*Salvia hispanica*) have become one of the most popular "superfoods" in the health food community. Sprouted chia powder was implicated as the cause of *Salmonella* outbreak in 2014, with 31 cases reported in United States and 63 cases in Canada. Eight products containing sprouted chia powder have been recalled over the past several years due to *Salmonella* contamination.

Purpose: To evaluate and compare performances of the U.S. Food and Drug Administration (FDA)-developed quantitative real-time PCR (qPCR) and commercial VIDAS UP & Easy assays for the detection of *Salmonella* in four preenrichment media: lactose broth (LB), universal preenrichment broth (UPB), modified BPW (mBPW), and buffered peptone water (BPW).

Methods: Chia powder test portions (25 g each) were soaked in 225 ml portions of LB, UPB, mBPW, and BPW for 24 h at 35°C. The FDA Bacteriological Analytical Manual *Salmonella* culture method was followed after preenrichment. The aerobic plate count of chia powder was 3 log CFU/g and *Salmonella* level on the day analysis was 0.46 MPN/g. The qPCR was performed on 24 h preenriched culture on ABI Fast 7500. A four-way comparison among culture method, qPCR, VIDAS Easy, and VIDAS UP using BPW with or without *Salmonella* supplement was conducted. Automated DNA extraction techniques (Qiagen One-for-all and ABI PrepSEQ) were compared with manual extraction methods (boiling and InstaGene).

Results: The qPCR was as sensitive as the culture method in all media. However, lower Ct values from UPB, mBPW, and BPW as compared to LB indicated more *Salmonella* growth in these three broths than in LB after 24 h preenrichment. The qPCR, VIDAS Easy, and VIDAS UP were equivalent to culture method in BPW with or without supplement. However, BPW produced more positive test portions than BPW with supplement. The automatic DNA extraction methods were as effective as manual extraction methods.

Significance: This research provides rapid methods for high-throughput applications for the detection of *Salmonella* in chia powder.

P2-182 Evaluation of PCR-based Methods for the Identification of Hemorrhagic Enteroaggregative *Escherichia coli* in Sprouts

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Introduction: Enteroaggregative *Escherichia coli* (EAggEC) and Shiga toxin-producing *E. coli* (STEC) have been recognized worldwide as causes of foodborne gastroenteritis over the past three decades. In 2011, a hemorrhagic EAggEC (EAHEC) O104:H4 strain, having acquired a gene encoding Shiga toxin 2, caused an outbreak originating from contaminated fenugreek seeds in Germany, resulting in about 4,000 cases of gastroenteritis, 855 cases of haemolytic uremic syndrome, and 53 deaths. The European Food Safety Authority Scientific Opinion 2013 and EU Regulation 209/2013 suggest STEC and EAggEC molecular detection criteria and zero tolerance for the pathogens in seeds and sprouts, respectively.

Purpose: This study was conducted to evaluate a real-time PCR method for detecting EAggEC contamination in sprouts.

Methods: Inclusivity tests were conducted using a real-time PCR kit targeting *agg*R and *aai*C that was provided by Diatheva Srl (Italy). Mung bean and alfalfa sprouts (25-g samples) were artificially contaminated at 1, 10, and 100 CFU (20, 10, and two replicates, respectively) of EAHEC O104:H4. Samples were enriched as described in the U.S. Food and Drug Administration's Bacteriological Analytical Manual, Chapter 4A. Culture enrichments were tested using real-time PCR kits provided by Diatheva, detecting *agg*R/*aai*C, *stx*/*eae,* and wzx_{O104}. For bacterial isolation enrichment, cultures were subjected to immunomagnetic separation (IMS) using anti-O104 magnetic beads and plated on modified rainbow Agar and CHROMagar STEC. Presumptive O104 colonies were confirmed by latex agglutination and end-point PCR (*stx*, *agg*R, and wzx_{O104}).

Results: The inclusivity and exclusivity was 100% using a panel of 49 strains. Using the commercial real-time PCR kits, the artificially contaminated samples were 60 to 80% positive when contaminated with 1 CFU, and 100% at 10 to 100 CFU. Microbiological detection and confirmation by latex agglutination and PCR gave similar results (Cohen's kappa value between 0.8 and 1).

Significance: This study demonstrates the efficacy of the real-time PCR method for the specific and sensitive detection of EAHEC from sprouts.

Poster

P2-183 Identification of a Single Selective Enrichment Media for the Simultaneous Recovery of *Salmonella* and *Escherichia coli* O157 from Ground Beef Samples

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Introduction: The prevalence of foodborne pathogens like *Salmonella* and *Escherichia coli* O157:H7 has increased in ground beef products, resulting in numerous outbreaks of public health concern and economic losses to the producers. A single enrichment media for isolation of both *Salmonella* and *E. coli* O157:H7 would help in minimizing the resources used during the screening process and reducing the cost of screening isolates.

Purpose: The purpose of this study was to identify a single enrichment media for the recovery of both *E.* O157:H7 and *Salmonella* simultaneously from ground beef products.

Methods: For this study, a total of 1,950 g of ground beef was inoculated with a cocktail of *E. coli* O157:H7 or *Salmonella* at a final concentration of 0.5 log CFU/g. Three different enrichment media (buffered peptone water with vancomycin, cefixime, cefsulodin [BPW VCC], modified tryptic soy broth [mTSB] with 8 mg/L novobiocin, and modified tryptic soy broth with acid digest of casein) were used for study. The three enrichment media were added in 1:4 ratio and incubated for 24 h at 42°C. After incubation, the samples were plated on selective media. XLT-4 Agar and MacConkey agar with sorbitol were used for detection of *Salmonella* and *E. coli* O157:H7, respectively.

Results: Salmonella counts were found to be 7.37, 8.59, and 8.56 CFU/ml in BPW VCC, mTSB with novobiocin, and mTSB with casamino acids, respectively. *E. coli* O157:H7 counts were 7.66, 8.39, and 8.45 CFU/ml in BPW VCC, mTSB with novobiocin, and mTSB with casamino acids, respectively. Both *Salmonella* and *E. coli* O157:H7 were able to grow up to 8 log in mTSB with novobiocin and mTSB with acid digest of casein. However, mTSB with novobiocin showed better results for both pathogens. Thus, mTSB with 8 mg/L novobiocin and acid digest of casein can be used as enrichment media for both *Salmonella* and *E. coli* O157:H7, even at very low levels of contamination.

Significance: This study would help in recovering pathogens at very low concentrations, and isolation of both pathogens would help in reducing economic costs and time.

P2-184 Interlaboratory Validation of a Streamlined Method for the Enumeration of *Salmonella* and Shiga Toxin-producing *Escherichia coli* in Cattle and Poultry Manure Samples

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Introduction: Enumeration of viable *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC) from environmental samples, including raw manure, is labor-intensive and expensive. To better understand the epidemiology of these pathogens, a reliable method for detection, isolation, and quantification is required.

Purpose: This research validated a screening method to enumerate *Salmonella* and STEC from indigenous poultry and cattle manure samples in two regions of the United States.

Methods: A streamlined flow process was developed by using a combination of presence/absence pathogen screening followed by MPN determination. A 30-g manure sample was processed for presence/absence using modified enrichment and isolation methods for *Salmonella* (tryptic soy broth [TSB], buffered peptone water, Rappaport-Vassiliadis enrichment broth, tetrathionate broth, and XLT4), *E. coli* O157 (TSB, immunomagnetic separation, supplemented Rainbow agar, and MacConkey agar), and non-O157 STEC (TSB, mEHEC enrichment broth, Chromagar STEC). Simultaneously, 0.5 g of the sample in four replications (2 g) was transferred and serially diluted in TSB using a 48-well reservoir plate for MPN analysis. Enrichment steps for both screening (presence/absence) and MPN determination were continued simultaneously up to the isolation stage. The MPN reservoir plate was paused and stored at 4°C for one to four days, then resumed if presumptive positive colonies were found during presence/absence screening. Parallel spike trials were done using clinical and environment strains of *Salmonella*, STEC O157, and non-O157. Bacteria was inoculated in low, medium, and high concentrations ranging from <1 to 10^4 CFU/g in three replications.

Results: Spike trials showed the proposed MPN method results are significantly (P<0.0005) in agreement with the inoculation concentration (lower limit of detection=0.089 MPN/g). Also, holding the samples in the enrichment reservoirs for up to four days insignificantly affected the MPN result.

Significance: This approach offers a cost- and labor-effective method to conduct a large-scale, culture-based survey of pathogen levels in manure samples.

P2-185 Withdrawn

P2-186 Rapid and Reliable Detection of *Salmonella* in Animal Food Via Duplex Loop-mediated Isothermal Amplification with an Internal Amplification Control

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Introduction: Internal amplification controls (IACs) are commonly used by PCR methods to assess food matrix inhibitions. Despite showing great promise as a rapid and robust method for detecting *Salmonella* in animal food, loop-mediated isothermal amplification (LAMP) has inherent challenges in incorporating an IAC for multiplex detection.

Purpose: This study aimed to develop and evaluate a duplex *Salmonella*-IAC/LAMP assay in various animal food matrices. The assay employed a newer LAMP platform to differentiate two targets in one reaction by distinct annealing temperatures (T_m).

Methods: A duplex LAMP assay pairing a newly designed pUC19 (IAC) primer set with an established *Salmonella invA* primer set was developed and optimized on the OptiGene Genie II platform. Assay specificity and sensitivity were determined with 250 bacterial strains and a serially diluted *Salmonella* reference strain. Cattle feed, chicken feed, dry dog food, and dry cat food spiked with two low levels (0.2 to 2 and 2 to 5 CFU/25 g) of *Salmonella* were analyzed after overnight enrichment. Methods in the U.S. Food and Drug Administration's Bacteriological Analytical Manual (BAM) and a well-established quantitative PCR (qPCR) assay were run as a comparison.

Results: The assay was rapid, with positive results observed within 5 min. The two targets had distinctive T_m peaks of 90 and 87°C, allowing for clear differentiation. The assay was 100% specific and lower limit of detection was 1.6 cells per reaction in pure culture. In spiked animal food samples, the duplex LAMP assay was capable of detecting at the fractional level (0.2 to 2 CFU/25 g), with relative levels of detection comparable to both BAM and qPCR methods. The IAC demonstrated the effectiveness in assessing animal food matrix effects.

Significance: Animal feed and pet food contaminated with *Salmonella* is a significant public health and animal health concern. The duplex *Salmonella*-IAC/LAMP assay offers a rapid and reliable approach for routine screening of *Salmonella* in these diverse and complex animal food matrices.

P2-187 A Novel Selective Medium for Simultaneous Enrichment of Shiga Toxin-producing *Escherichia coli* and *Salmonella* in Ground Beef

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Introduction: Microbiological analysis of ground beef for contamination with both *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC) is performed by the United States Department of Agriculture Food Safety Inspection Service (FSIS) as part of its Performance Standards Verification Testing program. FSIS has established a zero-tolerance for STEC serotype O157:H7 and serogroups O26, O45, O103, O111, O121, and O145 because they are regarded as adulterants. The detection and isolation of these specific serogroups presents a technical challenge necessitating time-consuming and costly laboratory procedures that often exceed the technical capabilities of many small internal and reference laboratories.

Purpose: This study describes a method using a novel media that allows for simultaneous selective enrichment of STEC and *Salmonella* spp., providing isolation and detection from the same broth.

Methods: The method only involves direct plating from beef enrichments to detect suspect isolates, followed by immunoassay and PCR confirmation, rendering the isolation simpler and less costly than the current described methods.

Results: In a side-by-side comparison, significantly more (P<0.05) samples containing *E. coli* O157:H7 and STEC-O26, -O111, -O121, and -O145 and a non-different (P>0.05) number of samples containing STEC-O103 and -O45 were identified when enriching in the novel STEC broth, as opposed to modified tryptic soy broth (mTSB). Co-enrichment using six different *Salmonella* serovars showed numerically greater but not significant (P>0.05) positive samples using the novel STEC compared to mTSB.

Significance: Using this novel STEC broth resulted in primarily isolating STEC and *Salmonella* spp., while substantially suppressing the growth of other gram-negative *Enterobacteriacae* by 90%.

P2-188 Non-Cultural Confirmation of Presumptive Positive *Escherichia coli* O157:H7 Test Results Using the BAX System STEC Screening Assay

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Introduction: Confirmation strategies are constantly evolving to meet industry needs while also enabling producers to make accurate and timely decisions about releasing their products into commerce. Cultural confirmation protocols for *Escherichia coli* O157:H7 are time-consuming and difficult to execute, so there is a need to incorporate new technologies to improve reproducibility and accuracy when confirming a presumptive result.

Purpose: This study was designed to evaluate the ability of a real-time PCR method to accurately predict the confirmation of a presumptive *E. coli* O157:H7 PCR result using the virulence genes *stx* and *eae*.

Methods: A total of 320 samples across six food matrices (beef trim, ground beef, iceberg lettuce, raw milk, flour, and whey protein) were fractionally inoculated with *E. coli* O157:H7 and tested after enrichment using the real-time PCR assay for *E. coli* O157:H7. All presumptive positive results were then tested for *stx* and *eae* using the real-time STEC Screening assay. All results were culturally confirmed using the appropriate reference method.

Results: The real-time PCR assay for *E. coli* O157:H7 detected 194 positive samples with 100% agreement to culture. Using the same lysate, the real-time PCR assay for Shiga toxin-producing *E. coli* (STEC) Screen verified that all *E. coli* O157:H7 positive samples contained *stx* and *eae*.

Significance: The results of these validation studies demonstrate no significant statistical difference between the BAX System Real-Time PCR Assay for STEC Screen and the reference culture method. Food producers and testing laboratories can therefore use this assay as a non-cultural method to act on presumptive positive *E. coli* O157:H7 results in a more accurate and efficient manner.

P2-189 Development of a Sensitive Single-tube Nested PCR Assay for Rapid Detection of *Campylobacter jejuni*

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❖ ***Developing Scientist Competitor***

Introduction: Campylobacter jejuni is a leading cause of foodborne illness in Hawaii, as well as throughout the United States, where it accounts for approximately 1.3 million illnesses each year. Outbreaks of *C. jejuni* are usually associated with contaminated raw milk and undercooked chicken. Conventional detection methods for this pathogen often require several days to obtain results and do not supply information rapidly enough to allow appropriate actions to protect the public.

Purpose: This study aimed to develop a sensitive single-tube nested PCR assay for rapid detection of *C. jejuni*.

Methods: Both outer and inner primer sets were designed based on the hippuricase gene (*hip*O) of *C. jejuni*. The two PCR annealing temperatures were optimized among a range from 50 to 70°C. The effect of outer primer (0.05, 0.1, 0.5, and 1.0 pmol) and inner primer (5, 10, 20, and 40 pmol) concentrations on the production of two amplicons was evaluated. The established assay was tested with DNA extracted from three *C. jejuni*, two *C. coli*, *C. lari*, and seven non-*Campylobacter* species. Serial dilutions of *C. jejuni* DNA were used as templates to investigate its sensitivity compared with PCR based on inner primers. The developed assay was also applied to detect *C. jejuni* in artificially contaminated ground chicken homogenate.

Results: The optimal concentration and annealing temperature were determined as 0.1 pmol for outer primers at 65°C and 40 pmol for inner primers at 55°C. The single-tube nested PCR assay only generated amplicons from *C. jejuni* DNA. The detection limit of this assay was determined to be single copies of target DNA extracted from the pure culture and 10 CFU/ml of *C. jejuni* in ground chicken homogenate, which was about 100-fold lower than that of PCR based on inner primers.

Significance: This single-tube nested PCR assay would enable more sensitive detection of *C. jejuni* within shorter time and thus improve the safety of food supply.

Poster

P2-190 Quantitative Detection of *Listeria monocytogenes* in Cheese and Bean Sprouts Using Droplet Digital PCR

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Introduction: Listeria monocytogenes is an important foodborne pathogen often associated with food processing environments and RTE foods. Rapid and accurate quantitative detection of *L. monocytogenes* is critical in risk assessments in order to ensure public safety.

Purpose: In the present study, we compared the performance of quantitative PCR (qPCR) and droplet digital PCR (ddPCR) in quantitative detection of *L. monocytogenes* in pure bacterial culture and different food matrices.

Methods: Ten-fold serial dilutions from 2×10^{3} to 2×10^{-1} CFU/µL of *L. monocytogenes* were used to spike bean sprouts and fresh cheese samples. qPCR and ddPCR amplification reactions were carried out using the DNA-binding dye chemistry EvaGreen. The results were analyzed by linear regression analysis using standard plate count as the gold standard.

Results: qPCR had a broader dynamic range than ddPCR. Both PCR formats were able to detect and quantify accurately ($P>0.05$) in most of the samples processed by the boiling method and the DNA extraction kit until the concentration reached 2.75 copies/reaction. However, ddPCR showed a better precision (less variation among replicates) than qPCR.

Significance: Without the need for external standards, the superior precision of ddPCR could make it a valuable tool for quantitative detection of foodborne pathogens.

P2-191 Wyss Accelerated Sample Enrichment Technology for Food Safety Testing

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Introduction: Reducing the cost and improving the speed of food safety and quality tests will have a positive impact on producers and consumers. Although many assay techniques provide fast results, there is often a need for lengthy enrichment procedures to provide enough contaminant organisms for accurate test results. We developed a rapid capture technology that uses Fc-Mannose-Binding Lectin (FcMBL), an engineered protein that binds to a broad range of contaminants, including fungi (*Aspergillus* spp., *Candida* spp.), bacteria (*Escherichia coli, Salmonella* Typhi) and viruses (e.g., hepatitis C) to quickly isolate target organisms from a variety of food matrices.

Purpose: Demonstrate rapid capture and detection of yeast and mold contaminants in yogurt. Assess performance and ease-of-use of the capture technique in a commercial production facility. Compare performance to current practices of enrichment via conventional culture methods.

Methods: FcMBL-coated magnetic beads were used in a sample preparation step to extract spoilage yeast and mold microorganisms to detect contamination in 30 yogurt samples in a factory setting. Captured spoilage microorganisms were then concentrated into a buffer solution and these cleaned, enriched samples were analyzed using a commercially available rapid optical system for the detection of microbial contamination using a pH-mediated colorimetric readout. Results were compared to 30 control samples tested using industry standard technology.

Results: In a pilot study screening for yeast and mold contamination in a yogurt production facility, the FcMBL-based technology reduced the time to test results from 7 days to 2 days and improved test accuracy from 93% (28 of 30) to 100% (30 of 30 samples) compared to the existing standard test methodology.

Significance: Reducing the time required to enrich samples for routine safety and quality testing will shorten quarantine protocols and reduce handling costs. Providing cleaner test samples from complex food matrices will improve the sensitivity and selectivity of conventional and emerging assay technologies.

P2-192 Comparison and Recovery of Extended Spectrum β-Lactamase *Escherichia coli* on MacConkey Agar Acquired from Global Sources

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❖ ***Developing Scientist Competitor***

Introduction: The increased global prevalence of extended spectrum β-lactamase (ESBL) *Escherichia coli* is worrisome. Developing countries need a reliable, readily available, and cost-effective media to detect these pathogens. Typically, MacConkey agar (MA), available globally, is used with ceftriaxone (AXO) or cefotaxime (TOX) to detect ESBL *E. coli*. For global, harmonized surveillance systems, the quality and reproducibility of bacteriologic media is critical for data comparison.

Purpose: To evaluate the quality and reproducibility of MA from global manufacturers to detect ESBL *E. coli*.

Methods: MA was purchased from eight manufacturers representing seven countries (United States [U.S.]-1, U.S.-2, China, India, Italy, France, Canada, and United Kingdom [UK]), prepared with 4 µg/ml TOX or AXO and stored at 4°C. Reference strains ATCC EC25922 (negative control), presumptive positive (pp) ESBL EC13457 (+TEM/CMY-2), and ESBL EC10455 were evaluated on MA. U.S.-1 with 2 or 4 µg/ml TOX was evaluated for detection of ESBL *E. coli* from poultry cecal samples (n=30); all isolates were confirmed *E. coli* using matrix assisted laser desorption ionization-time of flight and susceptibility tested using the Vitek Sensititre.

Results: Recovery of ppEC13457 on MA from China, U.S.-2, Italy, and UK were reduced by 3 to 4 log CFU/ml on MA with TOX. Conversely, recovery of pp ESBL EC13457 on MA with AXO was consistent across MAs except for U.S.-2. There was no difference in recovery of ESBL EC10455 across MAs and antimicrobials. Marked phenotypic differences in colony size and color were apparent by manufacturer for both ESBL strains. From ceca, 70% (21 of 30) and 66.6% (20 of 30) were presumptive for ESBL *E. coli* on MA U.S.-1 with 2 or 4 µg/ml TOX, respectively. No ESBL *E. coli* were confirmed from MA+2 µg/ml TOX; 9 of 20 pp *E. coli* were confirmed ESBL on MA+4 µg/ml TOX by Sensititre.

Significance: The quality and reproducibility of MA varied by manufacturer; phenotypic differences on MA agars were striking. MA (U.S.-1) with 4 µg/ml TOX is recommended for detection of ESBL *E. coli*.

Poster

P2-193 An Evaluation of *Salmonella* Supplement in Ready-to-Use Tablets

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Introduction: Rapid methods for the detection of food pathogens are widely adapted and preferred, as they provide same-day screening for pathogen-negative samples without the need for plate confirmation, which require two to three days for final reporting. Time-to-result is important, but equally important to any laboratory is workflow, ease-of-use, and confidence that the assay can be correctly performed by laboratory staff.

Purpose: The purpose of this study was to evaluate a single selective enrichment for *Salmonella* detection using two different forms of the same formulation of selective supplement, a ready-to-use tablet and a rehydrated lyophilized supplement.

Methods: Two sets of seven varied food matrices (dried pet food, spinach, non-fat dried milk, ground beef, ground chicken, lettuce, and peanut butter) in both 25- and 375-g sample sizes, in duplicate, were challenged with either *Salmonella* Typhimurium or *Salmonella* Salford in buffered peptone water. One set of samples was tested using the ready-to-use tablet and a second set of samples was evaluated with rehydrated lyophilized supplement. All sample enrichments were plated to selective/differential media for confirmation, regardless of the screening method result.

Results: Salmonella grew sufficiently in the selective enrichment in ≤20 h and was detected in both the enzymatic immunoassay and PCR systems for all 56 sample/method combinations (25 g, 375 g, and both forms of supplement, with the exception of lettuce). *Salmonella* growth in lettuce was intermittent and only one replicate each of the 25- and 375-g samples was culturally confirmed.

Significance: These data demonstrate the equivalence of both forms of supplement for the screening of implicated products for the presence of *Salmonella* spp. The new *Salmonella* supplement tablet provides significant ease-of-use and workflow benefits to the user, including no pipetting or rehydration steps and no alcohol hazards.

P2-194 Multianalyte Lateral-flow Immunoassays Using Universal Protein G-Liposomal Nanovesicles for the Detection of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes*

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Introduction: A novel universal reagent for immunoassays, protein G-liposomal nanovesicles, has been developed and successfully used in an immunomagnetic bead sandwich assay for the detection of *Escherichia coli* O157:H7.

Purpose: To demonstrate the universal characteristic of protein G-liposomal nanovesicles, we designed a multianalyte lateral-flow assay for *E. coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* using protein G-liposomal nanovesicles.

Methods: Lateral-flow assays were used to optimize the protein G density on the liposome surface, determine the amount of IgG binding to protein G-liposomal nanovesicles, and to detect *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes*.

Results: We selected 0.1 mole percent of protein G tagged on the liposomes to make protein G-liposomal nanovesicles for lateral-flow assays, and 20 ng of IgG was chosen to be added per ml of protein G-liposomal nanovesicles in order to make the protein G-immunoliposomes. In the lateral-flow assay for *E. coli* O157:H7, the LODs is 10^{5} CFU/ml for both in the pure cultures and also in the presence of 10^{6} CFU/ml *Salmonella* and 10^{6} CFU/ml *L. monocytogenes*. This assay can be completed in about 30 min.

Significance: These results demonstrated that protein G-liposomal nanovesicles can be successfully used in the lateral-flow assay for *E. coli* O157:H7. However, it was not feasible to detect *Salmonella* and *L. monocytogenes* in the lateral-flow assays, presumably because of the low affinities of the antibodies for these pathogens.

P2-195 ST73 *Escherichia coli* Strain 0.1229 Amplifies *Stx*2a Production of O157:H7

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❖ ***Developing Scientist Competitor***

Introduction: The severity of infection caused by the foodborne pathogen *Escherichia coli* O157:H7 can vary between individuals infected with the same strain. Shiga toxin 2a (Stx2a), one of O157:H7's virulence factors, is phage-encoded and toxin expression is enhanced by DNA-damaging agents. Previous work demonstrated that Stx2a production also increases when *E. coli* O157:H7 is co-cultured with specific non-Shiga toxin-producing *E. coli* strains.

Purpose: This research aimed to determine the mechanism by which *E. coli* strain 0.1229 induces Stx2a production by *E. coli* O157:H7.

Methods: A co-culture protocol was used where an O157:H7 strain, PA2, was grown with whole cells or cell-free supernatant of various commensal *E. coli* isolates at 37°C, and Stx2a was quantified by enzyme-linked immunosorbent assay. Illumina and PacBio sequencing platforms were used for whole genome sequencing, one-step recombination was used to knock out gene(s), and plasmid curing was accomplished using ethidium bromide. Three separate experiments were conducted and analyzed using analysis of variance.

Results: Five of 13 *E. coli* strains amplified Stx2a production when co-cultured with strain PA2. *E. coli* strain 0.1229 was unique among this collection in that increased Stx2a production (33.7±1.20 µg/mg when co-cultured with PA2 compared to 6.1±0.82 µg/mg for PA2 alone) was also observed by growing PA2 in cell-free supernatant of 0.1229 (32.6±1.34 µg/mg of Stx2 versus 4.17±1.34 µg/mg for PA2 in LB media). *E. coli* 0.1229 was found to carry at least three plasmids, and curing a 96kb plasmid reduced Stx2a production when co-cultured with PA2 to 3.38±0.19 µg/mg. Knockouts of a known DNA-damaging bacteriocin encoded on this plasmid did not affect the ability of this strain to amplify Stx2a production.

Significance: This study may uncover a new mechanism by which commensal *E. coli* could impact the clinical outcome of an O157:H7 infection.

P2-196 Acid Treatment of Sprouts Enhances Detection of Shiga Toxin-producing *Escherichia coli* in Enriched Sprout Microbiome

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Introduction: Metagenomic analysis provides a rapid method to detect Shiga toxin-producing *Escherichia coli* (STEC) in food, as strain isolation need not be performed prior to pathogenicity determination. However, detection sensitivity in sprouts is decreased due to the high microbial load and presence of other co-enriching *Enterobacteriaceae* in the sprout microbiome. The exceptional acid resistance of *E. coli* may be a phenotype that can be leveraged to increase the relative abundance of STEC during enrichment.

Poster

Purpose: In this study, the impact on the sensitivity of detecting STEC in sprouts using metagenomics was assessed after incorporating changes in the enrichment protocol utilizing low-pH media.

Methods: Sprouts spiked with either STEC O157:H7 or STEC O104:H4 were soaked in modified buffered peptone water with pyruvate media at pH 7, 3, or 2 for 30 min prior to enrichment at pH 7 according to the U.S. Food and Drug Administration Bacteriological Analytical Manual protocol. Shotgun metagenomic sequencing was performed on DNA extracted from the enrichments, and the data were analyzed for microbial community composition and number of reads mapping to STEC virulence and serotyping loci.

Results: Microbiome analyses of samples enriched for 5 h revealed that the acid soak partially depleted specific *Enterobacteriaceae* that co-enrich with STEC, resulting in a greater relative abundance of STEC in the microbial community after enrichment for 24 h. Given the same number of total reads per sample, acid treatment resulted in at least a 10-fold greater number of reads mapping to loci used for STEC serotype and virulence characterization, and this enhancement in detection sensitivity was greater for sprouts soaked at pH 2 compared to pH 3.

Significance: The improved enrichment method permits metagenomic detection of low STEC contamination levels in sprouts without requiring additional sequencing depth, thereby offering a more practical detection and characterization method for risk assessments or traceback investigations.

P2-197 Isolation and Characterization of Extraintestinal Pathogenic *Escherichia coli* from the Skin of Retail Chicken Meat

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Introduction: Extraintestinal pathogenic *Escherichia coli* (ExPEC) are a diverse type of foodborne pathogens that are commonly found in poultry meat and other foods and associated with ulcerative colitis, sepsis, meningitis, and urinary tract infections in humans. ExPEC are also a pathogen of significance for poultry and companion animals. They are often resistant to multiple antibiotics, and food isolates cause disease in animal model systems.

Purpose: The purpose of this study was to determine the incidence and prevalence of ExPEC on retail chicken skin parts and determine their genotype (e.g., virulence factors and antibiotic resistance) using PCR and whole genome sequencing (WGS). A major objective is to build a genomics database for ExPEC types isolated from foods (e.g., poultry meat).

Methods: E. coli isolated from the skin of retail poultry parts ranged from 0 to ca. 500 CFU/g. Of ca. 324 isolates screened for ExPEC status 36 (11.1%) were confirmed as ExPEC by PCR.

Results: Of those ExPEC, 33 contained *iutA* and *kpsMII* as the primary virulence factors. WGS revealed resistance to multiple antibiotics (e.g., aminoglycoside [aac(3)-VIa, aadA1] and sulphonomide [sul1, sul2]), additional virulence factors (e.g., *air*, *astA*, *cma*, *eilA*, *gad*, *iss*, *iha*, *iroN*, *mchF*, and *tsh*), and 12 plasmid types.

Significance: Future detection, control, and elimination of ExPEC in products ranging from poultry to pet feed has the potential to reduce veterinary and human illness.

P2-198 Whole Genome Sequence and Pulsed Field Gel Electrophoresis Analysis of Environmental *Listeria monocytogenes* Isolates from an Ice Cream Processing Facility

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Introduction: Listeriosis is the third leading cause of foodborne illness and has recently been shown to be associated with human outbreaks due to ice cream contamination. Approximately 16% of people who contract listeriosis will die each year. The most recent *Listeria* outbreak in ice cream resulted in 10 hospitalizations and three deaths across four states. The presence of *Listeria monocytogenes* in ice cream processing facilities poses a difficult challenge for public health and food safety.

Purpose: To characterize *L. monocytogenes* strains isolated from an ice cream processing facility.

Methods: Pulsed-field gel electrophoresis (PFGE) was performed on 18 *L. monocytogenes* environmental strains using the PulseNet protocol PNL04. Whole genome sequencing (WGS) was also performed on these isolates using the GenomeTrakr protocol. Genomic DNA was extracted using the QIACube instrument, genomic libraries were prepared using the Nextera XT kit, and sequencing was performed using the MiSeq instrument. WGS was analyzed using the CLC Genomics Workbench and the National Center for Biotechnology Information (NCBI) pathogen database.

Results: The 18 isolates yielded five different PFGE patterns with the primary enzyme AscI and three different patterns with the secondary enzyme ApaI. WGS analysis showed that all 18 isolates were closely related to a serovar of 1/2b, wgMLST 5, and a best match to a *L. monocytogenes* strain NC_021824. Furthermore, the 18 isolates were found to be associated with three different single nucleotide polymorphism (SNP) clusters.

Significance: Our data demonstrated that environmental strains of *L. monocytogenes* from an ice cream processing facility were closely related to each other but not to any other food, environmental, or clinical isolate in the NCBI or PulseNet database. All of the isolates were associated to the same K-mer group PDG000000001.779. Two ice cream environmental isolates, SRR5758448 and SRR5758406, had the same PFGE pattern name GX6A16.0020/GX6A12.2899, yet did not fall within the same SNP cluster. Whole genome sequencing data can provide a more discriminatory analysis for subtyping.

P2-199 Phylogenic Relationships between Bacteria Found in Cultured Food Starters and Dietary Supplement-associated Species

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Introduction: Cultured foods have become a large component of the consumer-driven market, which now includes the sale of starter microbial cultures for in-home production of products like kefir, yogurt, and tempeh. Although these products claim to confer health benefits, the identification and characterization of most beneficial microbial ingredients has not been well-documented.

Purpose: The objective of this work was to determine the phylogenic relationships among species commonly isolated from dietary supplements and cultured food starter kits.

Methods: A culture-independent metagenomic approach was used to obtain whole genome sequencing (WGS) data from whole products. Single-colony isolates were grown on media and sequenced for phylogenic comparison. A novel K-mer analysis was used to determine the relative abundance of each present species, as well as strain identification from different isolates. Sequences were compared to those in the National Center for Biotechnology Information (NCBI) pathogen database, including the ones generated from our ongoing post-market surveillance of dietary supplements (BioProject PRJNA336518). Single nucleotide polymorphism (SNP) analysis of core genes established phylogenic relationships among the isolates from the starter kits and closest relatives found in dietary supplements.

Results: Several microbial species were shared among cultured food starters and dietary supplements, including the genera *Lactococcus*, *Lactobacillus*, *Streptococcus*, and *Pediococcus*. However, other species were found only in the cultured food starter products: *Lactococcous lactis* sbsp. *cremoris, Leuconostoc mesenteroides*, *Weissella cibaria*, and *Leuconostoc pseudomesenteroides*; they will be included in our NCBI BioProject. Phylogenic SNP analysis demonstrated a varying degree of genotypic diversity among species found in starter kits versus close relatives commonly found in dietary supplements and fermented products.

Significance: WGS is a powerful analytical post-market surveillance tool for cultured food starter kits and dietary supplements. Enriching our genomic database with microbial sequences from these food commodities will enable a more in-depth analysis of the safety of such food products.

P2-200 Prevalence and Distribution of Efflux Pump Complex Genes in *Cronobacter sakazakii* Using Whole Genome and Pan-genomic Datasets

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✦ ***Undergraduate Student Award Competitor***

Introduction: Cronobacter spp. are foodborne pathogens that cause serious disease in neonates, infants, and adults. Microorganisms express various transmembrane complexes known as efflux pumps (EPs) that influence their survival under stressful growth conditions. Although EPs are important in resistance to antibiotics, disinfectants, and preservatives, there is little information about them among *Cronobacter* spp.

Purpose: To better understand the prevalence and distribution of efflux pumps among strains of *Cronobacter* spp.

Methods: Previously, 143 *Cronobacter* strains representing all seven species were analyzed using a novel pan-genomic DNA microarray (MA) containing 37 EP probe sets. Selected EP loci common among all species as determined by MA were used to design PCR primers for the presence of *kef*, *RND*, and *cmeB*. A survey of the genomic landscape of *Cronobacter sakazakii* strain BAA-894 for these EPs was conducted by analyzing its genome with Geneious 9.1.5 suite.

Results: Homologs of 13 families of efflux pumps were identified using the U.S. Food and Drug Administration *Cronobacter* DNA microarray. PCR analysis showed that *kef*, *RND*, and *cmeB* EP loci were present in 115 strains tested. In *C. sakazakii* BAA-894's genome, *kefBCFG* loci were located in four regions associated with protein and cobalt-magnesium anion efflux. Five *RND* gene clusters were identified and were associated with copper-zinc and multidrug efflux and represented by *CusA*/*CzcA* and *MexE*-multidrug efflux loci. Two genomic regions associated with *cmeB* loci were identified and were involved in sugar transport.

Significance: Persistence of *Cronobacter* spp. in stressful growth environments may involve the transport of a variety of substrates by EPs. The genomic landscape around *kef, RND*, and *cmeB* EP loci suggests that they could be involved in important physiological processes including protein, heavy metal, and sugar efflux. Understanding the mechanisms of *Cronobacter* adaptation in a wide range of environments could lead to development of methods to control the contamination of foods by these organisms.

P2-201 Characterization of Plant-associated *Cronobacter sakazakii* Strains Using Molecular, Whole Genome, and Pan-genome Sequence Analyses and Zebrafish Infectivity Studies Identifies Clinically Relevant and Virulent Sequence Types

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Introduction: There is a growing body of evidence suggesting that eukaryotic plants may be the ancestral host for *Cronobacter* species. *Cronobacter sakazakii*, the primary pathogen, continues to be isolated from ready-to-eat produce, flours, cereals, nuts, and spices; several surveillance studies have shown that *C. sakazakii* can also contaminate food manufacturing environments, posing a risk to susceptible consumers. To date, very little is known about the phylogenomic and virulence traits possessed by plant-associated *C. sakazakii* strains.

Purpose: To understand the phylogeny and virulence of plant-associated *C. sakazakii* strains using molecular and genomic analyses.

Methods: Ninety-three *C. sakazakii* strains were obtained from various plant-derived foods and food manufacturing environments located in the United States, Middle East, Asia, and Europe. The strains were characterized using PCR, DNA microarray (MA), multi-locus sequence typing (MLST), and whole genome sequencing (WGS) analyses. WGS was conducted using Illumina's MiSeq platform and Nextera XT library kit. To assess virulence, Zebrafish infectivity (ZI) studies were performed on selected strains.

Results: PCR analysis showed that the strains possessed the virulence plasmid, pESA3, and 98% (91 of 93) were positive for the *Cronobacter* plasminogen activator gene. Twenty percent (19 of 93) of the strains possessed the filamentous hemagglutinin gene cluster. Combinatorial MLST and MA analyses showed that these strains clustered according to ST with more than 20 STs among six phylogenetically related clades, including the malonate-positive ST64 and clinically relevant ST1, ST4, ST8, ST12, and ST13 clones. WGS analysis resulted in comparative annotations, clarified genome-wide nucleotide polymorphisms, and elucidated distinct clades representing strain clusters from different sources. ZI studies showed that the plant-associated *C. sakazakii* strains are as virulent as other *Cronobacter* species.

Significance: Finding virulent *C. sakazakii* strains of clinically relevant STs which were associated with plant-based foods suggests that these foods can serve as potential transmission vehicles and supports widening the scope of continued surveillance of this important foodborne pathogen.

Poster

Poster

P2-202 Phylogenomic Analyses of Type II Toxin-antitoxin Genes in the Foodborne Pathogen *Cronobacter sakazakii* Using Sequence-based Bioinformatics

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Introduction: Cronobacter spp. are considered an opportunistic group of foodborne pathogens that can survive in low-moisture environments and can cause both intestinal and systemic human disease. However, little is known about the mechanisms that *Cronobacter* employ to survive and persist in such environments. Toxin-antitoxin (TA) loci play a role in bacterial stress, as well as in the stabilization of horizontally acquired elements such as plasmids. The phylogenetic landscape of TA loci among *Cronobacter* is poorly understood.

Purpose: To understand the phylogenetic relatedness among toxin-antitoxin genes present in *Cronobacter sakazakii* strains.

Methods: A local database was created by downloading 22 TA FASTA sequences given by TAfinder and known to be present in *C. sakazakii* strain BAA-894. These sequences were blasted against 300+ *C. sakazakii* genomes previously sequenced as part of the Center for Food Safety and Applied Nutrition's GenomeTrakr project. A PythonV shellscript was generated to extract TA FASTA sequences present in these *C. sakazakii* genomes that were used for further phylogenomic analyses by Clustal Omega and CLC. Five common toxin genes were selected for PCR analysis and primers were designed to detect toxin alleles: ESA_00258, ESA_00804, ESA_01887, ESA_03838, and ESA_04372.

Results: BLAST analysis showed that not every *C. sakazakii* strain possessed all 22 TA loci. Interestingly, some strains contained either the toxin or the antitoxin component, but not both. PCR analysis of 63 *C. sakazakii* strains using primers designed to detect these five common toxin genes gave variable results, possibly due to single nucleotide polymorphisms associated with some of the sequences. Phylogenetic studies showed that for the most part, TAs follow species evolutionary lines, except for a few toxins possessed by some *C. sakazakii* and *C. malonaticus* strains, demonstrating that some TA alleles share a common phylogeny.

Significance: Understanding the phylogeny of TAs among *Cronobacter* spp. is essential to designing future studies and realizing the physiological mechanisms of stress adaptation and persistence of *Cronobacter* in low-moisture food matrices.

P2-203 Diversity among Bacterial Isolates from Naturally Fermented Foods and Their Relatedness to Live Microbes in Dietary Supplements

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Introduction: Live beneficial microbes have long been used in the production of fermented foods. With the advent of the "eat local" mentality, small artisan businesses have begun to sell their fermented products from local produce sources. The identity and characterization of specific strains used and found in these fermentations has not been well-established.

Purpose: To determine the identity of bacterial strains isolated from naturally fermented foods and their genetic diversity when compared to those found in live microbial dietary supplements.

Methods: A culture-independent metagenomic approach was used to obtain whole genome sequence (WGS) data from fermented products using the Illumina MiSeq platform. In addition, single colony isolates were sequenced and identified using a novel bioinformatic K-mer method of analysis. Sequences were compared to National Center for Biotechnology Information (NCBI) reference genomes that include sequences from microbes isolated from dietary supplements (BioProject PRJNA336518).

Results: One hundred isolates from fermented products were sequenced. For *Lactobacillus brevis* and *Lactobacillus plantarum* species, single nucleotide polymorphisms (SNPs) were determined for 27 *L. brevis* and 32 *L. plantarum* isolates relative to a NCBI reference genome, and phylogenetic relationships were determined using core 1.8 Mb and 2.0 Mb genomes, respectively. Despite considerable diversity of isolates (10,000 SNPs) within and across fermentations, isolates from multiple sources were found that were similar to strains found in commercial dietary supplements. WGS of isolates of two species not well-represented in genomic databases, *Pediococcus parvulus* and *Lactobacillus saniviri*, was also performed in order to improve species determinations using our K-mer database.

Significance: WGS analysis of live microbials from fermented foods aids in the safety assessment, manufacturer labeling, and identification of ingredients for these products.

P2-204 PerC Homologue pchE Controls *Escherichia coli* O157:H7 Biofilm Expression

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Introduction: Escherichia coli serotype O157:H7 causes hemorrhagic colitis that can progress to hemolytic uremic syndrome (HUS). Shiga toxin(s) and the locus of enterocyte effacement (LEE) are the most important virulence factors. Transcriptional regulator Ler encoded in LEE operon 1 controls most LEE genes, including the enterocyte adhesion protein, *espA*. Ruminant fecal shedding is the primary transmission source, and biofilm formation, dependent on curli fimbriae encoded by *csgA*, enhances bacteria survival prior to host infection. Prophage-encoded LEE regulators, such as the five *pch* genes (A-E) and *glrA* control virulence, but their role in biofilm formation is unclear.

Purpose: Investigate the role of LEE regulators in the control of O157:H7 biofilms.

Methods: O157:H7 strain 20R2R and isogenic deletion mutants, 20R2RΔ*ler* and 20R2RΔ*espA*, were transformed with recombinant plasmids expressing LEE regulators and tested for 30°C biofilm formation. Expression of curli regulator, *csgD*, and *csgA* were determined using quantitative reverse transcription PCR and RNA harvested from 20R2R expressing each of the *pch* regulators. Gene deletions were constructed by RedET recombination, protein fusions were constructed in pSE380, and biofilms were assayed by crystal violet retention. Two separate experiments, each testing two independent samples of each strain, were analyzed by analysis of variance ($P<0.05$).

Results: pchA and pchB reduced 20R2R biofilm formation >40 and >15% respectively, dependent on *ler.* pchE reduced biofilm formation >2.8-fold, independent of *ler.* Expression of *csgD* and *csgA* was increased ≥2-fold by the pchABCD genes but were reduced nearly 2-fold by pchE.

Significance: Three (A, B and C) of the five *pch* genes of serotype O157:H7 enhance LEE virulence properties by stimulating *ler*; however, p*chD* and pchE functions are unknown. These studies show that the *pch* regulators affect biofilm formation and that pchE is a strong biofilm repressor, likely by reducing *csg* expression. pchE provides a new target for biofilm reduction strategies.

P2-205 The Effects of Microbiome on the Abundance of *Vibrio parahaemolyticus* and *Vibrio vunlificus* in Oysters

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Introduction: Oysters are a reservoir of microbes, including human pathogenic bacteria such as *Vibrio parahaemolyticus* and *Vibrio vulnificus*, and consumption of improperly handled or raw oysters poses a public health concern. Studies suggest that the abundance of *V. parahaemolyticus* and *V. vulnificus* may be influenced by several biotic factors, including the oyster microbiome or bacteria inhabiting the oyster and overlying water.

Purpose: To investigate the effects of microbiome on the abundance of *V. parahaemolyticus* and *V. vulnificus* in oysters.

Methods: A total of 80 individual oysters and 48 water samples were collected between May and October 2016 from two sites (Manokin River and Chester River) in the Chesapeake Bay, Maryland, United States. Genomic DNA from oyster homogenates and water were extracted using the DNeasy Blood and Tissue Kit. Then, 16s rRNA and shotgun sequencing were used to characterize the microbiomes of oysters and water. Real-time PCR (qPCR) was also used to determine the abundance of *V. parahaemolyticus* and *V. vulnificus* in the same oyster and water samples.

Results: The oysters' microbial community was not reflective of the overlying water, as shown by the differences ($P<0.05$) in the relative abundances and composition of both entities. *Pelagibacteraceae*, *Synechococcus,* and *Enterobacteriaceae* were the predominant groups in both oyster and water samples. Several observed operational taxonomic units accrued different relative abundances among individual oysters from the same site, as well as between the sites. Individual oysters also contained differential *V. parahaemolyticus* (49%) and *V. vulnificus* (88.8%) abundance using qPCR. No correlation was found between the oyster microbiome and *Vibrio* spp.

Significance: The bacterial diversity observed in the oyster and water microbiomes suggests that the oysters contained a varied and dynamic bacterial community that was spatially and temporally dependent. These data will be the first of their kind and may elucidate a mechanism for changes in the prevalence of bacterial pathogens among oyster populations.

P2-206 Gold Nanoparticle-based Colorimetric Detection of Nucleic Acids Using Loop-mediated Isothermal Amplification Coupled with Differential Centrifugation

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❖ ***Developing Scientist Competitor***

Introduction: We demonstrated a novel approach for colorimetric DNA detection using gold nanoparticles (AuNPs) as hydrodynamic separators coupled with differential centrifugation. This approach relies upon the change in the sedimentation rate of AuNPs when conjugated to DNA amplicons. Isothermal nucleic acid amplification (LAMP) results in the formation of unique DNA amplicons that are large enough to prevent the sedimentation of conjugated AuNPs at a specific centrifugal force. In contrast, free nanoparticles are readily centrifuged and the solution color changes to colorless, enabling accurate and quantitative detection of the targeted DNA.

Purpose: We aim to develop an ultrasensitive, on-site bacterial detection method.

Methods: In the present study, *sdfI* gene of *Salmonella* was used as a model sequence. The targeted DNA was isothermally amplified using LAMP technique into large-sized looped amplicons, followed by interacting with specifically DNA-engineered AuNPs to form large Au-DNA assemblies. After differential centrifugation, the color intensity of large Au-DNA assemblies was determined using UV-vis spectrometry for the accurate quantification of the targeted DNA. The detection sensitivity and specificity of *sdfI* gene were also assessed in water and milk samples. To validate our results, conventional PCR was performed as the reference method.

Results: The developed method could specifically detect *Salmonella*. The decline of the red color intensity of AuNPs was linear to the concentration of the targeted DNA from 1.2×10^1 copies/ml to 1.2×10^7 copies/ml, and the detection limit was as low as 120 copies/ml (S/N=3). By controlling the reaction volume and AuNPs concentrations, the results from our developed assay can be determined by the naked eye without the need of any instrument.

Significance: This simple platform could be used to establish inexpensive and sensitive assays for in-field detection of pathogenic bacteria in both food industries and clinical settings.

P2-207 The Effect of the Previous Life Cycle Phase on the Proteomic and Transcriptomic Profiles of *Salmonella* Typhimurium DT104 in Brain Heart Infusion Broth and Ground Chicken Extract

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❖ ***Developing Scientist Competitor***

Introduction: Salmonella Typhimurium DT104 (DT104) is a foodborne pathogen previously isolated from poultry products that can survive long-term starvation and harsh conditions; however, adequate information is not available on the molecular mechanisms underlying its survival and persistence in poultry environments.

Purpose: To determine gene and protein expression profiles of *Salmonella* Typhimurium DT104 in brain heart infusion (BHI) and ground chicken extract (GCE) to identify potential intervention targets.

Methods: Protein expression of DT104 cells in early stationary phase (ESP, 24 h) to early death phase (EDP, 720 h) and in ESP and EDP log phase cells (after reinoculation) in BHI and GCE were compared. Additionally, gene expression based on RNA-Seq data on ESP and EDP log phase cells after reinoculation in BHI and GCE was investigated. The transcriptional profiles to identify genes with significant transcriptional changes were compared (</=4-fold; $P<0.05$). A *t* test was applied to determine whether the mean of three biological replicate ratios for each protein differed significantly.

Results: We measured the expression of 581 genes (98 up-regulated, 483 down-regulated) from cells in EDP (7.5 h in BHI) and 398 genes (138 up-regulated, 260 down-regulated) in EDP (7.5 h in GCE). Despite limited overlap between RNA-Seq and proteomics data; together the results provided information for understanding the adaptations of DT104 in GCE. In the EDP, expression of heat shock response, DNA protection, and catalase-peroxidase genes may have played a vital role in protecting DT104 from starvation and harsh conditions. In GCE, acid shock response and the tripartite efflux system (EmrAB-TolC) genes were up-regulated, possibly aiding in DT104 survivability. Thus, RNA-Seq and proteomics data can be used in combination to provide a comprehensive analysis of DT104 survival under different conditions.

Significance: The information may be useful for identification of genes that can be employed as potential targets for interventions to control DT104.

P2-208 Clinical and Retail Meat *Salmonella* Typhimurium Var. O5 Isolates That Match by PFGE and Drug Resistance Can be Distinguished by Whole-Genome Sequencing

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❖ ***Developing Scientist Competitor***

Introduction: Non-typhoidal *Salmonella* are a prominent cause of foodborne illness and often display antimicrobial resistance (AMR). The standard methods employed to investigate outbreaks include pulsed-field gel electrophoresis (PFGE) and AMR surveillance, but whole-genome sequencing (WGS) has recently emerged as an alternative tool that can be routinely utilized.

Purpose: The aim of this study was to assess the role of WGS in integrated surveillance for antimicrobial resistant *Salmonella* from clinical and retail meat sources. Here, we utilized WGS to retrospectively resolve the genetic relatedness and AMR profiles of a historic collection of PFGE-matched and multi-drug resistant retail meat and clinical *Salmonella* Typhimurium var. O5- isolates.

Methods: Thirty-eight *Salmonella* Typhimurium var. O5- (five retail meat and 33 clinical) isolates, subdivided into two subsets based on PFGE pattern, were sequenced on an Illumina MiSeq. SNVPhyl was used to identify variants and PhyML was used to construct maximum likelihood phylogenetic trees. AMR genes were identified using publicly available databases and resistance plasmids were characterized using PacBio sequencing, PlasmidFinder, and BRIG.

Results: The retail meat isolates within *Salmonella* Typhimurium var. O5- subset 1 and subset 2 were separated from all PFGE-matched clinical isolates by 41 to 96 and 21 to 81 single nucleotide polymorphisms, respectively. In total, there was a 100% correlation (38 of 38) between AMR phenotype and genotype for β-lactams, chloramphenicol, sulfonamides, and tetracycline antimicrobials, whereas there was a 74% correlation (28 of 38) for streptomycin. AMR was chromosomally mediated in subset 1 and plasmid-mediated in subset 2.

Significance: These data suggest that historic isolates that match by PFGE and AMR could in fact be different strains, further underscoring the importance of WGS; nonetheless, epidemiological data would still be necessary to confirm such findings. Furthermore, these results improve upon the understanding of the prevalence and transmission of AMR in humans and, importantly, food-derived *Salmonella*.

P2-209 Allelic Variants of *Shigella sonnei* Genes Predict Phylogenetic Global Lineages

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❖ ***Developing Scientist Competitor***

Introduction: As countries become more industrialized, *Shigella sonnei* has emerged as the main disease-causing *Shigella* species. Previous analysis by Holt et al. of *S. sonnei* using single nucleotide polymorphism (SNP) detection resulted in four clusters referred to as the Global Lineages. These Global Lineages appear to be based on SNP acquisition or deletion, not geographic origin, and the majority of *S. sonnei* isolated presently fall within Lineages 2 or 3.

Purpose: This research focused on identifying lineage-specific genes from *S. sonnei* whole genome sequences that define Lineages 2 or 3, followed by using these genes to predict the Global Linages of various *S. sonnei*.

Methods: Twenty-two *S. sonnei* isolates from the Pennsylvania Department of Health collection were sequenced using an Illumina MiSeq, assembled using SPAdes, and SNPs were called via the SNVPhyl phylogenetics pipeline. Genes with lineage-specific SNPs were identified and then used to predict the Global Lineage of 40 additional *S. sonnei* whole genome sequences acquired from the National Center for Biotechnology Information Pathogen Detection Database. The prediction results were then compared to the SNVPhyl-generated maximum likelihood phylogenetic tree to determine the accuracy of the prediction genes.

Results: Twenty-two lineage-specific genes were identified; four of these genes were specific to Lineage 2 specific and 18 were specific to Lineage 3. When tested against other *S. sonnei* genome sequences, they accurately identified whether the isolates segmented into Lineage 2 or 3 based on gene identity patterns (98% accuracy, 39 of 40 isolates). These genes also allow the distinction of *S. sonnei* strains from other species of *Shigella* and *Escherichia coli*.

Significance: Whole genome sequencing of foodborne pathogens has become an integral part of outbreak tracking and human health. Consequently, the need for accurate and efficient characterization methods is vital, and these lineage prediction genes could be used to quickly identify and classify *S. sonnei* isolates.

P2-210 Antibiotic Resistance Genes on Lettuce and Radishes Field-grown in Soils Amended with Manure or Compost from Antibiotic-treated Cattle

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Introduction: Cattle are commonly treated with antibiotics, which can survive digestion and potentially promote the spread of antibiotic resistance in the environment. Manure or composted manure is often used as a soil amendment for crop production. There is a need to understand the potential for manure-derived soil amendments, particularly those from antibiotic-treated cows, to affect the "resistome" (i.e., antibiotic resistance genes [ARGs]) on fresh produce, particularly vegetables eaten raw.

Purpose: The purpose of the study was to determine the extent to which prior antibiotic use history and composting of cow manure influences the resistome of radish and lettuce surfaces when grown at field-scale.

Methods: Lettuce and radishes were planted in fields amended with one of the following: raw manure from antibiotic-treated dairy cows (cephapirin and pirlimycin); compost generated from manure from antibiotic-treated or untreated cows; or chemical fertilizer at recommended application rates (12 plots, n=3). Lettuce and radishes were harvested at maturity. DNA was extracted from lettuce leaf or radish taproot surfaces, subjected to shotgun metagenomic sequencing, and compared against the Comprehensive Antibiotic Resistance Database to identify ARGs.

Results: More than 590 ARGs from 21 antibiotic classes were identified from un-assembled reads. Overall, the ARG profiles of vegetables grown in manure-amended soils were distinct from vegetables grown in compost or control soils (R=0.29, P<0.03). Radish surfaces contained genes from all 21 antibiotic classes across the four amendment types. Surfaces of lettuce grown in manure contained genes from 21 antibiotic classes, three of which were unique to manure-grown lettuce. Lettuce grown in control plots contained ARGs from 16 antibiotic classes.

Significance: Composting has established benefits for reducing pathogen loads. Composting may also be an important strategy to reduce some ARGs on fresh produce, but differences in the resistomes of lettuce and radishes suggest the extent of soil contact should be considered.

P2-211 16S rRNA Gene Sequence Analysis of Bacterial Microbiota Fluctuations in Cold-smoked Salmon Stored at 4°C for 30 Days

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Introduction: Smoked salmon is colonized by spoilage-associated bacteria that are present during the cold-smoking process, and some compete for nutrients with bacterial pathogens.

Purpose: This study employed targeted amplicon sequencing to identify and characterize bacterial population dynamics of cold-smoked salmon stored at 4°C for 30 days.

Methods: Eight vacuum-packed, retail samples of cold-smoked salmon (A through H) were opened and stored at 4°C. Samples of 25 g each were tested nine times over a 30-day period. Each time point sample was immersed in buffered enrichment broth for 30 min, and aliquots were collected for aerobic plate counts and sequencing.

Results: Aerobic plate counts on day 1 were undetectable, but reached 9 to 10 log CFU/gm after 30 days at 4°C. *Brochothrix* predominated in all samples on day 1 at proportional abundances, averaging 54±2.37%, followed by *Carnobacterium* (24±2.69), *Pseudomonas* (14±1.14%) and *Psychrobacter* (0.79±0.29). After 30 days at 4°C, *Brochothrix* and *Carnobacterium* proportional abundances decreased in all samples, reaching lows of 19 and 1%, respectively. In six samples, *Pseudomonas* abundances increased 3- to 5-fold, while *Psychrobacter* abundances remained low (0.69%). However, in samples E and H, *Psychrobacter* abundances increased to 45 and 54%, with a concomitant decrease in abundances of *Pseudomonas* to 5 and 0.86%, respectively, indicating that these two taxa may directly compete with one another. *Lactococcus*, *Enterococcus*, and *Leuconostoc* proportional abundances were maintained at or below 1% through day 9, and these genera died off at day 14.

Significance: Targeted amplicon sequencing revealed a succession of the microbiota consistent with spoilage. Additionally, sample to sample variability was observed, as well as direct microbial competition between certain taxa. Understanding bacterial population dynamics in smoked seafood is important for maintaining quality and safety, as they can impact the survival and persistence of foodborne pathogens.

P2-212 Status of Selected Virulence Genes in Antibiotic-resistant and Sensitive *Salmonella* Clinical Isolates from Tennessee

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Introduction: The widespread use of antibiotics has significantly contributed to the surge of antibiotic resistance (AR). For pathogenic bacteria, both AR and virulence are necessary for survival under adverse conditions. Pathogens like *Salmonella* has evolved to acquire AR and have increasingly been found in the food supply chain. It is possible that AR can influence virulence of some bacteria, yet the relationship between AR and virulence is not well-understood or extensively studied.

Purpose: The purpose of our study was to explore the relationship between different virulence determinants in antibiotic-resistant and susceptible *Salmonella* isolates from Tennessee, United States.

Methods: The virulotyping of 220 *Salmonella* isolates was done by targeting 13 virulence genes (*avrA*, *ssaQ*, *mgtC*, *siiD*, *sopB*, *gipA*, *sodC1*, *sopE1*, *spvC*, *bcfC*, *invA*, *sefA*, and *pefA*) and a gene for Class 1 integron. The AR of the isolates was tested using the Clinical and Laboratory Standard Institute broth microdilution method using the Sensititre system.

Results: Nearly half of the *Salmonella* isolates were pan-susceptible. Approximately 60% of the resistant isolates were found to confer resistance to more than three antibiotics. The distribution of virulence genes was found to be similar for antibiotic resistant and susceptible isolates. Virulence genes *invA*, *bcfC*, and *ssaQ* were present in almost all isolates. Two non-SPI genes, *sodC1* and *spvC*, were found at higher frequencies in antibiotic-resistant isolates compared to susceptible isolates. Virulotypes varied across AR and antibiotic-susceptible isolates. The frequency of Class 1 integron gene was lower in resistant isolates.

Significance: This study sheds light on the relationship between bacterial virulence gene distribution and AR. Understanding the nature, magnitude, and association of virulence and AR of *Salmonella* may provide useful information to develop strategies to manage the severity of salmonellosis in humans.

P2-213 Virulence Factors and Acquired Antimicrobial-resistance Genes of Shiga Toxin-producing *Escherichia coli* Isolated from Meat-processing Plants in Honduras

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) can cause severe illness in adults and children alike. Life-threatening complications can occur if infected by STEC, including hemolytic uremic syndrome and thrombotic thrombocytopenic purpura.

Purpose: Determine virulence factors and acquired antimicrobial resistance gene patterns of presumptive STEC isolates from meat processing plants in Honduras.

Methods: DNA extraction of 64 presumptive STEC isolates was performed using the GenElute Bacterial Genomic DNA kit. DNA was quantified with a Qubit Fluorometer and diluted to 0.25 to 0.40 ng/µl. DNA libraries were prepared following the Nextera XT DNA Library preparation kit; libraries were then run through the TapeStation 2200 and quantified for pooling. DNA libraries were pair-ended and sequenced on an Illumina MiSeq platform. Raw reads were assembled using SPADES Genome Assembler. Virulence factors and acquired antimicrobial resistance genes were determined using the Center for Genomic Epidemiology pipelines. Chi square test was used to compare virulence factors and resistance genes using R software (v3.3.2).

Results: Resistance genes to 15 different antimicrobial classes were evaluated, and 36.7±8.8% of the isolates were deemed resistant to at least one antimicrobial class. From the resistant isolates, 45.5±15.0% were resistant to at least two or more antimicrobial classes. β-lactam, fosfomycin, and fluoroquinolone resistance were significantly higher than all antimicrobial classes. Twenty-two different virulence factors were found in the isolates. Presence of *gad*, *iss*, and *IpfA* were significantly higher than the rest of virulence factors.

Significance: Virulence factors found are involved in attachment and adhesion, known and predicted effectors, type III secretion system, LEE island non-effectors, and regulators. Understanding the antimicrobial resistance patterns and infection systems of pathogens can be key for adequate treat-

Poster

ment of infections. Proper monitoring procedures of STEC prevalence in meat processing environments in Honduras is required to mitigate the risks of their presence in finished products.

P2-214 SeqSero2: Rapid and Improved *Salmonella* Serotype Determination Using Whole Genome Sequencing Data

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Introduction: Launched in 2015, SeqSero is a bioinformatics pipeline for *Salmonella* serotype determination using whole genome sequencing (WGS) data. The original SeqSero is widely used, despite being relatively slow (2 to 4 minutes per genome for raw reads), unable to differentiate subspecies, and not optimal for draft assemblies.

Purpose: To optimize the SeqSero algorithm and develop SeqSero2, which is rapid (seconds per genome), subspecies-cognizant, and tolerant of low-quality assemblies.

Methods: Unique K-mers of serotype determinant alleles were identified and used to predict O and H antigen types from both raw reads and assemblies. Unique K-mers for *Salmonella* subspecies were determined from a curated collection of reference genomes to enable subspecies identification. In addition, a hybrid approach of reads-mapping and micro-assembly was used to allow optional retrieval of serotype determinant alleles from WGS data.

Results: Evaluated using 894 genomes representing 79 serotypes common in the United States, SeqSero2 was 20 times faster than the original SeqSero, while registering equivalent accuracy for raw reads and much improved accuracy for assemblies from 91.5 to 99.7%. The ability to determine subspecies eliminated the ambiguity in serotype determination for 93 genomes analyzed by the original SeqSero where distinct serotypes of different subspecies shared the same antigenic formula. Furthermore, SeqSero2 demonstrated the potential to detect third-phase H antigens and contamination of WGS data from different serotypes.

Significance: By significantly accelerating and improving *Salmonella* serotype determination from WGS data, SeqSero2 further strengthens the readiness for WGS-based routine surveillance of *Salmonella*.

P2-215 Evaluation of a High-throughput Next Generation Sequencing Assay for Rapid Detection of Spoilage Indicators via Microbiome Analysis

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Introduction: Microbiologists have traditionally relied on culturing techniques to measure the microbial composition of foods and make deductions about spoilage potential and shelf life. Although culturing methods are a powerful indicator tool, these methods have limitations, such as media bias and extensive incubation periods. This research explores the application of next generation sequencing (NGS) technologies to rapidly identify spoilage indicators without the need for culturing or incubation. This assay will reduce processing time and enable identification of the complete microbial community within a food sample simultaneously, rather than testing for a single target microorganism in one assay.

Purpose: The purpose of this study was to evaluate the use of NGS technologies to characterize the microbial composition of raw ground chicken and chicken wings throughout shelf life to identify known spoilage indicators, as well as discover potential new indicators.

Methods: Raw ground chicken and chicken wings were purchased from eight different retail stores for analysis in this study. DNA was extracted from each sample and multiplex (mPCR) was used to amplify universally accepted 16s rRNA regions of the bacterial genome with markers covering 85% of the 16s region. The PCR products were sequenced using the Illumina MiSeq platform.

Results: A signature profile of the microbial community throughout shelf life of raw ground chicken and raw chicken wings was characterized and used to identify known spoilage indicators, as well as potential new indicators. It was found that there was significantly more *Brochothrix* ($P<0.01$), *Janthinobacterium* ($P<0.05$), *Psychrobacter* ($P<0.05$), and *Shewanella* ($P<0.05$) species in expired raw chicken wings as compared to non-expired wings. Expired ground chicken contained significantly more *Pseudomonas* ($P\leq0.05$) than non-expired product.

Significance: This method for spoilage detection and microbiome characterization provides reduced processing time compared to culture methods and provides identification of the microbial community within a sample, rather than detection of a single target in one assay. This assay will allow food processors to make more timely decisions regarding the quality of their product and any changes in sanitation practices to correct deficiencies.

P2-216 Biofilm and Virulence Gene Profiling of *Listeria monocytogenes* Strains Isolated from Environmental and Clinical Sources in Korea

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Introduction: Listeriosis is a foodborne disease caused by an opportunistic bacterial pathogen, *Listeria monocytogenes*, that is abundant in the natural environment. Biofilm formation *in vivo* confers resistance to antimicrobial agents and *in vitro* formation increases resistance and risk of transmission of the pathogen. Virulence factor is regarded as an important marker for the discrimination between pathogenic and nonpathogenic *Listeria* species.

Purpose: This study investigated the detection of *Listeria*-specific, biofilm, and virulence genes of *L. monocytogenes* using PCR and DNA quantification by Ultra-Fast LabChip real-time PCR.

Methods: An assay was evaluated for rapid detection and quantification of *L. monocytogenes* strains. A total of 21 strains of *L. monocytogenes* were isolated from different environmental (smoked salmon, pig, cow, and soil at a pig farm) and clinical (patient's blood) sources and analyzed for the presence of *Listeria*-specific, virulence, and biofilm genes.

Results: L. monocytogenes DNA concentrations ranged from 10^1 to 10^5 copies/µl. *Listeria*-specific genes were detected in 21 strains, but virulence genes were not detected in FI1 and FI3 strains, which were isolated from the soil at the pig farm.

Significance: This result was of importance in the prevention of major *L. monocytogenes* contamination because *L. monocytogenes* can establish biofilms and survive in the food processing environment.

P2-217 Bacterial Microbiota of Wooden Boards Used for Aging Semi-soft Cheese

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Introduction: Cheese aging often relies on adventitious microorganisms present in the environment to aid in development of cheese flavors, aromas, and other organoleptic characteristics. In addition, this microflora might provide a competitive advantage against potential pathogens. A variety of wooden boards are often used for cheese aging. There is little information regarding what microbiota is present on these boards.

Purpose: The purpose of this study is to identify bacteria in the microbiota of pine boards used to ripen semi-soft cheese using next-generation sequencing of the 16S rRNA V4 region.

Methods: For this study, wooden boards used to ripen semi-soft cheese were obtained from local cheese production facilities. Wood shaving samples were collected at a depth of 0.2 cm from the board surface using a sanitized drill bit. Sterile phosphate-buffered saline was added to the shavings and stored overnight at -20°C. Total genomic DNA was extracted using phenol:chloroform and quantified. Primers specific to the V4 region of the 16S rRNA gene were used to amplify bacterial DNA by PCR. Illumina-based sequencing on a MiSeq was used to obtain maximum sequencing depth and coverage. Data were processed using mothur software and further analyzed usingn R software.

Results: Our preliminary results suggest that there are at least 10 operational taxonomic units in these samples. Sequences belonging to genera *Brevibacterium*, *Brachybacterium*, and *Staphylococcus* were found in relatively high abundance at 0.2-cm depth in the boards. The presence of these genera was not unexpected, as they are thought to contribute to the flavor and other characteristics of cheese.

Significance: Wooden boards are commonly used in cheese aging and are thought to contribute to the desired aroma and flavor of the product. Identifying the microbiota associated with wooden boards used for cheese aging will improve our understanding and perhaps allow manipulation of the microbiome to enhance desired characteristics of cheese quality and safety.

P2-218 Microbial Community of Naturally Fermented Soymilk and Soymilk-Kefir Produced from Sprouted Soybeans under Optimized Sprouting Conditions

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❖ ***Developing Scientist Competitor***

Introduction: Spontaneous fermentation of soy-based foods is characterized by a vast microbial community of lactic acid bacteria (LAB) and yeast. Fermentation of soymilk has been achieved in recent times by the use of pure microbial strains; however, natural fermentation of soymilk has been sparsely studied.

Purpose: This study describes the microbial community of spontaneous fermentation of soymilk and soymilk-kefir from sprouted soybeans and their respective controls (fermented soymilk from unsprouted soybeans) using molecular-based techniques.

Methods: Soymilk produced from optimized conditions of soybean sprouting (12 h soaking and 52 h germination) were allowed to ferment naturally for up to 48 h and undergo kefir fermentation by the addition of 5 g wet kefir grains/100 ml soymilk for 24 h. Eighteen samples were collected at 6-h intervals (0 to 48 h for naturally fermented soymilk and 0 to 24 h for soymilk-kefir) from each fermentation lot and their LAB and yeast loads were enumerated after growth on suitable media. Further microbial characterization was carried out using Rep PCR coupled with 16S and 26S rRNA sequencing.

Results: Higher LAB loads were obtained for all sprouted fermented soymilk and sprouted soymilk-kefir samples compared with the controls. Increase in LAB counts were recorded as fermentation time increased for all naturally fermented soymilk samples; however, LAB decreased with increase in fermentation time for soymilk-kefir samples. Yeast growth was found only in soymilk-kefir fermentations, and higher loads were obtained in sprouted soymilk-kefir. LAB species detected from sequencing include *Weissela cibaria*, *Lactococcus lactis*, *Leuconostoc lactis*, and *Leuconostoc messenteroides*, while yeast species were *Saccharomyces cerevisae*, *Pischia fermentans* and *Torulospora delbrueckii*.

Significance: The microbial diversity of spontaneous fermentation of soymilk and soymilk-kefir was established through this study. Isolated cultures could be useful for controlled fermentation of soymilk.

P2-219 Development of Colorimetric Loop-mediated Isothermal Amplification (LAMP) Assay Using Molecular Beacon Horseradish Peroxidase-mimicking for the Rapid Detection of *Vibrio* spp.

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Introduction: Foodborne *Vibrio* infections are on the rise globally, even as numbers of infections from other foodborne bacterial pathogens are decreasing. Therefore, the need for a rapid, sensitive, and convenient method for detection of *Vibrio* spp. has increased.

Purpose: The objective of this study is to develop a colorimetric loop-mediated isothermal amplification (LAMP) assay using a molecular beacon, horseradish peroxidase-mimicking DNAzyme (HRPzyme), for rapid and sensitive detection of *Vibrio* spp.

Methods: Two pairs of primers targeting the hemolysin gene and a molecular beacon were designed and synthesized. The optimization of the colorimetric LAMP assay was performed by determining several key factors such as concentration of molecular beacon and incubation temperature and time. After optimization, sensitivity and specificity of the method were investigated. To validate the method, artificially contaminated flatfish samples with known amounts of *Vibrio* spp. (10^0, 10^1, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, and 10^8 CFU/ml) were analyzed by the LAMP assay.

Results: The colorimetric LAMP assay optimized at 58.8°C showed a detection limit of 1 fg/ml DNA (hemolysin gene) and was confirmed to be specific to *Vibrio* spp. The time required to complete the method was within 1 h. The cut-off value of the method for the artificially inoculated flatfish samples was 1×10^1 CFU/g.

Significance: The method was successfully applied for rapid detection of *Vibrio* spp. in fish and is therefore a useful tool for the study of *Vibrio* spp. in food samples. This study suggests that the LAMP assay is expected to be used as a point-of-care molecular diagnostic technology because it does not require any expensive instruments such as a thermocycler or detector.

P2-220 Whole Genome Analysis of *Salmonella S*erovars Isolated from Produce Irrigation Water from the State of Georgia

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Introduction: In collaboration with academic institutions, our group has made efforts to investigate the prevalence, diversity, and distribution of *Salmonella enterica* in surface water in the southern and central regions of Georgia, United States. These efforts led to the isolation of a diverse set of *Salmonella* strains from environmental water, collected over three separate surveillance activities. To our surprise, strains of *Salmonella enterica* Newport were the dominant serovar recovered (29 of 55) from one study and were also frequently isolated in the other two studies.

Purpose: The purpose of this investigation was to compare and determine relatedness among isolates of *Salmonella* Newport from three distinct surveillance activities, which all took place in agricultural environments from a discrete geographical area, namely the rivers and surface waters of southern and central Georgia, United States, and to compare these genomes to *Salmonella* Newport isolates from other geographical areas.

Methods: Isolates of *Salmonella* Newport (*n*=41) were compared by reference-based whole genome single nucleotide polymorphism (SNP) analysis, average nucleotide identity, and tetranucleotide frequency to determine relatedness. Representative strains from each identified cluster were compared by reciprocal BLASTP analysis to determine unique and clade-specific genomic features.

Results: SNP analysis on the genomes separated the isolates into two major clades, one with 16 strains (lineage II) and the other with 25 strains (lineage III). All isolates of lineage II were recovered from a single investigation and single source. All those strains harbor a large plasmid, which is highly homologous with the antibiotic resistance plasmid, pSN254, from the *Salmonella* Newport strain SL254. The lineage III clade was further separated into three subclades, with two of them comprising isolates from more than one study. Comparative genomics confirmed these results, with sub-clade members possessing group-specific, as well as inter-group specific, prophage and other mobilome elements.

Significance: Findings in this study suggest that strains of lineages II and III of *Salmonella* Newport can persist in some geographic areas for an extended period of time.

P2-221 Genotypic and Phenotypic Mechanisms of Biofilm Formation by Emetic Toxin-producing *Bacillus cereus* Strains

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Introduction: Bacillus cereus is widespread in nature, and readily found in soil and a variety of foods. It can form biofilms on different biotic and abiotic surfaces and acts a source of contamination. Therefore, biofilm formation by *B. cereus* is food safety concern.

Purpose: The objectives of this study were to examine biofilm formation abilities of *B. cereus* emetic strains and to determine phenotypic characteristics to understand their biofilm formation behaviors.

Methods: A total of 11 emetic strains and two reference strains of *B. cereus* were used in this study. Biofilm formation analysis was quantified by crystal violet assay. Characteristics of biofilm formation, including cell surface hydrophobicity, antibiotic susceptibility, resistance to disinfectants, and swarming motility test, were performed. In addition, expression levels of two genes (*spo0A* and *abrB*) involved in the regulation of cell differentiation, mobility, and biofilm formation were determined.

Results: Crystal violet assay results indicated that the biofilm formation behavior was highly variable depending on strain and surface. Phenotypic characteristics of planktonic cells such as cell surface properties and swarming abilities were positively correlated with biofilm formation behavior. Expression levels of *spo0A* and *abrB* were positively correlated with biofilm formation, further confirming the role of swarming mobility in *B. cereus* biofilm formation.

Significance: Results of this study indicate that multiple factors are involved in biofilm formation by emetic toxin-producing *B. cereus.*

P2-222 Rapid Discovery of an Emerging Contamination Event in Nut Butter Using Whole Genome Sequencing

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Introduction: Next-generation whole genome sequencing (WGS) is now used regularly by government and state food safety investigators as a high-resolution molecular epidemiological tool for delimiting the scope of foodborne outbreak events and for the tracking of genetically homogenous bacterial strains during foodborne contamination events.

Purpose: Making a genomic link between food sources and clinical isolates is essential for effective traceback to a contamination source. Here, WGS was applied to *Salmonella enterica* serovar Braenderup to examine the relationship between several environmental swab samples previously associated with a nut butter facility and several reported cases of salmonellosis in 2014.

Methods: To this end, we relied on the GenomeTrakr foodborne pathogen WGS network and database; we downloaded and analyzed the genomes of 26 strains of *Salmonella* Braenderup from disparate clinical and environmental sources, the latter of which included several that were isolated from a nut butter facility in the United States. These genomes were combined with other ecologically diverse *Salmonella* Braenderup strains and subjected to comparative genomic analysis using maximum likelihood.

Results: Analysis of all 292 variable high-quality single nucleotide polymorphism (SNP) positions revealed a single monophyletic and highly clonal lineage (separated by ≤5 SNPs) of *Salmonella* Braenderup that contained environmental swab isolates from the nut butter facility, as well as clinical isolates and several food contamination events. As expected, unrelated environmental reference isolates of *Salmonella* Braenderup were phylogenetically disparate to the nut butter clade, falling at least 32 SNPs away. It is noteworthy that the link between illnesses and the processing facility was established by 15 diagnostic SNPs from WGS, even though a common food vehicle was not identified at this point.

Significance: Taken together, these findings support applicability of WGS in delimiting contamination sources, even in rare cases where a food vehicle remains elusive. Moreover, these data underscore the utility of this technology in revealing otherwise undetected genotypic differences essential to the tracing of bacterial pathogens as they emerge in the food supply.

P2-223 Genometrakr Proficiency Testing for Foodborne Pathogen Surveillance

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Introduction: Pathogen monitoring is becoming much more robust as sequencing technologies become more affordable and accessible worldwide. This transition is especially apparent in the field of food safety, which has demonstrated how whole genome sequencing (WGS) can be used on a global scale to protect public health. GenomeTrakr coordinates the WGS performed by public health agencies and other partners by providing a public database with real-time cluster analysis for foodborne pathogen surveillance.

Purpose: As growing numbers of public health labs use WGS technology to support enforcement decisions, it is essential to have confidence in the quality of the data being used and the downstream data analyses which guide these decisions. Routine proficiency tests, such as the one described here, have an important role in ensuring the validity of both data and procedures.

Methods: GenomeTrakr ran an annual internal proficiency test through 2015 that is now harmonized with PulseNet. In 2015, the GenomeTrakr proficiency test consisted of eight isolates of common foodborne pathogens; participating laboratories were required to follow a protocol to culture these and perform WGS. Resulting sequence data were evaluated for proper annotation, sequence quality, and applicability to downstream bioinformatics analyses.

Results: Overall, this exercise revealed the degree of variation which should be expected in sequence data produced across a diverse network of laboratories. Illumina MiSeq sequence data collected for the same set of strains across 21 different labs exhibited high reproducibility, while revealing a narrow range of technical and biological variance.

Significance: The numbers of SNPs reported for sequencing runs of the same isolates across multiple labs support the robustness of our cluster analysis pipeline in that each individual isolate cultured and resequenced multiple times in multiple places are all easily identifiable as originating from the same source. Subsequent proficiency tests confirm these results.

P2-224 Comparative Genomic Analysis of *Salmonella enterica* subsp. *enterica S*erovar Senftenberg Isolates from Recurrent Outbreaks

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Introduction: Pistachios have been linked to two multistate outbreaks of *Salmonella* infections in 2013 and 2016 and involved in product recalls in 2009 and 2013. *Salmonella enterica* serovar Senftenberg has been commonly isolated from pistachios since 2009.

Purpose: In this study, whole genome sequencing (WGS) data from 69 *Salmonella* Senftenberg isolates were analyzed to provide insight into evolutionary relationships and persistence among strains linked to events of salmonellosis over the seven-year period.

Methods: The sources of these isolates comprised 41 pistachio (2005 to 2016), 18 other food commodities (1941 to 2016), three clinical (1964 to 2016), and seven environmental (2011 to 2016) strains. Thirty-eight out of the 41 pistachio isolates were obtained from the United States, and one each from Canada, Turkey, and Lebanon. Genomes of three strains isolated from pistachios were completely closed with long-read sequencing technology and used as reference to create a cgMLST scheme containing 2,696 core genes using Ridom SeqSphere$^+$. Sequence assemblies were imported and typed using the cgMLST scheme.

Results: The phylogeny illustrates that the 2016 outbreak involved direct descendants from the 2009 and 2013 events, rather than being an independent contamination event. These isolates clustered together sharing only a few single nucleotide polymorphism differences between them and were distinct from other food isolates and pistachio products from outside the United States. Interestingly, pistachio isolates from a separate 2013 outbreak associated with environmental contamination were genetically different from the clonal strain. Notably, the outbreak strain harbored a *ClpB* gene that was significantly different than the ATCC reference strain. Part of a stress-induced multi-chaperone system, it is involved in the recovery of the cell from heat-induced damage.

Significance: The data suggests a prominent clonal strain of *Salmonella* Senftenberg is persistent in the pistachio production supply chain. The mechanisms of persistence of this clonal strain is of high importance to public health and may allow for clearance of this strain.

P2-225 Transcriptomic Analysis of *Listeria monocytogenes* Adaptation on Fresh-cut Produce

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Introduction: In recent years, *Listeria monocytogenes* has been frequently implicated in foodborne outbreaks involving fresh-cut produce. However, molecular understanding of *L. monocytogenes* adaptation on fresh-cut produce is still limited.

Purpose: To investigate global transcriptomic responses of *L. monocytogenes* during its adaptation on fresh-cut produce.

Methods: Fresh-cut celery, apple, cucumber, cantaloupe, and tomato samples were inoculated with 10^9 CFU of *L. monocytogenes* strain F8027 that was previously isolated from celery. Samples were stored at 4°C for 48 h. A reference sample was prepared by subjecting the same strain to phosphate buffered saline (PBS) under the same storage conditions. Total RNA of *L. monocytogenes* were extracted, purified, reverse transcribed to cDNA, and sequenced on an Illumina MiSeq platform. Genes and functional pathways that showed differential expression on fresh-cut produce samples in comparison with the reference were identified.

Results: A total of 118 and 78 genes were up- and down-regulated in all five fresh-cut produce samples. Major functional categories of commonly up-regulated genes included membrane transport (*n*=18), transcriptional regulation (*n*=10), and amino acid metabolism (*n*=11) such as that of arginine, lysine, cysteine, methionine, and histidine. Major functional categories of commonly down-regulated genes were phosphotransferase system (*n*=12) and carbohydrate metabolism (*n*=33), including that of fructose, mannose, starch, sucrose, pentose phosphate, pentose, and glucuronate. The transcrip-

Poster

tome in response to apple diverged from other produce samples by featuring the largest numbers of genes that were uniquely up- or down-regulated in this fruit.

Significance: The information obtained from this study will contribute to elucidating molecular mechanisms of *L. monocytogenes* adaptation on fresh-cut produce.

P2-226 Mitochontrakr: Mitochondrial Genome Assemblies of Insects Commonly Known to Infest Foods

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Introduction: Complete mitochondrial genomes are useful references for a wide variety of population genetic, phylogenetic, and comparative genomic analyses. There is unanimous consensus that some insect species are important vectors of foodborne pathogens. Although targeted amplification techniques are being developed to identify insects in foods and food ingredients, there are always cases when more information is valuable to distinguish between closely related insect contaminants. Thus, reference collections of fully sequenced mitochondrial genomes can provide new amplification targets, as well as sufficient information to distinguish between closely related species.

Purpose: To generate a high-quality reference collection of insect mitochondrial genomes for use with targeted and target-independent sequence-based detection methodologies.

Methods: Fifty-two insect species belonging to 19 families and six orders were used for genomic DNA extraction using the DNeasy blood and tissue kit and sequenced on the Illumina NextSeq platform. Denovo assembly and iterative mapping were performed using NovoPlasty 2.6.2 and MITObim. Circularized assemblies were annotated using MITOS and Geneious.

Results: Fifty-two near complete mitochondrial genome assemblies and contigs have been annotated and submitted to the National Center for Biotechnology Information (NCBI) for future reference relatives.

Significance: This collection of complete mitochondrial genome sequences from insects and their close relatives known to commonly contaminate foods has been made publicly available at the NCBI under the header MITOchonTrakr. The publicly available data include annotated assemblies, raw sequencing data, and authenticated reference specimens. This collection should enable a variety of future target-based and target-independent detection assays.

P2-227 Anaerobic Physiological Pre-enrichment Improves *Salmonella* Yield from Naturally Contaminated Papayas and Allows Detection and Subtyping Using Metagenomics

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Introduction: Food samples generally demand selective culture steps for pathogen identification. Expedited source tracking can be achieved using variations of the U.S. Food and Drug Administration's Bacteriological Analytical Manual pre-enrichment protocols in conjunction with quasi-metagenomics (shotgun sequencing of enrichments and genomic analyses).

Purpose: To develop a physiologically selective broth that allows *Salmonella* to thrive anaerobically utilizing tetrathionate and glutathione and improve speed and precision of serovar level discrimination based on genomic data from pathogens growing in complex food matrices.

Methods: Fifteen papayas from a naturally contaminated lot from Mexico in August 2017 were analyzed for *Salmonella*. Papayas were pre-enriched aerobically in modified buffered peptone water or anaerobically in tryptone broth supplemented with glutathione and tetrathionate (TT), then transferred to TT broths. In parallel, aerobic plate counts, *Salmonella* quantitative PCR (qPCR), and MPNs, as well as microbiome samples, were described for H0 and H24 of enrichments. Shotgun sequencing was used to subtype *Salmonella* from microbiome samples, annotated by CosmosID and CFSAN single nucleotide polymorphism pipelines.

Results: Nine samples were positive for *Salmonella*. Average total bacterial load was 2×10^6 CFU/g of papaya at H0 and increased to 6×10^8 CFU/g with 1.7 to 7 *Salmonella* log MPN at H24, which correlated with qPCR results. The microbiome of papayas was diverse, with numerous enteric species. Proportional abundance of *Salmonella* ranged from undetectable to 2.5% following pre-enrichment. One sample, TT enrichment following anaerobic pre-enrichment, supported 42% proportional abundance of *Salmonella*, with 71 to 79% reads mapping to *Salmonella*. Anaerobic pre-enrichment favored *Enterococcus* spp. (31 to 43%) and led to reduced proportional abundance of *Pseudomonas* (8 to 53% in aerobic compared to 0 to 2% for anaerobic), as well as certain enteric species such as *Providencia rettgeri* (7 to 18% in aerobic compared to >1% for anaerobic) when transferred to selective TT broths.

Significance: Improving the selectivity of *Salmonella* enrichment from foods and coordinating this with sequencing approaches facilitates expedited source attribution.

P2-228 Genetic Context of Antimicrobial-resistant *Escherichia coli* at the Livestock-Wildlife Interface

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Introduction: Previous studies show that livestock production is spatially correlated with increased prevalence of antimicrobial resistance (AMR) within the gut bacteria of associated wildlife. Thus, impacted wildlife potentially maintain, reintroduce, and disseminate AMR bacteria across food production landscapes. However, the genetic context underlying the apparent similarities of AMR phenotypes at the livestock-wildlife interface remains unclear.

Purpose: Here, the genetic context of phenotypically similar AMR *Escherichia coli* harboring priority AMR phenotypes that were collected from cattle and raccoons was investigated.

Methods: Phenotypic testing was performed on more than 1,000 bacteria isolated from cattle and wildlife feces collected at six livestock facilities in Colorado. From these isolates, two groups of *Escherichia coli* isolates resistant to cephems (n=8) and cephems/fluoroquinolones (n=3) were selected for whole genome sequencing analyses on the basis of identical antibiograms (18 different antibiotics tested) and presence in the feces of both raccoons and cattle on multiple farms. Complete closed and polished genomes (PacBio/HiSeq sequencing) were acquired for each isolate, and *in silico* (Parsnp, CARD-RGI, etc.) analyses were used to evaluate phylogenetic relationships and AMR phenotypes.

Results: Three main clades of AMR *E. coli* isolates were identified, with the most highly conserved clade containing isolates from both cattle and raccoons. The variable genomes of isolates was primarily attributable to differential repertoires of Inc-like plasmids and prophage mosaics. Similarities between cattle and wildlife isolates were further highlighted by conserved AMR determinants. In total, 59 AMR determinants were detected, with 47 of these found in all isolates tested. Identical plasmid-encoded CMY-2 AmpC β-lactamases were present in six isolates. Similarly, conserved mutations in *gryA* and *parC* were linked to fluoroquinolone resistance.

Significance: Genetic conservation between AMR isolates from cattle and wildlife suggest a complex AMR livestock ecology that has inputs from multiple sources.

P2-229 Identification of a New Shiga Toxin-producing *Escherichia coli* O26:H11 Stx2 Single Nucleotide Polymorphism Clonal Complex in the United States

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Introduction: Shiga toxin-producing *Escherichia coli* O26:H11 (STEC O26) has emerged as an important human pathogen capable of causing enterohemorrhagic diarrhea and hemolytic uremic syndrome (HUS). O26 strains carrying Stx2 are associated with an increased risk of HUS in comparison to strains that contain Stx1. Recently, single nucleotide polymorphism (SNP) and sequence typing (ST) of STEC O26 strains predominantly isolated from Europe revealed that Stx2 strains could be classified into one of four clonal complexes (CC), with SNP-CC1 being identified as a new European clone.

Purpose: Characterize STEC O26 Stx2 SNP-CC strains in the United States.

Methods: High-molecular weight DNA were used to make size-selected PacBio libraries and sequenced on a Pacific BioSciences RS II sequencing platform. Reads were assembled using HGAP v3.0 and contigs were trimmed and circularized in Geneious. Geneious, Mauve, and VirulenceFinder were used to analyze the genome sequence data.

Results: Strains from the United States were classified into three of the four SNP-CC previously identified for European STEC O26, and additionally into a SNP-CC that had not been previously identified. The strains from this newly identified SNP-CC differed from the other SNP-CC by integration of a Stx-phage between *yfdC* and *argW* and a large virulence plasmid that ranged from 147 to 173 kb in size. The plasmid contained the virulence factors *ehxA*, *toxB*, and *espP*, but was missing the catalase/peroxidase *katP* gene. The increased size of the plasmid could be attributed in part to the inclusion of the *efa1/lifA* gene, as well as 20 additional genes not previously found on the typical O26:H11 virulence plasmid.

Significance: We report the identification of a new Stx2 SNP-CC in the United States. Phylogenetic analysis suggests this new SNP-CC arose through convergent evolution in STEC O26 by acquiring mobile genetic elements.

P2-230 Comparative Analysis of Genome and Methylome of a Multidrug-resistant *Campylobacter jejuni* strain YH002 from Retail Beef Liver

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Introduction: Armed with multiple virulence factors and rising antimicrobial resistance (AMR), *Campylobacter jejuni* continues to be a major cause of foodborne gastroenteritis worldwide.

Purpose: In this study, we report the comparative genomic and methylome data of *C. jejuni* YH002.

Methods: C. jejuni YH002 was isolated from retail beef liver by passive filtration method. The PacBio RS II Single Molecule Real-Time (SMRT) sequencing system and the MiSeq System were used for genome sequencing of the strain. The whole genome sequence was assembled, annotated, and analyzed with various bioinformatics software.

Results: C. jejuni YH002 has a 1,820,488 bp genome comprised of a chromosome (1,774,584 bp, GenBank accession numbers CP020775) and a plasmid (45,904 bp, CP020776). Annotation of the genome revealed novel features, including an integrated intact phage element, multiple AMR genes, virulence factors, and a Phd-Doc type toxin-antitoxin system. Phenotypic tests of AMR showed that *C. jejuni* YH002 was resistant to tetracycline and amoxicillin, which correlates with the AMR genes found in the strain. Comparative analysis of cell motility at genotypic and phenotypic levels identified discernible patterns of amino acid changes and could explain variations of motility among *C. jejuni* strains. Analysis of DNA methylomes of *C. jejuni* strains revealed non-uniform methylation patterns among the strains, although type I and type IV restriction-modification (RM) systems were observed. Interestingly, a novel methylation motif (CGCG**A**) of type II RM system was found in the organism.

Significance: These results provide important clues to the genetic mechanisms of antimicrobial resistance, cell motility, and DNA modifications in *C. jejuni*.

P2-231 A Comparison of In Silico Methods to Serotype *Salmonella* enterica Isolates from Food and Agricultural Environments

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Introduction: Salmonella is the leading foodborne bacterial pathogen in Canada and the United States. More than 2,500 serotypes of S*almonella enterica* are known, and these serotypes have been defined according to the Kaufmann-White-Le Minor (KWL) classification scheme; however, misinterpretation of serotypes can result from weak or non-specific antibody agglutination reactions and loss of antigen expression. *In silico* serotyping methods have become increasingly popular as the cost of whole genome sequencing (WGS) continues to decrease.

Purpose: The objective of this study was to compare *in silico* serotyping methods SISTR and SeqSero to KWL serotyping of 128 *S. enterica* isolates of food and agricultural origin.

Methods: Traditional serotyping was performed using antisera-based agglutination assays according to standard KWL practices. Whole genome sequencing (WGS) was performed on 128 *S. enterica* isolates on an Illumina MiSeq platform with 300-bp paired-end libraries and 30X coverage, and the

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raw reads were assembled using the A5 pipeline. *In silico* serotyping was performed using SISTR and SeqSero, and a WGS tree based on core genome SNPs was constructed using HARVEST and edited using iTOL to compare KWL and *in silico* methods.

Results: SISTR identified 88% of isolates correctly (compared to the KWL serotype) and 12% incorrectly (identified the wrong serotype compared to KWL), of which 8% of incorrect results were for serotypes outside the top 100 illness-causing serotypes in Canada. SeqSero identified 68% of isolates correctly and 8% incorrectly. Furthermore, SeqSero produced 24% ambiguous (reported more than one serotype) results and could not identify the serotype of rough *S. enterica* isolates. The WGS core genome SNP tree demonstrated that isolates from individual serotypes clustered together, highlighting the genomes that produced incorrect and ambiguous *in silico* results.

Significance: These results indicate that SISTR is a reliable and accurate method for molecular serotyping of common foodborne serotypes of *S. enterica*.

P3-01 Phenotypic and Genotypic Detection of Methicillin Heat-resistant *Staphylococcus aureus* in Pasteurized Camel Milk Distributed in Saudi Arabia

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Introduction: Antibiotic- and heat-resistant bacteria in camel milk are a potential public health problem. *Staphylococcus aureus* is a significant opportunistic pathogen both in humans and dairy animals, especially camels.

Purpose: Phenotypic and genotypic characterization of methicillin-resistant staphylococcal strains recovered from pasteurized camel milk distributed in the retail markets of Saudi Arabia.

Methods: A total of 100 samples of pasteurized camel milk were assessed between March and May 2017. PCR assay was employed for the detection and verification of methicillin-resistant strains by using methicillin-resistant primer genes (*mec*A) and nucleotide sequencing.

Results: Out of 20 isolates of *Staphylococcus aureus,* about 10 isolates were resistant to cefoxitin (30 µg) and identified as methicillin-resistant bacteria. The resistance ratio reach 60% for many other different antibiotics through antimicrobial susceptibility tests. Basic Local Alignment Search Tool (BLAST) analysis of the gene sequence showed that the *S. aureus* resistant strain had 96 to 100% similarity to *S. aureus* strain CS100 penicillin binding protein 2a (*mecA*) gene. Heat resistant *S. aureus* was detected in some methicillin-resistant isolates and can resist up to 80°C. *D*-values (decimal reduction times) were determined for the 10 isolates at 80°C in brain heart infusion medium and were 0.7 to 1.0 min. There was no difference between total protein profile for *S. aureus* and methicillin/heat-resistant *S. aureus* isolates when samples were analyzed on SDS-PAGE.

Significance: This study provides much needed data on antibiotic and thermal resistance of *S. aureus* detected in pasteurized camel milk. In the present case, it is recommended that pasteurization temperature should be more than 80°C for camel milk.

P3-02 Evidence of *Bacillus cereus* Spores as the Target Pathogen in Thermally Processed Extended Shelf-life Refrigerated Foods

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Introduction: Many extended shelf life (ESL) refrigerated foods are given a "nonprot bot cook" (i.e., 90 C for 10 min) to yield a 6-log reduction of nonproteolytic *Clostridium botulinum* spores and ensure food safety. Recently, certain psychrotrophic outbreak strains of *Bacillus cereus* have become a point of interest, as spores of these strains have been described to be more resistant than those of nonproteolytic *C. botulinum*.

Purpose: Evaluate thermal resistance of six outbreak strains of *B. cereus* and compare their resistance with thermal resistances of nonproteolytic *C. botulinum.*

Methods: Spores of six outbreak *B. cereus* strains (BC 005-BC 010) acquired from Unilever Foods were prepared using a nutrient agar (NA) method, diluted in ACES buffer to 10^5 to 10^6 CFU/ml and dispensed into a sterile NMR tube, heat-sealed, and subjected to temperatures ranging from 80 to 91°C in an oil bath. *B. cereus* survivors were determined by plate count using NA after incubation at 30°C for 48 h.

Results: Thermal *D*-values of six outbreak *B. cereus* strains decreased as process temperature increased from 80 to 91°C. At 80°C, the *D*-values ranged from 183 to 454 min. Thermal *D*-values for these six strains ranged from 12 to 23.2 min at 91°C. Among the six strains evaluated, BC009 had highest thermal *D*-value at 91°C (23.2 min), which is significantly greater than thermal *D*-values of nonproteolytic *C. botulinum* types B and F strains (Kap9-B, Ham-B, 610-F) obtained in our previous study. Thermal *D*-values of these nonproteolytic *C. botulinum* strains were <1 min at 87°C.

Significance: Results indicate that outbreak *B. cereus* spores are more resistant to thermal processing than nonproteolytic *C. botulinum* spores based on their *D*-values. The data obtained in this study may suggest the use of *B. cereus* spores as potential non-select agent surrogates for establishing thermal processes for ESL foods.

P3-03 Survival of *Escherichia coli* O157:H7 in Spent Sprout Irrigation Water

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Introduction: Spent irrigation water has been tested in compliance programs in order to detect *Escherichia coli* O157:H7 in sprouts. However, little is known about the survival of *E. coli* O157:H7 in spent sprout irrigation water.

Purpose: This study examined the survival of *E. coli* O157:H7 in spiked, spent sprout irrigation water during storage by real-time PCR using three most probable number (MPN) procedures.

Methods: Spent alfalfa sprout irrigation water was inoculated with ~1,000 *E. coli* O157:H7 cfu/ml and stored refrigerated (4°C) for three days. Levels of the pathogen were determined daily using three-tube MPN procedures in triplicate. Three enrichment procedures were used: (1) modified buffered peptone water + pyruvate (mBPWp) for 5 h at 37°C, followed by addition of acriflavine (A), cefsulodin (C), and vancomycin (V), and further incubated at 42°C to further enhanced selectivity (U.S. Food and Drug Administration Bacteriological Analytical Manual [BAM]); (2) mBPWp with CV held at 42°C with shaking; and (3) mBPWp with CV held at 42°C without shaking. After enrichment, wash-spin-boil preparations were prepared and real-time PCR detection of *E. coli* O157:H7 was done on the ABI 7500 Fast platform, screening for *stx*1, *stx*2, and O157*wzy* gene targets.

Results: All three MPN enrichments showed that the inoculated *E. coli* O157:H7 survived refrigerated storage for three days. At the onset of storage, the levels of *E. coli* O157:H7, as determined by the BAM procedure, using method (2) and method (3) were 3.24, 3.27, and 3.29 log MPN/ml, respectively. After 3 days at refrigeration temperatures, the levels were found to be 3.22, 3.44, and 3.37, respectively. No significant difference ($P>0.05$) was observed among the *E. coli* O157:H7 levels determined by the three enrichment procedures.

Significance: These results demonstrate that spent sprout irrigation water samples contaminated with *E. coli* O157:H7 can be shipped refrigerated to laboratories within a three-day timeframe without the organism dying off during transport.

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P3-04 Antibiogram and Phylogenetic Relatedness of Non-O157 Shiga Toxin-producing

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) is a major foodborne bacterial pathogen that causes sporadic human infections or outbreaks globally. Previously, we proposed that STEC O91:H14 are the predominant STEC serovars associated with asymptomatic infections or mild diarrhea in humans in South Korea.

Purpose: In this study, we hypothesized that a certain pathogenic clone(s) of STEC O91:H14 isolates are present in South Korea, reflecting a geographical difference in their distribution.

Methods: A total of 17 STEC O91:H14 isolates were collected from human patients (n=14) or retail meat samples (n=3) during 2003 to 2014 and investigated for their genetic relatedness by multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), and *stx* subtyping. Virulence potentials of these isolates were further evaluated phenotypically and genotypically.

Results: MLST analysis demonstrated that all isolates belonged to the sequence-type (ST) 33 STEC lineage, implying that they originated from a single prototype strain. Using PFGE analysis, however, such ST33 STEC O91:H14 isolates were divided into three pulsotypes (designated A, B, and C) with a cut-off value of 80% similarity. Interestingly, all STEC isolates in the pulsotypes A and B harbored the elastase-activated Stx2d gene ($stx2_{dact}$) with or without stx_1, while those in the pulsotype C carried the stx_1 gene (with or without stx_{2b}). None of the isolates had the $stx2_{dact}$ gene.

Significance: These results imply that a low-pathogenic clone of STEC O91:H14 isolates with ST33 is prevalent in South Korea.

P3-05 Identification and Characterization of Two Novel Staphylococcal Enterotoxins

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Introduction: Staphylococcal superantigens (SAgs), also known as staphylococcal enterotoxins (SEs), play a very important role in clinical infections and food poisoning caused by *Staphylococcus aureus*. Recently, *Staphylococcus argenteus* and *Staphylococcus schweitzeri* were recognized as novel species close related to *S. aureus*, and these three species were redefined as the *S. aureus complex* (SAC). Our previous study indicated that *S. argenteus* and *S. schweitzeri* harbor most of SE genes identified in *S. aureus* with significant divergence.

Purpose: To identify and characterize two novel staphylococcal enterotoxins among SAC.

Methods: Recombinant SE/26 and SE/27 were expressed and purified to assess the superantigenic activity by examining cell proliferation and cytokine production of human peripheral blood mononuclear cells (PBMCs) and mouse splenocytes.

Results: The two putative SE genes were found located in the upstream of pathogenicity island *v*Saβ and the deduced amino acid sequences showed <66.7% identity with those of known SEs. In the *S. aureus* strains, mRNA encoding SE/26 and SE/27 were highly expressed in the early stationary phase of cultivation, whereas a high level of expression was found in *S. argenteus* and *S. schweitzeri* strains at the log phase. SE/26 and SE/27 exhibited superantigenic activity in human peripheral blood mononuclear cells (PBMCs) and mouse splenocytes. Interestingly, SE/26 variants from different species showed different sensitivity to human PBMCs, which correspond to the primary bacterial species hosts.

Significance: We identified SE/26 and SE/27 to be two novel SE toxins and suggested that some SEs evolve along with the bacteria when the bacteria adapt the hosts' immune systems.

P3-06 Microbiological Growth Assessment of *Staphylococcus aureus* and *Bacillus cereus* in Biscuit Dough Systems Using Simulated 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 *Bacillus cereus*, potentially resulting in heat-stable enterotoxin formation. Conditions of non-continuous dough systems may allow the growth of *S. aureus* and *B. cereus* during routine production.

Purpose: The purpose of this study was to evaluate the potential growth of *S. aureus* and *B. cereus* in biscuit dough during simulated production conditions (time/temperature) to provide scientific support for appropriate preventive controls for the manufacturing facility's food safety plan.

Methods: Dough samples were individually inoculated with multiple strains of *S. aureus* and *B. cereus* to achieve a target level of ~10^2 CFU/g and stored at 15.5 and 18.3°C. Samples were enumerated for *S. aureus* and *B. cereus* using U.S. Food and Drug Administration-approved microbiological methods at several time points between 0 and 14 days. *S. aureus* enterotoxin testing was conducted using VIDAS SET2. The food safety limit was defined as 10^5 CFU/g for both *S. aureus and B. cereus*. Analysis of variance was performed to understand the effect of storage temperature and time on the growth profile of *S. aureus* and *B. cereus*. The level of significance used was 5%.

Results: Enumeration of *S. aureus* presented significant challenges due to high background microflora. Therefore, enterotoxin testing results were used to corroborate if *S. aureus* levels exceeded the proposed food safety limit. Data analysis indicated growth of *S. aureus* and *B. cereus* did not exceed ($P<0.05$) the food safety limit at both temperatures. This may be attributed to "competitive inhibition" due to the presence native ingredient microflora.

Significance: The study findings indicate no significant food safety risk associated with the current production practices. The data generated in this study provide scientific basis for the facility's food safety plan.

P3-07 *Clostridium perfringens* Has New Roles Other Than Its Well-known Role in Foodborne Illness

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Introduction: Clostridium perfringens is a major pathogen that causes foodborne illness and is frequently isolated from foods such as meat products and fermented foods However, the role of *C. perfringens* in the human intestine is only partially known.

Purpose: The objective of this study was to identify the new role of *C. perfringens* in the human intestine.

Methods: Phosphate buffer solution (PBS) or *C. perfringens* were orally injected into five-week-old (YOUNG) and 12-month-old C57BL6/J (ADULT) mice. Gene expression levels related to aging factors (inflammation [*TNF-α, IL-1β*, and *IL-6*] and oxidative stress [*SOD1, SOD2, SOD3, GSR, GPx3,* and *CAT*] responses) were evaluated in the brain, small intestine, and liver. In addition, brain damage factors (*BAX1, HMGB1, C/EBPβ, C/EBPδ, CHOP,* and *APP*) were examined.

Results: In general, the gene expression levels of aging factors were significantly increased ($P<0.05$) in mice treated with *C. perfringens* compared to PBS-treated mice, and the levels were higher ($P<0.05$) in CP-ADULT than in CP-YOUNG mice. For brain gene expression levels, apoptosis derived from

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cell damage-related genes (*BAX1* and *HMGB1*) and brain disorder-related genes (*C/EBPβ, C/EBPδ, CHOP,* and *APP*) increased ($P<0.05$) in mice treated with *C. perfringens* compared to PBS-treated mice, regardless of age. For protein expression levels, oxidative stress response proteins (GPx and SOD2) and cell damage-related proteins (HMGB1) significantly increased ($P<0.05$) in mice treated with *C. perfringens*. In addition, C/EBP protein was significantly increased ($P<0.05$) in CP-YOUNG mice, compared with PBS-YOUNG mice, and β-amyloid level was increased in mice treated with *C. perfringens* compared with PBS-treated mice, regardless of age.

Significance: These results indicate that *C. perfringens* in the small intestine cause inflammation, oxidative stress, apoptosis, and brain damage, which may result in brain disorders. Therefore, even though *C. perfringens* sometimes does not cause rapid foodborne illenss, the pathogen should be destroyed in order to prevent it from causing chronic symptoms in brain.

P3-08 Genetic Characterization of 60 Proteolytic *Clostridium botulinum* Strains Using Pulsed-field Gel Electrophoresis and High-throughput Sequencing

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Introduction: Foodborne botulism is caused by the consumption of the most potent protein neurotoxin, botulinum neurotoxin (BoNT), which is produced by the heterogeneous species *Clostridium botulinum*. Proteolytic *C. botulinum* continues to be the leading cause of foodborne botulism outbreaks in the United States.

Purpose: The objective of this study was to genetically characterize proteolytic *C. botulinum* foodborne isolates and their mobile genetic elements, focusing on BoNT subtype classification and toxin gene cluster location.

Methods: A total of 60 proteolytic *C. botulinum* strains (44 serotype A, 16 serotype B) were analyzed using pulsed-field gel electrophoresis (PFGE), Southern hybridization using BoNT-specific probes, and high-throughput sequencing (HTS) using an Illumina MiSeq.

Results: The type A toxin gene was chromosomally located in 43 subtype A1 strains and one subtype A2 strain. The type A1 toxin gene was located in a ha-/orfx+ cluster in four strains (CC1A, CC4A, CJ4-1, CJ5-1). Six BoNT/B1, three BoNT/B2, one BoNT/B5 and six BoNT/B7 subtype toxin genes were identified in the 16 type B strains examined. The BoNT/B2 gene was chromosomally encoded in three strains (169B, 185B and 213B). The BoNT/B1 strain Camp5B carried a large plasmid (~217 kb) as identified by PFGE; however, Southern blot analysis and HTS indicated BoNT/B1 was on the chromosome. Strain CSIRO2 contained subtype toxins B5 and F2 located on a plasmid that shared similarity to plasmid pCBG. Nine strains carried an ~160 kb plasmid that encoded either BoNT/B1 (four strains) or BoNT/B7 (five strains). Two larger plasmids (~260 kb) were identified and carried either BoNT/B1 (strain 15580P1-B) or BoNT/B7 (strain Garlic B).

Significance: The genetic characterization of proteolytic *C. botulinum* and its BoNTs is imperative to the development of improved methods used to detect the more than 40 subtypes of BoNTs in foods, and countermeasures used in the rapid treatment of foodborne botulism.

P3-09 Genetic Characterization of 15 Nonproteolytic *Clostridium botulinum* Type B and E Strains Using Pulsed-field Gel Electrophoresis and High-throughput Sequencing

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Introduction: Group II nonproteolytic *Clostridium botulinum* are psychrotrophs and produce the deadly botulinum neurotoxin (BoNT) types B, E, and F. Although nonproteolytic strains produce spores that are less heat-resistant than proteolytic *C. botulinum*, their ability to grow at refrigeration temperatures makes them a concern in extended-shelf life refrigerated foods.

Purpose: The objective of this study was to genetically characterize nonproteolytic *C. botulinum* foodborne isolates and their mobile genetic elements, focusing on BoNT subtype classification and toxin gene cluster location.

Methods: A total of 15 Group II *C. botulinum* strains (seven serotype B, eight serotype E) were analyzed using pulsed-field gel electrophoresis (PFGE), Southern hybridization analysis using digoxigenin-labeled BoNT-specific probes, and high-throughput sequencing using an Illumina MiSeq.

Results: All serotype B strains contained the BoNT/B4 subtype toxin cluster located on plasmids of ~50 kb. These plasmids showed similarity to other class I plasmids of nonproteolytic serotype B strains, including pCLL. Previous reports have shown that BoNT/E is rarely plasmid-borne; however, it was observed in the present study that five serotype E strains (066B-E, Igr7-E, D70-E, 2570-E, and Kal-E) carried a BoNT/E3 subtype toxin cluster on plasmids of ~135 kb. Only three serotype E strains (Belgium-E and Birmingham-E, subtype BoNT/E3; Saratoga-E, subtype BoNT/E7) analyzed carried the toxin gene on the chromosome. Both the BoNT/E3 and BoNT/E7 toxin gene clusters were inserted into a split *rarA* gene on the chromosome in strains Saratoga-E, Belgium-E, and Birmingham-E.

Significance: Botulinum neurotoxin genes seem to be encoded within plasmids more commonly in nonproteolytic Group II than in proteolytic Group I *C. botulinum* strains. Understanding the diversity of BoNT genes and their mobility permits the development of improved toxin detection strategies and provides vital knowledge on the evolutionary and phylogenetic understanding of this important foodborne pathogen.

P3-10 Transcriptomic Analysis of Arginine-induced Botulinum Neurotoxin Repression in *Clostridium botulinum* Strain ATCC3502 Using RNA Sequencing

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Introduction: Foodborne botulism is caused by the consumption of foods in which diverse strains of botulinum neurotoxin-producing *Clostridia* have grown and produced botulinum neurotoxins (BoNTs). Arginine has been shown to repress BoNT production in *Clostridium botulinum* strain ATCC3502, although the genetic mechanism of regulation is unknown.

Purpose: The objective of this study was to perform a transcriptomic analysis of strain ATCC3502 using RNA sequencing to determine the genetic mechanism of BoNT/A repression in the presence of arginine.

Methods: C. botulinum strain ATCC3502 was grown in toxin production medium (TPM) with and without arginine. Samples were removed during the log, stationary, and lytic growth phases. Total RNA was extracted and the mRNA was converted to cDNA and sequenced using the TruSeq Stranded mRNA sample preparation kit (Illumina) and sequenced on an Illumina MiSeq. SDS-PAGE and Western blotting were also performed on the samples to measure BoNT/A production throughout growth.

Results: The BoNT/A and toxin complex genes were down-regulated two-fold during the log phase when *C. botulinum* strain ATCC3502 was grown in the presence of arginine. However during the lytic phase, BoNT/A and toxin complex genes were up-regulated eight-fold and 25-fold, respectively, despite a 1,000-fold repression of BoNT/A production. Hypothetical protein, CBO2309, was upregulated in both the log (156-fold) and lytic phase (eight-fold). BLASTp analysis indicated CBO2309 contains an ubiquitin-associated domain commonly involved in protein degradation. The transcriptional regulator for BoNT/A, botR, was down-regulated 59-fold during log phase, but appeared unaffected during later stages of growth. Additional positive regulators of BoNT/A, namely CodY and Spo0A, were down-regulated 30-fold and three-fold, respectively, during the lytic phase.

Significance: An understanding of the regulation of *C. botulinum* growth and BoNT formation is pivotal in defining requirements for BoNT formation in foods and elucidating the biological functions of BoNTs for the bacterium.

P3-11 Validation of the 3M Petrifilm Rapid Yeast and Mold Count Plate for the Enumeration of Yeast and Mold in a Variety of Food in Canada

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Introduction: Yeast and mold enumeration is a quality indicator test that is widely used in the food industry. An alternative method for yeast and mold enumeration, 3M Petrifilm Rapid Yeast and Mold Count Plate method, is compact and easy to use. The colonies on the alternative method can be differentiated and are ready for enumeration in as little as 48 h, compared to five days with traditional methods**.**

Purpose: The objective of this study was to evaluate the performance of an alternative yeast and mold enumeration method compared to the Health Canada compendium of analytical methods MFHPB-22 in a variety of foods.

Methods: A total of 15 matrices from five different categories (dairy, chocolate and bakery, multi-component foods, dried cereals, fruits, nuts, seeds, and vegetables and pet foods) were tested with the alternative method and MFHPB-22. Each matrix was inoculated with a target organism to achieve the different levels of contamination: uninoculated, low (10^2 to 10^3 CFU/g), medium (10^3 to 10^4 CFU/g), and high (10^4 to 10^5 CFU/g). The alternative method plates were incubated at 25 and 28°C for 48 and 60 h.

Results: Linear regression analysis was conducted to compare the alternative method results to the reference method. The mean difference between the candidate and reference method, the transformed results, and its 95% confidence interval were calculated. The results indicate that the alternative method performed satisfactorily and is comparable to the Health Canada method MFHPB-22 for enumeration of yeast and mold in food within 48 to 60 h of incubation (low Sr=0.08328, medium Sr=0.06835, and high Sr=0.05647).

Significance: The alternative method showed acceptable performance and complies with Canadian requirements of the Microbiological Methods Committee. The 3M Petrifilm Rapid Yeast and Mold Count Plate is an acceptable alternative method to the food industry to analyze samples for yeast and mold in a shorter time, thereby enabling faster product release.

P3-12 Comparing the Lytic Activity and Genetic Makeup of Bacteriophages Targeting Shiga Toxin-producing

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❖ ***Developing Scientist Competitor***

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) have been associated with numerous outbreaks and recalls, making them a dangerous group of pathogens. One strategy to control them is the use of phage-technology, involving bacteriophages that could infect and lyse specific host bacteria. Bacteriophage isolates, varying in genetic makeup, differ in their lytic activity. However, information is limited on the relationship between bacteriophage genetics and lytic activity. Understanding the role genetics play in the lytic activity of a STEC-specific phage could lead to the development of better intervention strategies.

Purpose: To compare the genetic makeup of newly isolated STEC-specific bacteriophages and their lytic activity against various STEC serotypes.

Methods: Previously isolated bacteriophages (P1, P2, P3, P4, P7, P9, P13, and J25) were tested for lytic activity using spot-on-lawn assay against multiple isolates of their specific STEC serogroups (O157, O26, 145, and O45). Bacteriophage lysates of selected phages were used for DNA extraction using the phenol-chloroform method and digested using restriction enzymes. The digested fragments were then cloned, transformed into *E.coli* XO-1-Blue, and sequenced. MEGA software was used to compare the genetic data of bacteriophages and create a phylogenetic tree.

Results: Genetic comparisons revealed that phages P1, P2, P5, P13, and J25 belong to the *Myoviridae* family. Phages P3, P4, and P7 belong to *Siphoviridae* and P9 belongs to *Tectiviridae*. Lytic activity of the phages was determined by the clarity of their lytic spots and categorized as: High (very clear), Moderate (clear), and Low (turbid). All phages showed high lytic activity except P3, P4, and P7, which showed moderate activity. Phages belonging to the *Myoviridae* family showed a high lytic activity, along with phage P9, which belonged to *Tectiviridae*.

Significance: Understanding the genetic makeup of bacteriophages could help streamline identification and characterization of STEC-specific bacteriophages, leading to further advances in the use of phages as biocontrol agents.

P3-13 Extremely Heat-resistant *Escherichia coli* among Cattle and Beef

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Introduction: Extremely heat-resistant (XHR) *Escherichia coli* survive exposure of 60°C (140°F) for 60 min and in cooked (71°C, 160°F) ground beef patties. Whole genome sequencing of XHR *E. coli* identified a 14-kb genetic locus of heat resistance (LHR) that imparts the XHR phenotype. The prevalence of these *E. coli* among cattle and beef is unknown.

Purpose: Establish the prevalence of XHR *E. coli* among cattle at harvest and determine if *E. coli* previously isolated from beef are XHR.

Methods: A multiplex PCR assay was developed to detect the intact LHR in feces collected from fed (n=538), cull dairy (n=425), and cull beef (n=475) cattle arriving at nine beef-processing plants across the United States. Bacteria from the feces samples were heat-treated (80°C, 20 min) to isolate XHR *E. coli.* Archived *E. coli* O157:H7 (n=81), non-O157 adulterant Shiga toxin-producing *E. coli* (STEC; n=50), other STEC (n=97), and other *E. coli* (n=232) isolated from beef were screened for the LHR and XHR phenotype.

Results: The intact LHR appeared present in 28.6, 14.4, and 11.4%, and absent from 24.7, 36.2, and 62.7% of fed, cull dairy, and cull beef cattle feces samples, respectively. The remainder of samples contained incomplete LHRs. The multiplex PCR cannot distinguish *E. coli* containing an intact LHR from mixtures of *E. coli* possessing non-intact LHRs. This is likely why only 10 strains with an intact LHR were isolated from the 1,151 feces samples. The intact

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LHR was found in 2.4% of the *E. coli* isolated from beef. None were recognized pathogens; however, one *E. coli* O157:H7 and one adulterant STEC-O103 contained an incomplete LHR.

Significance: Portions of the LHR appear widespread, but few XHR *E. coli* with an intact LHR can be isolated from cattle and no pathogenic *E. coli* from beef have been found to possess an intact LHR.

P3-14 A Comparison of the Prevalence of Antibiotic-resistant Bacteria Found in Ground Beef from Conventionally and Naturally Raised Cattle

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❖ ***Developing Scientist Competitor***

Introduction: Ground beef may harbor a wide variety of bacteria including spoilage, pathogenic, and non-pathogenic bacteria. Repeated antibiotic use in food animal production may allow for resistant organisms to proliferate and dominate the space around them. As the topic of antibiotic resistance becomes increasingly popular among consumers and regulatory agencies, the meat industry must strive to better understand the relationship between antibiotic resistance and consumer meat products.

Purpose: The objective of this study is to compare the prevalence of antibiotic-insusceptible bacteria found in ground beef samples processed from conventionally and naturally (never administered antibiotics) raised cattle.

Methods: One hundred ground beef samples were collected from each city (Fort Collins, Colorado; Seattle, Washington; Atlanta, Georgia), with 50 from conventionally raised cattle and 50 from naturally raised cattle. Each sample was cultured for the presence of generic *Escherichia coli*, tetracycline-resistant (TET^r) *E. coli,* 3rd generation cephalosporin-resistant (3GC^r) *E. coli*, *Salmonella,* generic *Enterococcus,* erythromycin-resistant (ERY^r) *Enterococcus*, and methicillin-resistant *Staphylococcus aureus* (MRSA). Statistical analyses were performed using a Pearson's chi-squared test with Yates' continuity correction in R software (version 3.4.2).

Results: Culture results suggest that the number of conventional ground beef samples harboring TET^r *E. coli* (52.0%, 78 of 150) is statistically different (P<0.05) than natural ground beef samples (29.3%, 44 of 150). 3GC^r *E.* coli detection was different between the two treatments (P<0.05), despite minimal detection in conventional (2.5%, 3 of 150) and natural (8.2%, 12 of 150) samples. ERY^r *Enterococcus* was also detected more frequently (P<0.05) in conventional samples (59.3%, 89 of 150) compared to natural samples (44.7%, 67 of 150). *Salmonella* and MRSA were detected too infrequently to effectively perform statistical analysis.

Significance: This survey of resistance in ground beef samples purchased at the retail level will assist in guiding future scientific endeavors towards a better understanding of antibiotic-resistant foodborne infections.

P3-15 A Novel Role of Foodborne *Clostridium difficile* in Intestine

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Introduction: In recent years, *Clostridium difficile* has been considered a foodborne pathogen. In addition, recent research data showed that the pathogen is related to liver disease. Hence, the pathogenicity after infection through food consumption should be studied rather than acute symptoms of foodborne illnesses.

Purpose: The objective of this study was to elucidate the new role of *C. difficile* on initiation and aggravation of liver injury after oral infection.

Methods: Four-week-old male C3H/HeN mice were divided to four groups. To induce liver injury, diethylnitrosamine (DEN) was intraperitoneally injected once a week for DEN-treated groups with phosphate buffered saline (DEN+PBS), and *C. difficile* (DEN+CD), and non-DEN-treated groups were only provided with PBS and *C. difficile* (CD) for nine weeks. On the day of euthanization, serum and organs were removed for bacterial translocation, serum liver enzymes, transcriptome and Western blot analyses.

Results: The positive rates of *C. difficile* in the mesenteric lymph nodes, an indicator of bacterial translocation, for CD and DEN+CD groups were 30 and 75%, respectively, but 20% in both PBS and DEN+PBS group. In the livers, *C. difficile* of DEN+CD group was also found in 50% of positive MLNs. In analysis of serum liver enzymes, ALT and AST levels were significantly increased (P<0.05) in DEN+CD, compared to DEN+PBS. In transcriptome analysis, the elevated gene expression of *IL-1β*, *HB-EGF*, *EGFR*, *TGF-α*, *PCNA*, *desmin*, *HMGB1*, and CRP were observed in CD and DEN+CD groups, but not in PBS and DEN+PBS groups. At protein levels, PCNA and IL-6 levels were increased in CD and DEN+CD groups, respectively, compared to PBS and DEN+PBS groups.

Significance: These results indicate that even though infection of *C. difficile* through food consumption causes acute gastroenteritis, the infection may also cause initiation and aggravation of liver injury.

P3-16 Survival of *Salmonell*a and *Escherichia coli* O121 in Flour during 270 Days of Storage and Evaluation of Storage Time on Heat Resistance in Flour and Muffin Batter

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Introduction: Low-moisture bakery ingredients, such as flour, can become contaminated with Shiga toxin-producing *Escherichia coli* and *Salmonella*; and moreover, these pathogens can survive in dry environments for extended periods. It is important to study the survival and thermal resistance characteristics of these organisms during prolonged ingredient storage.

Purpose: To study the survival of a 4-strain *E. coli* O121 and a 7-serovar *Salmonella* cocktail in flour during 270 days of storage at ~25°C, and to compare D- and *z*-values of *E. coli* O121 and *Salmonella* cocktails in flour and muffin batter prepared from inoculated flour on days 1, 30, 90, 180 and 270.

Methods: Individual cultures were grown as lawns on tryptic soy agar, harvested using 0.1% peptone solution, and mixed in equal proportions to get *E. coli* or *Salmonella* cocktails. Flour was then mist inoculated separately with the *E. coli* O121 or *Salmonella* cocktail, dried back to the original pre-inoculation moisture level, and stored in air-tight plastic bags. D-values of each pathogen in flour and muffin batter were determined using thermal-death-time disks. The study was designed as a randomized complete block with three replications as blocks.

Results: *E. coli* O121 and *Salmonella* cocktail populations in inoculated flours on day 1 were 7.6 and 7.8 log CFU/g, respectively, and decreased to 2.2 and 3.5 log CF/g, respectively, by day 270. D-values of *E. coli* O121 and *Salmonella* cocktails in flour on day 1 were 20.3 and 41.7, 10.2 and 24.3, and 3.5 and 8.0 min at 70, 75 and 80°C, respectively; whereas, the D-values in muffin batter were 47.5 and 37.6, 10.7 and 8.4, and 0.5 and 0.8 min at 60, 65 and 70°C, respectively. Overall, thermal resistance of both *E. coli* O121 and *Salmonella* decreased during extended flour storage.

Significance: This study proved that *E. coli* O121 and *Salmonella* can survive in flour for over 270 days at significant levels. Commercial baking processes should be validated to ensure eradication of these pathogens in final baked products.

P3-17 Validation of Simulated Commercial Baking of Cheesecake to Control *Salmonella*

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Introduction: Pathogens such as *Salmonella* can contaminate and survive in raw flour for extended periods; therefore, thermal lethality processes in bakery operations are important in order to ensure the safety of finished products. However, published validation studies on baking to control *Salmonella* in specific bakery products are scarce.

Purpose: To validate a simulated commercial baking process for cheesecake to control *Salmonella* and to determine *D*- and *z*-values of a seven-serovar *Salmonella* (Hartford, Newport, Senftenberg, Tennessee, Typhimurium, and two pet food isolates) cocktail in cheesecake batter.

Methods: Cheesecake batter was prepared using flour mist inoculated with the *Salmonella* cocktail (~8 log CFU/g). Cheesecake batters were evenly spread onto crust mixtures in aluminum pans and baked at 148.9°C (300°F) for 50 min, followed by 30 min of ambient air cooling (B+C). *D*-values of the cocktail in cheesecake batters were determined using thermal-death-time disks and temperature-controlled water baths. Studies were designed as randomized complete blocks with three replications as blocks (a=0.05), and statistical analyses were conducted using analysis of variance and SAS software version 9.3.

Results: The mean internal temperature of cheesecake increased from 17 to ~97°C at the end of 50-min baking time. The *Salmonella* population in cheesecake decreased by >5 log CFU/g by 37.5 min of baking and was completely eliminated after 50 min of baking (as determined by enrichment). The pH and water activity of cheesecake at B+C were 4.86 and 0.943, respectively. *D*-values of the *Salmonella* cocktail at 55, 58, and 61°C were 27.4, 13.8, and 4.9 min, respectively, whereas the *z*-value of the *Salmonella* cocktail was 8.2°C.

Significance: This study validated a baking process for cheesecake; however, additional research should be conducted if recipe and/or baking parameters are modified. The *D*- and *z*-values determined in cheesecake batter can be used by the bakery industry to evaluate the expected lethality of their baking processes.

P3-18 Evaluation of Pesticide Residues on *Beta vulgaris* spp., *Brassica oleracea* var. *capitata,* and *Solanum tuberosum* in Bloemfontein, South Africa

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Introduction: Pesticides poisoning caused by residues left on vegetables is a substantial and increasing problem. It is alarming to find the presence of banned pesticides residues on vegetables. This implies that there are gaps in the pesticide laws which regulate or prohibit the importation, sale, acquisition, disposal, and or use of certain pesticides in South Africa, as declared in the Fertilizers, Farm Feeds, Agricultural Remedies, and Stock Remedies Act No.36 of 1947.

Purpose: To assess the level of pesticide residues in staple vegetables. This includes an estimation of human health risk associated with the consumption of pesticide-contaminated vegetables and the identification of gaps in the pesticide legislation in South Africa.

Methods: The analysis for pesticide residues in staple vegetables was done using the application of liquid chromatography-tandem mass spectrometry.

Results: All samples of swiss chard, cabbage, and potatoes contained pesticide residues above the maximum residues limits set by Codex Alimentarius (0.1 mg/kg), but others were unauthorised for use in some vegetables. The results indicated that the samples could pose health hazards to the consumers. Therefore, effective monitoring of pesticide residues in food items is required.

Significance: There is a critical information gap regarding pesticide residues on produce consumed. Without a more thorough sampling program, it is not possible to accurately reduce risks posed by residues in vegetables. The analysis highlights the need for additional investigation and for monitoring, enforcement, and other interventions to improve food safety and reduce pesticide exposures in fresh produce in Bloemfontein.

P3-19 Transmission of Human Enteric Pathogens from Artificially Inoculated Flowers to Vegetable Sprouts/Seedlings Developed Via Contaminated Seeds

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❖ ***Developing Scientist Competitor***

Introduction: Seeds contaminated with bacterial pathogens were found to be the primary cause of sprout-associated outbreaks of human gastrointestinal infections.

Purpose: This study was undertaken to determine if cells of selected *Salmonella enterica* and enterohemorrhagic *Escherichia coli* (EHEC) strains, artificially inoculated onto the flowers of vegetable plants, will result in contamination of the sprouts/seedlings that develop from seeds produced by the inoculated flowers.

Methods: Seeds of alfalfa, fenugreek, lettuce, and tomato flowers were inoculated with cells of selected *S. enterica* or EHEC strains. A total of 906, 715, 1,237, and 1,276 mature seeds produced by lettuce, tomato, alfalfa or fenugreek flowers inoculated with *Salmonella* were collected as 48, 94, 109, and 116 composite samples (367 in total), respectively. Correspondingly, 934, 640, 1,827, and 1,027 seeds produced by the four respective types of flowers after inoculation with *E. coli* were divided into 42, 81, 162, and 107 composite samples (392 in total), respectively. Seeds in each composite sample were surface-decontaminated with NaOCl solution and germinated at 25°C in the dark for 5 days. Subsequently, pathogen populations on the sprouts/seedlings developed from each composite seed sample were determined by plate count assay.

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Results: The overall *Salmonella* detection percentage from vegetable sprouts/seedlings developed from the 367 composite seed samples was 2.7%, while none of the sprouts/seedlings grown from the 392 composite seed samples with prior *E. coli* inoculation tested positive for the pathogen. One of the 94 tomato seedling samples contained 4 *Salmonella* CFU/seedling and five samples tested positive by enrichment (6.4%). Two (1.8%) out of 109 alfalfa and 116 (1.7%) fenugreek composite samples tested positive for *Salmonella* with enrichment. However, none of the lettuce seedlings tested positive for *Salmonella* or *E. coli*, even after enrichment.

Significance: This study suggests that under controlled environmental conditions, human pathogens inoculated onto flowers of vegetable plants can result in the contamination of sprouts/seedlings via seeds produced by the inoculated flowers. However, the frequency of sprout/seedling contamination was low and could be affected by characteristics of the pathogens and plant species tested.

P3-20 Effect of Routine Sanitation and Surface Material on the Shift in Microbial Communities in Fresh Produce Processing Environments

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Introduction: Multiple foodborne disease outbreaks have been reported to be associated with fresh produce caused by cross-contamination during processing. However, little is known about the effect of routine sanitization on the shift in diverse microbial communities in food production environments.

Purpose: To investigate the shift in bacterial communities on environmental surfaces in fresh produce processing facilities as affected by the sanitization process, surface materials, and sampling time.

Methods: In this study, environmental samples were collected from 10 surface sites in a fresh-cut produce processing plant before and after the daily routine sanitization in March and June 2017. Live bacteria population was evaluated using culture method and quantitative real-time PCR (qPCR) after propidium monoazide treatment. The microbiota on surface samples were analyzed by 16S rDNA amplicon sequencing using MiSeq and Qiime2.

Results: Bacterial populations on surfaces from five sample sites (group A, including floor 1, two mezzanines, and two drain covers) significantly decreased (>0.7 log CFU/cm^2) after sanitization. Bacteria on the other five sample sites (group B, including floor 2, two doors, and two walls) were not efficiently removed by sanitization. Proteobacteria was the dominant phylum, and two *Cupriavidus* and *Ralstonia* spp. were determined to be the residential bacteria in the environment. In group A sample sites, the population of *Pseudomonas* spp., as well as other psychrophilic bacteria, were significantly reduced after sanitization. Bacteria on metal surfaces can be more efficiently removed compared to other materials. Exogeneous bacteria, such as several *Pseudomonas* spp., might be introduced into the processing environment in between the two sampling events.

Significance: This study provides insight on the dynamics of microbial communities in produce processing environments, which can benefit further research on the prevention of foodborne pathogens and spoilage.

P3-21 Dynamics of Microbial Communities on Spinach Irrigated by Ground Water, Reclaimed Water, and Roof-harvest Water

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Introduction: Irrigation water, particularly if applied overhead, could be an important source of bacterial contamination to fresh produce. The colonization, survival, and proliferation of exogenous bacterial pathogens can be strongly influenced by the microbiota of the produce.

Purpose: To investigate the dynamics of bacterial communities on spinach after irrigation by ground water (Cr), reclaimed waste water (Wa), and roof-collection water (Rf).

Methods: Spinach was irrigated with three types of water (Cr, Wa, Rf) for two weeks (once per week) in a randomized complete block design with two replicates (plot) for each treatment. Spinach before and after irrigation and water samples were collected for bacterial plate count, quantitative PCR, and 16S rDNA sequencing survey analyses.

Results: The average bacterial population densities on spinach (plate count=6.50±0.04 log CFU/g; qPCR estimation=7.40±0.10 log 16S copies/g) were significantly higher than that in tested irrigation water (3.61±0.12 log CFU/ml; 4.94±0.13 log 16S copies/ml). The composition of bacterial communities in Cr changed between the two sampling weeks, while the composition was not significantly shifted in Wa and Rf. The dominant bacterial taxa on spinach were not significantly affected during the two-week irrigation; however, certain bacterial species were transmitted to spinach through irrigation. No *Shigella*, *Salmonella*, *Listeria*, or *Campylobacter* spp. were detected in this study. *E. coli* was detected in ground water in both weeks, but not in Wa and Rf water. The populations of *E. coli*, *Bacteroides fragilis*, and *Veillonella* spp. on spinach were significantly decreased in week 2.

Significance: This study provides knowledge on the microbial ecology of diverse bacterial communites on spinach after irrigation by different types of water, which can benefit further studies on the interaction of microbes on produce and the prevention of foodborne pathogens and plant disease.

P3-22 Association of Tulane Virus with Bacterial Cell Components in Suspension

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Introduction: Human noroviruses (hNoVs) are shown to interact with histo-blood group antigens (HBGAs) on mucosal epithelial surfaces. Additionally, research suggests that association with gastrointestinal tract (GIT) bacteria is a co-factor for viral infection. Moreover, these interactions can occur prior to virus entry into the host such as within food preparation environments. It is hypothesized that bacterial cell components (BCC), including peptidoglycan (PG), lipopolysaccharide (LPS), and extracellular polymeric substances (EPS), may increase viral receptor binding and infectivity.

Purpose: This study aims to investigate the association between Tulane virus (TuV) — a hNoV surrogate — and GIT bacterial cell components at room temperature (RT).

Methods: Initial experiments were conducted using 100 µl of 10^7 PFU/ml TuV incubated individually with 100 µl BCC for 2 h at RT. BCC included *Enterobacter cloacae* LPS, commercial (*Escherichia coli* O111:B4) LPS, *Pantoea ananatis* LPS, *Bacillus cereus* PG, *B. cereus* EPS, and *E. cloacae* EPS. Experiments with whole cell lysates utilized 100 µL of 10^5 PFU/ml TuV added to 100 µL of lysed bacterial cells (*E. cloacae* or *B. cereus*) with incubation at RT for 2 h. TuV plaque assay was used to determine the virus concentration. TuV association with cell lysate was visualized by transmission electron microscopy.

Results: Data suggest that TuV interaction with BCCs enhances infectivity as determined by an increase in PFU compared to phosphate buffered saline (PBS) control. Differences in treatments and control were observed across all time points; however, after 1 h, *E. coli* LPS+TuV showed the greatest log increase (0.18 log) in PFU compared with PBS+TuV control ($P < 0.0001$). For whole cell lysates, data show that TuV binding and infectivity increases by 0.2 log after 2 h ($P \leq 0.0034$) when associated with lysed cells compared to PBS+TuV control.

Significance: These data show a positive interaction of TuV with BCCs with respect to enhanced host cell binding and/or infectivity. Understanding these interactions will allow further studies on the role these relationships play in enteric virus life cycle—prior to as well as after host entry. In addition, characterizing virus-bacteria relationships within our food systems will aid in development of targeted prevention and control strategies to enhance food safety.

P3-23 Thermal Resistance of *Listeria monocytogenes* in Low-moisture Foods Using a Dry Inoculation Procedure

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Introduction: Survival and heat resistance of *Listeria monocytogenes* and other organisms in low-moisture foods (LMF) are an underdeveloped area of applied research. New techniques and survival data in LMF matrices will benefit the development of effective food safety plans.

Purpose: The aim of this study was to investigate the thermal resistance and survival of *L. monocytogenes* in LMF foods using a dry inoculation procedure.

Methods: A three-strain cocktail of *L. monocytogenes* was inoculated onto talc powder to create dry inoculums. The inoculums were used to contaminate a model LMF peanut paste. The heat resistance of *L. monocytogenes* was determined using Thermal Death Time (TDT) studies at 75°C. For each trial (two replicates), samples were heated using copper plates in a water bath. Plates were removed from the water bath at predetermined intervals up to 50 min. Survivors were enumerated using 0.6% tryptic soy agar with yeast extract. Log reduction data (log CFU/g) for *L. monocytogenes* and Salmonella (as a reference from a prior study) were plotted against time, and the Weibull model was used to predict minimum time to 4-log reduction. The log-linear model was also applied to determine the *D*-value of the organism at 75°C using the linear portion of the line (3 to 50 min).

Results: *L. monocytogenes* had comparable log reduction to Salmonella in a model peanut paste. Predicted minimum time to 4-log reduction derived from Weibull model also indicated that both organisms have similar heat resistance. However, *D*-values calculated using the linear portion of inactivation curves appear to demonstrate a higher heat resistance for both organisms. Therefore, more replications are required to compare and elucidate appropriate models.

Significance: Research studies comparing this or similar dry inoculums are needed to support the findings of this pilot study. Further advances in methodology will help the industry produce safe food, comply with regulations, and protect public health.

P3-24 Genetic Determinants of *Salmonella enterica* Critical for Biofilm Formation on Abiotic Surfaces and Attachment to Vegetable Seeds

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Introduction: Salmonella is a bacterial pathogen frequently involved in foodborne illnesses, including fresh produce-borne outbreaks of human gastrointestinal infections.

Purpose: This study was undertaken to identify the genetic determinants critical for *Salmonella* attachment and biofilm formation.

Methods: Escherichia coli SM10 lambda *pir*, with a kanamycin resistance marker on mini-*Tn10 (mini-Tn10:lacZ:kan^r)*, an ampicillin resistance maker on the mini-*Tn10*-bearing suicidal plasmid pLBT, and a streptomycin sensitive marker on SM10 chromosome (*amp^r, kan^r*, *strep^s*) was used as a donor, and three *Salmonella* strains (*amp^s, kan^s*, *strep^r*) were used as recipients in a transposon mutagenesis study. The donor and each recipient were co-incubated overnight on tryptic soy agar at 37°C. Mutant colonies (*amp^s, kan^r*, *strep^r*) were subsequently selected. A single-band degenerate PCR product, amplified from each mutant genome using oligonucleotide primers derived from the end of min-*Tn10* and restriction enzyme *Eco*R I- or *Pst* I-recognizing sequence, were sequenced using the Sanger sequencing technology. Obtained DNA sequences were compared against those deposited in the Genbank using BLAST search.

Results: It was found that cells of *Salmonella* mutants accumulated either significantly more or less biofilms on polystyrene surfaces than their parent cells. The biofilm mass correlated positively with pathogen attachment to fenugreek and alfalfa seeds with a R^2 value of 0.97 and 0.67, respectively. Sequencing analysis of the degenerate PCR products revealed that the mini-*Tn10* on pLBT had inserted into the *cdg*, *trx*, *fadI*, or *rxt* on *Salmonella* chromosomes.

Significance: The study identified some of the genes critical for *Salmonella* attachment and biofilm formation. The proteins and enzymes encoded by the identified genes could be the likely targets for control of pathogen attachment and biofilm formation in future antimicrobial interventions.

P3-25 Effects of Different Moisture and Temperature on *Salmonella* Survival in Poultry Fat

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Undergraduate Student Award Competitor

Introduction: Fats products have historically been thought to have water activity too low to harbor pathogens. However, it has been recently reported that high moisture levels in fats may lead to *Salmonella* presence and growth. Limited research on strategies to eliminate pathogens in these environments is available, and the mechanisms contributing to microbial presence and growth are not yet well-understood.

Purpose: The purpose of this research was to evaluate the effects of moisture levels and storage temperatures on the growth and survival of *Salmonella* in poultry fat and to determine death rate kinetics.

Methods: The effects of four moisture levels (0, 0.5, 1, and 3%) and two temperatures (48 and 76°C) were evaluated in poultry fat inoculated with a low (~10^5 CFU/ml) and high (~10^8 CFU/ml) *Salmonella* serovar cocktail (Newport, Thompson, and Infantis). Samples were stored for seven days at 48 and 76°C. Remaining population was evaluated daily. One ml of each sample was enriched in Rappaport-Vassiliadis broth, plated on XLD agar, and incubated at 37°C for 24 h. Death rates were calculated for each temperature and moisture level.

Results: A 4-log CFU/ml reduction in *Salmonella* population was observed with 0.5 and 1% moisture level at 48°C after 5 days ($P<0.05$) with a death rate of 4.8 and 17.3 d^{-1}, respectively. A 2-log CFU/ml reduction was recorded at 3% moisture as compared to the control ($P<0.05$) after 5 days, with a slower death rate (18.9 d^{-1}). When products were challenged at 76°C, counts were below detectable limits after 24 h.

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Significance: The findings of this research identified the significant effect of moisture and temperature in poultry fat contaminated with *Salmonella* and underlined the need to use time-moisture-temperature data to minimize microbial growth during transportation and storage.

P3-26 Evaluation of Whole Genome Sequencing Web-based Methods and Bead-based Molecular Methods for the Serotyping of *Salmonella* Isolated from Food and Environmental Samples

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Introduction: Salmonella serotyping using antisera has been essential to surveillance and outbreak investigations. There are more than 2,500 known *Salmonella* serovars. Keeping a full inventory of antisera is not practical, as they are expensive and those use for rare antigens often expire before they can be used. Recent methods have been developed that target the genes encoding the antigens recognized by traditional serotyping following the White-Kauffmann-Le Minor scheme. These include bead-based *Salmonella* molecular serotyping (SMS) methods and Web-based serotyping using publicly available internet tools and whole genome sequencing (WGS) data.

Purpose: The goal of this study was to evaluate WGS Web-based serotyping of *Salmonella* isolates using SeqSero and *Salmonella in silico* typing resource (SISTR) and compare the results to SMS and traditional serotyping.

Methods: A previous study of 572 isolates compared SMS to traditional serotyping. The *Salmonella* isolates were recovered from food, pet food, or environmental samples by U.S. Food and Drug Administration regulatory laboratories or were reference strains. Illumina MiSeq-generated WGS data for many isolates from the SMS study are publicly available in the GenomeTrakr depository maintained at the National Center for Biotechnology Information (NCBI). The sequences were downloaded from NCBI and analyzed by SeqSero and SISTR. The results were compared to SMS and traditional serotyping.

Results: Of the 330 isolates analyzed to date, the number of isolates considered correctly identified or as expected were 322 (97.6%) by SMS, 320 (97.0%) by SeqSero, and 326 (98.8%) by SISTR. The ability of each method to narrow to a single serovar was SISTR (98.2%), SeqSero (78.4%) and SMS (45.3%).

Significance: The WGS Web-based methods provided comparable results to SMS and traditional serotyping and should considered valuable tools, enabling a more targeted approach to antiserum testing and confirmation. Genomic-based methods should aid in the identification of rough, non-motile, or weakly agglutinating isolates, reducing the rates of misidentification.

P3-27 Synergistic Antimicrobial Efficacy of Essential Oils against *Escherichia coli* O157:H7 and Their Application Potential in Lettuce

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Introduction: Essential oils (EOs) are rich in phytochemicals and have shown promising antimicrobial activities. However, application of a single EO into a food matrix often results in reduced or lost antimicrobial efficacy.

Purpose: This study aimed at evaluating the synergistic antimicrobial activities of EO compounds to investigate their antimicrobial mechanisms and to validate their efficacy in a food model.

Methods: The MICs of seven EO compounds were determined against *Escherichia coli* O157:H7, and their synergistic activities were evaluated using checkerboard method. The antimicrobial efficacy of the best EO combinations were measured using time kill assay, and antimicrobial mechanisms were investigated using flow cytometry. Anti-*E. coli* activities of these EO combinations were validated in lettuce at 4°C.

Results: The MICs of seven EO compounds range from 0.63 to >10 mg/ml. All EO combinations showed additive effect, with thymol/eugenol (Thy/Eug), carvacrol/eugenol (Car/Eug), and trans-cinnamaldehyde/eugenol (TC/Eug) showing the strongest activities. Enhanced killing efficiency was observed in EO combinations (≥6.8-log CFU/ml reduction) than single compound (reduction of 0.7 to 4.9 log CFU/ml) *in vitro*. Flow cytometry results suggested that eugenol enhanced antimicrobial activity of the partner compound by increasing membrane permeability. When evaluated on lettuce, Thy/Eug and Car/Eug combinations showed stronger activities than TC/Eug and single Car. Both Thy/Eug and Car/Eug caused an initial microbial reduction of 1.4 to 1.5 log CFU/g compared to control, and a final microbial load that was 1.7 log CFU/g lower than control by the end of 7-day storage at 4°C.

Significance: The results suggested that synergistic antimicrobial activities of EOs, such as Thy/Eug and Car/Eug, could be explored as a more effective means for controlling *E. coli* O157:H7, both *in vitro* and on lettuce.

P3-28 Survival of *Listeria monocytogenes* in Dual-species Biofilms with *Pseudomonas fluorescens* at Different Colonization Sequences during Desiccation and Disinfection

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Introduction: L. monocytogenes could persist in food processing plants in biofilms, possibly in mixed-species biofilms, which are more resistant than those of a singled species. Colonization sequence in biofilms between different species might impact initial attachment and biofilm maturation, affecting biofilm structure and cell resistance to environmental stresses.

Purpose: The objective of this study was to investigate the impact of different inoculation sequences of *L. monocytogenes* and *P. fluorescens* in mixed-species biofilms on survival of *L. monocytogenes* during desiccation and disinfection.

Methods: Dual-species biofilms with different colonization sequence (*L. monocytogenes* + *P. fluorescens*, *P. fluorescens* + *L. monocytogenes,* and co-culture of these two species) were formed on stainless steel coupons in tryptic soy broth at 15°C for 48 h. Cells in biofilms were exposed to desiccation stress at 43% relative humidity for 21 days or treated with 100 ppm benzalkonium chloride for 15 min. Biofilm structures were also observed by confocal laser scanning microscope.

Results: The populations of *L. monocytogenes* was 0.6 to 1.0 log CFU/cm^2 higher after 21 days when *P. fluorescens* was the first colonizer than those in biofilms with other colonization sequences. During sanitation treatment, *L. monocytogenes* in dual-species biofilms showed lower inactivation rate when *P. fluorescens* was colonized first compared with biofilms with other sequences. Confocal images showed flat and thin-layer structure of both *L. monocytogenes* single-species and dual-species biofilms when it was the first colonizer. On the other hand, when *P. fluorescens* was colonized first on the surface, the dual-species biofilms displayed multi-layered structure with higher thickness and roughness than biofilms in other sequences.

Significance: This study provides valuable information about survival of *L. monocytogenes* in dual-species biofilms with *P. fluorescence* in different colonization sequences during desiccation and sanitation. Highlighting the presence of *P. fluorescence* in biofilms could protect *L. monocytogenes* cells from desiccation stress and sanitizer treatment.

P3-29 Antimicrobial Activity of 405-Nm Light-emitting Diode in the Presence of Riboflavin against *Listeria monocytogenes* on the Surface of Smoked Salmon

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Introduction: The use of light-emitting diodes (LEDs) has emerged as a promising food preservation technology over the last decade. However, little information is available on the antibacterial efficacy of 405 nm LED with or without a photosensitizer in controlling foodborne pathogens on RTE fish products.

Purpose: The aims of this study were to determine the efficacy of 405 nm LED with or without riboflavin on the inactivation of *Listeria monocytogenes* in phosphate buffered saline (PBS) and on the surface of smoked salmon at different storage temperatures and to evaluate its impact on quality of the illuminated smoked salmon.

Methods: Bacterial suspension (10^6 CFU/ml) in PBS containing riboflavin (0 to 100 μM) was illuminated with 405 nm LED at 25°C for 1 h. For food application, the inoculated smoked salmon (10^3 CFU/cm^2 of *L. monocytogenes* cocktail culture) was treated with riboflavin (0 to 100 μM), followed by LED illumination for 22 to 48 h at 4, 12, and 25°C. Color change and lipid peroxidation of the illuminated smoked salmon were analyzed.

Results: LED illumination alone inactivated 0.9 log CFU/ml of *L. monocytogenes* in PBS for 1 h, while riboflavin-based LED illumination reduced *L. monocytogenes* by 6.2 log CFU/ml for 30 min at 25°C. LED illumination with and without riboflavin inactivated *L. monocytogenes* on the smoked salmon by 1.6 to 2.2 log CFU/cm^2 for 48 h at 4 and 12°C, while the number of non-illuminated control cells remained unchanged during storage. At 25°C, non-illuminated control cells on the smoked salmon grew to 6.1 log CFU/cm^2 for 22 h, whereas LED illumination with and without riboflavin inactivated cell populations to 2.1 to 2.4 log CFU/cm^2. However, LED illumination with and without riboflavin caused color change and lipid peroxidation.

Significance: Although LED illumination caused quality change in the smoked salmon, this study demonstrates the potential of 405 nm LEDs to preserve smoked salmon products, reducing the risk of listeriosis.

P3-30 Ultrasound-induced Bacterial Cell Death Exhibits Physical Disruption and Biochemical Apoptosis

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❖ ***Developing Scientist Competitor***

Introduction: Acoustic cavitation on cells is one of the major applications of ultrasound technology. The physical effect of ultrasound treatment on microorganisms is well-studied, but its potential influence on gene expression regulation system of cells has not yet been fully explored.

Purpose: The purpose of this study was to demonstrate the physical disruption of ultrasound-induced bacterial cell death and reveal the relationship between bacterial apoptosis and biochemical processes.

Methods: Escherichia coli was selected as the model microbe to undertake ultrasound treatment using defined ultrasonic frequency (20 kHz), power intensities (25.5 and 255 W/cm^2), and duration times (0, 5, 15, and 25 min). The sublethal and lethal effects, variations in membrane potential and ion channels, physical disruption, and bacterial apoptosis during ultrasound treatment were investigated.

Results: Slight variations in membrane potential and ion exchanges across membrane induced by low-intensity ultrasound increased the membrane permeability of *E. coli*; this reversible sublethal effect can preserve the viability of *E. coli* and meanwhile be beneficial for bioprocess application. In comparison, high-intensity ultrasound resulted in irreversible lethal effect on *E. coli*, which can be applied in the field of microbial sterilization. In addition, both low- and high-intensity ultrasound induced either physical destruction or triggered genetically encoded apoptosis of *E. coli*. Accumulation of reactive oxygen species and decrease of adenosine triphosphate might be related to the physiological and biochemical hallmarks of apoptosis, including exposed phosphatidylserine and activated caspases in *E. coli*.

Significance: The significance of our research is to identify genetically encoded bacterial apoptosis in response to environmental stimuli caused by ultrasound treatment. The result provides novel insight into the mechanisms of non-thermal physical treatment on the inactivation of bacteria and lays the foundation for further research on cell signaling and metabolic pathways in apoptotic bacteria.

P3-31 Detection of *Salmonella* from Manure and Soil Samples Collected from Multiple Commodity Farms

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Introduction: The presence of *Salmonella* in produce production environments may lead to the contamination of produce and ultimately produce-related human salmonellosis. A clear understanding of pathogen migration along the farm-to-fork continuum and the temporal fluctuations of enteric pathogens, including *Salmonella* populations in produce, is essential for food safety.

Purpose: In this study, production samples (manure and soil) were screened for *Salmonella* to evaluate possible contamination when manure (fertilizer) was applied to the soil.

Methods: Environmental samples were obtained from 12 farms fertilized with dairy and poultry manure over a 12-month period. The samples were pre-enriched in buffered peptone water (BPW) and incubated overnight at 37°C. Samples were then transferred and incubated overnight at 42.5°C in selective enrichment broths namely, Rappaport-Vassiliadis and tetrathionate broths. The enrichments were plated on xylose-lysine-tergitol-4 agar plates. The plates with typical colonies were further examined for the presence of *Salmonella* using the colorimetric gram-negative card and Vitek 2 Compa system (v. 5).

Results: *Salmonella* was recovered from seven of the 12 farms evaluated. The presence of *Salmonella* was more prevalent on the dairy farms (n=5) compared to the poultry farms (n=2). Additionally, *Salmonella* was detected in the manure and soil from the dairy fertilized farms, which was not observed in the poultry farms. It appears that *Salmonella* was detected in the soil between one and four months after application of the manure.

Significance: Based on the results of this preliminary study, it appears that *Salmonella* was present in soil fertilized with contaminated manure. Additional investigations are needed to understand the link between the transfer of *Salmonella* throughout the food supply chain from farm to fork.

P3-32 Utilizing Rhamnose as the Primary Carbohydrate in Buffered *Listeria* Enrichment Broth Increases Post-enrichment *Listeria monocytogenes* Populations in Some Food Matrices

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Introduction: The purpose of selective enrichment is to achieve maximum growth of *Listeria monocytogenes* while minimizing or restricting growth of non-target organisms. The goal for including selective enrichment in a regulatory testing program is to increase the likelihood of recovering low levels of *L. monocytogenes* from food matrices which may have a much higher background of non-target organisms.

Purpose: This study evaluates the effect of substituting rhamnose for glucose in buffered *Listeria* enrichment broth (BLEB) on the resulting levels of *L. monocytogenes* following selective enrichment of select food matrices.

Methods: Analytical portions of uncooked bean sprouts, avocado pulp, shrimp, crab meat, and salmon were spiked (1 to 5 CFU/g) with *L. monocytogenes* (*n*=10 strains, three replications). Selective enrichment was performed using BLEB or rhamnose-substituted BLEB (BLEB-rhamnose) with the U.S. Food and Drug Administration Bacteriological Analytical Manual method. *L. monocytogenes* was enumerated using PALCAM agar.

Results: The median post-enrichment *L. monocytogenes* populations with associated inter-quartile range (IQR) were 9.2 (9.2 to 9.4), 6.6 (5.3 to 7.0), 8.7 (7.9 to 9.1), 4.4 (3.7 to 4.9), 5.2 (3.9 to 6.0), and 4.3 (3.3 to 4.7) log CFU/ml for matrix-free, bean sprouts, avocado pulp, shrimp, crab meat, and salmon, respectively, when BLEB was formulated using glucose. When BLEB-rhamnose was used, the median post-enrichment *L. monocytogenes* populations were 8.9 (8.4 to 9.0), 5.0 (3.6 to 6.0), 8.8 (7.9 to 8.9), 6.2 (5.1 to 6.9), 8.3 (6.8 to 8.7), and 6.3 (5.3 to 7.1) log CFU/ml for the same matrices, respectively.

Significance: The post-enrichment populations of *L. monocytogenes* varied markedly depending on matrix. Rhamnose-substituted BLEB increased *L. monocytogenes* post-enrichment in some matrices. *L. monocytogenes* enrichment population enhancement was more pronounced in fish and seafood matrices compared to other matrices included in the study. Currently there are no known specific selective agents that would allow growth of only *L. monocytogenes*. A matrix-specific approach to enhancing recovery of *L. monocytogenes* may be necessary to improve the capabilities of regulatory laboratories to recover this organism amidst a complex microflora.

P3-33 Effect of Hydrophobicity and Surface Charge of Abiotic Surfaces on Dynamics of Initial Phases of Bacterial Attachment

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❖ ***Developing Scientist Competitor***

Introduction: Bacterial adhesion to a surface is an initial prerequisite to bacterial fouling. Therefore, it is essential to understand how surface characteristics influence the bacterial adhesion process. Hydrophobicity and surface charge of the abiotic surfaces are two of the main physicochemical parameters affecting bacterial attachment. Self-assembled monolayers are selected as model surfaces because it is possible to eliminate the effect of roughness and texture on bacterial adhesion and make surfaces with similar surface coverage, but varying surface energies and chemistry. *Staphylococcus aureus* and *Escherichia coli* O157:H7 were selected as model bacteria in this study because they exhibit differing shapes (i.e., coccoid [circular] and bacilli [rod-shaped], respectively) and gram-reactions.

Purpose: The objective of this research is to understand the initial stages of bacterial adhesion onto abiotic surfaces of varying hydrophobicity and surface charge.

Methods: Bacterial pathogens *S. aureus* and *E. coli* O157:H7 as model gram-positive coccoid and gram-negative bacilli microbes were inoculated on substrates coated with molecularly smooth, self-assembled monolayers with precisely controlled surface coverage and chemistry. Interfacial properties of substrates and microorganisms were extensively characterized using laser Doppler electrophoresis, streaming potential technique, and contact angle goniometry; the numbers and distributions of adherent bacteria were quantified via high-resolution scanning electron microscopy.

Results: Bacterial adhesion was greatest on hydrophilic substrates with positive surface charge, followed by hydrophobic substrates with increasing hydrophobicity, and the smallest on hydrophilic substrates with negative surface charge. The time constant of adhesion was about two to four times greater for *E. coli* O157:H7 compared to *S. aureus*, indicating a lower number attached per unit time for *E. coli* O157:H7 but a comparable mass attached per unit time due to the difference in size of these species. A transition from a power-law to exponential time-dependency was observed upon changing substrates from hydrophilic surfaces with positive charge to hydrophobic surfaces and hydrophilic surfaces with negative charge.

Significance: Results suggest that understanding the effect of surface charge and hydrophobicity of abiotic surfaces is extremely important for the hygienic design of surfaces for the food industry.

P3-34 Biofilms Assessment of *Escherichia coli* and *Salmonella* Isolates from Poultry Farms in Ilorin, Kwara State, Nigeria

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Introduction: Biofilms are complex exopolysaccharides which have been implicated in a variety of diseases and are a great global public health concern. Studies on biofilm formation by isolates of *Escherichia coli* and *Salmonella* in Ilorin, Nigeria are limited.

Purpose: The objective of this study was to assess the biofilm forming potentials of *E. coli* and *Salmonella* isolates from poultry in Ilorin.

Methods: Biofilms of eight laboratory stock cultures, two each of *E. coli* (non-pathogenic), *E. coli* O157:H7, *Salmonella* Nagoya and *Salmonella* Enteritidis from poultry was developed in tryptose soya broth in 96-well polystyrene microtiter plates. The broth was supplemented with fructose or sucrose at 2 and 4%. Biofilm development was permitted at 37°C for 12, 24, and 36 h using a multifactorial study design. Un-inoculated broths and broths not supplemented with the sugar served as controls. Biofilm masses were quantified using the crystal violet binding assay. The experiment was done in three replicates.

Results: The mean biofilm mass (OD=optical density) for the *E. coli* isolates with 2% fructose was less (0.303±0.75) than 0.508±0.16 for 4% fructose. Similar results were obtained for 2 and 4% sucrose. However, the biofilm mass for the *Salmonella* isolates with 2% fructose was higher (0.716±0.18) than the 4% fructose (0.578±0.13). Conversely, there was a higher mass (0.53±0.14) with 4% sucrose when compared with 2% sucrose (0.222±0.17). *E. coli isolates* showed the least biofilm mass with 2% fructose, while 4% sucrose showed the highest biofilm mass. On the other hand, *Salmonella* isolates developed the highest biofilm mass with 2% fructose, while the least biofilm mass was obtained with 2% sucrose. However, in both the *E. coli* and *Salmonella* isolates, biofilm development increased steadily with incubation time.

Poster

Significance: Presence of fructose or sucrose had impact on biofilm formation by *E. coli* and *Salmonella* spp. from poultry in Ilorin, Nigeria.

P3-35 *Bacillus thuringiensis*: Navigating the Crossroads between Sustainable Agriculture and Food Safety

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Introduction: Trends in agriculture and environmental regulation are favoring biopesticide adoption over conventional, synthetic pesticides for crop protection; however, the intersection between phenotype and agricultural use pattern has associated *Bacillus thuringiensis*-based biopesticides with potentially pathogenic *Bacillus cereus* species. When considering the presence of *B. thuringiensis* biopesticides in food, the breadth of literature describing human exposure to commercial strains cannot be overlooked and is complimented with studies conducted using *B. thuringiensis* strains ABTS-185 and ABTS-351.

Purpose: Assess past and current research on the safety of commercial *B. thuringiensis* biopesticides to highlight the crossroads between microbial control in agriculture and food safety assessment.

Methods: A review of human exposure studies supplemented by whole genome sequencing, challenge testing in stored food, and studies conducted with the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) were executed to assess the safety of commercial *B. thuringiensis* residues in food.

Results: Health surveillance studies of large populations following aerial applications of commercial *B. thuringiensis* near populated areas, as well as studies of farm workers, did not reveal any impact to human health. Whole genome sequencing of commercial strains ABTS-351 and ABTS-1857 confirmed the absence of the *ces* gene required for cereulide synthesis and *CytK-1* which produces a variant of cytotoxin K. Furthermore, commercial *B. thuringiensis* strains did not germinate on fresh produce or purees or juices stored for up to 4 months. Commercial *B. thuringiensis* spores did not germinate during passage through a simulated gut model (SHIME), but exhibited reduced thermotolerance and slightly beneficial effects on colonic microbiota.

Significance: Combined, these studies support the low risk of commercial Bt strains ABTS-351 and ABTS-1857 and highlight the need for differentiation methods to provide a more accurate measure of *Bacillus cereus* group species in food safety testing. These methods can support the sustainable use of these commercial strains while still providing consumer protection.

P3-36 Characterization of Culturable Bacterial Communities on Romaine Lettuce Leaves: Application of a New Optical Scattering Technology

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Introduction: Romaine lettuce is susceptible to contamination by human pathogens which must compete with the resident bacteria. The composition and ecology of the bacterial communities of romaine lettuce can provide insights that protect against human pathogen invasion.

Purpose: BEAM technology was trained to determine if it can become a rapid and inexpensive method for characterization of culturable bacterial communities.

Methods: A total of 24 romaine lettuce heads (12 conventional, 12 organic) were evaluated. Leaves were blended and isolated colonies were identified by Sanger sequencing of the 16S rDNA. These colonies were used to test the BEAM technology developed by Purdue University, in which a 635-nm laser beam passes through the center of a colony and generates a scatter pattern that might be used for identification after building a classification library.

Results: Conventional lettuce had lower bacterial populations ($1.93\pm0.59\times10^5$ CFU/g) than organic ($1.60\pm0.77\times10^6$ CFU/g), $P<0.05$. Organic lettuce presented higher diversity of the culturable bacteria (42 genera) than conventional lettuce (22 genera). 645 colonies were clustered into 69 operational taxonomic units (OTUs), with *Pseudomonas* and *Arthrobacter* as the most common in both types. A total of 30 strains, representing 33% of total OTUs and 80% of total strains, were used to build a classification library. Training sets with positive predictive value above 90% were achieved, suggesting that BEAM technology can differentiate bacterial genera isolated from romaine lettuce with up to 10% overlap. Pure and mixed cultures of the same strains used in the training sets were evaluated to determine their correct classifications. Misclassification was up to 40% due to overlap among some classes. Therefore, BEAM technology can potentially differentiate bacterial genera and become a tool to characterize culturable communities. However, issues with similar scatter patterns from different bacterial genera need to be resolved.

Significance: BEAM might help future studies of bacterial communities associated with inhibition or promotion of the growth of human pathogens in fresh produce.

P3-37 Nutrient Starvation Enhances the Resistance of *Listeria innocua* to Atmospheric Cold Plasma and Decreases the Extent of Sublethal Injury in Survivors

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Introduction: Starvation stress can induce bacterial resistance to food processes. High-voltage atmospheric cold plasma (HVACP) is a novel non-thermal technology being explored to destroy foodborne pathogens and spoilage organisms. There are no published reports on the effect of physiological state on resistance of *Listeria* spp. to HVACP.

Purpose: This purpose of this study was to evaluate the effect of nutrient starvation on HVACP-resistance and sub-lethal injury in *Listeria innocua*.

Methods: Washed *L. innocua* cells that were cultured in tryptic soy broth with 0.6% (weight/volume) yeast extract (TSBYE; 35°C, 24 h), were starved in 0.85% weight/volume NaCl (saline; 25°C) for 10 days. Exponential- (EXP) and stationary-phase (STAT) *L. innocua* grown in TSBYE (35°C) and harvested after 2 and 24 h, respectively, served as controls. The *L. innocua* cells representing each physiological state were suspended in phosphate buffered saline (pH 7.2) to obtain an initial viable count of ~ 7.0 log CFU/ml and subjected to HVACP at 50 and 60 kilovolts (kV) for up to 100 s. *L. innocua* survivors were enumerated by plating aliquots of cell suspension on modified Oxford agar and tryptic soy agar with 0.6% yeast extract.

Results: Starved cells exhibited higher HVACP resistance ($P<0.05$) than EXP and STAT controls. HVACP (50 kV, 100 s) reduced viable counts of EXP, STAT, and starved cells from 7.2 to 3.57, 4.76, and 6.26 log CFU/ml, respectively. Starved survivors exhibited less sublethal injury ($P<0.05$). HVACP (60 kV, 100 s) completely inactivated EXP and STAT cells; however, starved survivors were 5.86 log CFU/ml ($P<0.05$).

Significance: This study demonstrates that nutrient starvation enhances the resistance of *L. innocua* to HVACP and should be considered as an important factor in the design of HVACP treatments to destroy pathogens such as *Listeria monocytogenes* in foods.

Poster

P3-38 Rapid Detection of *Listeria monocytogenes* in Natural Cheese and Meat Products by Loop-mediated Isothermal Amplification Bioluminescent Assay

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Introduction: Listeria monocytogenes is one of the most important foodborne pathogens, causing listeriosis through contaminated food. In Japan, *L. monocytogenes* the standard for natural cheese and unheated meat product is 100 CFU/g or less, and standard method (conventional plate count method) was published in 2014 as NIHSJ-08. In this study, detection of *L. monocytogenes* in food samples by a loop mediated isothermal amplification (LAMP) bioluminescent assay was compared to the Japan Standard method. The LAMP assay provides next-day results and is easy to use as compared to standard method, which takes three to four days.

Purpose: To evaluate a LAMP bioluminescent assay in comparison to the Japan Standard method (NIHSJ-08, culture method) for detecting *L. monocytogenes* using natural samples and artificially spiked samples.

Methods: A total of 90 samples (*n*=90), including 43 of natural cheese and 47 of meat products, were collected in Japan. Thirty-two samples (13 cheese and 19 meat products) were spiked with *L. monocytogenes* ATCC 7644 serovar 1/2c (500 CFU/25g). Twenty-five g of each sample was enriched in Demi Fraser broth at 37°C for 24 to 30 h and tested with the LAMP bioluminescent assay and the Japan standard method (NIHSJ-08).

Results: Among 58 natural samples tested, 57 were negative by both methods; one sample was positive by the LAMP bioluminescent assay and negative by the cultural method. In spiked samples, 13 (100%) out of 13 of natural cheese and 19 (100%) out of 19 of meat products were positive by the LAMP bioluminescent assay after 24 and/or 30 h of enrichment, respectively. The limit of detection for the LAMP bioluminescent assay was 10^3 to 10^4 CFU/ml after enrichment.

Significance: These data suggest that the LAMP bioluminescent assay can be used for detection of *L. monocytogenes* in natural cheese and meat products. The alternative LAMP bioluminescent method enabled reliable, rapi,d and automated detection of *L. monocytogenes* in natural cheese and meat products.

P3-39 Performance Evaluation of a Loop-mediated Isothermal Amplification Bioluminescent Assay for Rapid Detection of *Salmonella* spp. in Brazilian Poultry Matrices

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Introduction: Rapid *Salmonella* spp. detection is critical in order to guarantee food safety and to protect food company brands. Poultry and meat-associated products are recognized as a recurrent vehicle for *Salmonella* outbreaks. This pathogen contamination can result in public health problems and economic losses.

Purpose: To verify the specificity and sensitivity of a loop-mediated isothermal amplification (LAMP) bioluminescent assay (AOAC 2016.01 and NF 3M 01/16-11/16) for a variety of Brazilian meat matrices compared to ISO 6579-1:2017 and chromogenic agar.

Methods: A total of 93 poultry samples (raw poultry [raw chicken, raw chicken skin, mechanically separated chicken and turkey, chicken liver, wings, and legs [*n*=70]) and seasoned turkey breast (*n*=23) were artificially contaminated with *Salmonella* serovars (Typhimurium, Enteritidis, and Heidelberg) and analyzed with both methods. Each group was divided into: a) uninoculated, b) analyte-low inoculum (5 CFU/25g), c) analyte-high inoculum (>12 CFU/25g), d) analyte with interferents (>100 CFU of *Citrobacter freundii*), and e) only interferents (>100 CFU/25g).

Results: Compared to the traditional method, sensitivity and specificity of the LAMP bioluminescent assay was 100 and 97.8%, respectively. In the only sample which presented positivity in the alternative method and negativity in ISO 6579, it was possible to recover the analyte with chromogenic agar.

Significance: The alternative LAMP bioluminescent molecular method enabled reliable, rapid, and automated detection of Salmonella spp. in variety of meat samples.

P3-40 Performance of Rapid Enumeration Methods for Lactic Acid Bacteria and Yeast and Mold in Sauces and High-fat Food Products from Brazil

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Introduction: Spoilage microorganisms have the potential to cause significant losses in the food industry due to reduction of shelf life, recalls, and brand damage. For this reason, it is necessary to monitor these organisms using rapid and reliable methods.

Purpose: The purpose of this study was to verify correlation between two alternative methods, 3M Petrifilm Lactic Acid Bacteria Count Plate (LABCP) and 3M Petrifilm Rapid Yeast and Mold Count Plate (RYMCP), compared to their respective ISO methods for sauces and high fat matrices.

Methods: A total of 44 samples from Brazil (barbecue sauce, *n*=11; ketchup, *n*=11; margarine. *n*=11; and light mayonnaise, *n*=11) were artificially contaminated with a mixture of *Candida albicans* and *Zygosaccharomyces bailii* (300 CFU/g) and *Lactobacillus plantarum* and *Lactobacillus brevis* (500 CFU/g). The samples for lactic acid bacteria and yeast and mold evaluation were analyzed respectively with LABCP and ISO 15214:1998 and RYMCP (AOAC Official Methods of AnalysisSM 2014.05) and ISO 21527:2008-2.

Results: Compared to the reference methods, the alternative lactic acid bacteria and yeast and mold methods presented bias between 0.00 and 0.05 and 0.08 and 0.20, respectively, and *t*-paired presented a *P* value of 0.07 to 0.43 and 0.09 to 0.99 (both *P*>0.05), showing that in this study no significant differences were observed between the enumeration with Petrifilm methods and the corresponding ISO standards.

Significance: The alternative methods enabled reliable and rapid enumeration of lactic acid bacteria and yeast and molds in these samples as compared to their respective reference methods.

P3-41 Confirmation and Identification of *Salmonella* spp., *Cronobacter* spp., and Other Gram-negative Organisms by the Matrix-assisted Laser Desorption Ionization Biotyper Method: Collaborative Study

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Introduction: Confirmation and identification for pathogens following the biochemical test reference methods from the U.S. Food and Drug Administration (FDA), United States Department of Agriculture (USDA), and International Organization for Standardization (ISO) can take anywhere from three to seven days. This extended time can lead to delays in recalls and result in widespread outbreaks. The matrix assisted laser desorption ionization

(MALDI) Biotyper method is based on MALDI time of flight mass spectrometry, and is now perceived as an accurate and reliable alternative to reduce time to result to between 30 min and 2 h.

Purpose: The purpose of this AOAC OMA Collaborative Study was to compare the candidate method to the confirmation procedures from the FDA Bacteriological Analytical Manual (BAM) Chapters 5 & 29, USDA Food Safety and Inspection Service Microbiology Laboratory Guidebook 4.09 and ISO 6579:2002 & 22964:2017 reference methods, as well as evaluate the candidate method as an identification method.

Methods: The candidate method was assessed in an international collaborative study. For *Salmonella,* 15 collaborators in Europe participated. For *Cronobacter,* 14 collaborators in the United States participated. Within each data set, 24 blind coded isolates, including 16 target strains (either *Salmonella* or *Cronobacter*) and 8 additional gram-negative bacteria were evaluated. The following media were tested: tryptic soy agar (TSA), xylose lysine deoxycholate, and RAPID' *Salmonella* for *Salmonella* isolation, and TSA, ESIA, and chromogenic *Cronobacter* isolation medium for *Cronobacter*. All growths, regardless of organism, were evaluated from the select agars.

Results: Correct identifications and confirmations to *Salmonella* spp. and *Cronobacter* spp. were 100% from each agar type. For both non-*Salmonella* and non-*Cronobacter* organisms, a percentage of 100% were correctly identified with the MALDI Biotyper. For comparison, the reference procedures produced a correct identification rate of 96.6% for *Salmonella* and 95.5% for *Cronobacter*. For the non-*Salmonella* and non-*Cronobacter* organisms, percentages of 97.5 and 93.8% were obtained, respectively.

Significance: The results indicated that the alternative method produced equivalent results when compared to the confirmatory procedures specified by each reference method and demonstrated reliability as a rapid confirmation and identification method.

P3-42 Confirmation and Identification of *Listeria monocytogenes, Listeria* spp., and Other Gram-positive Organisms by the Matrix Assisted Laser Desorption Ionization Biotyper Method: Collaborative Study

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Introduction: Over the past decade, *Listeria monocytogenes* has been linked to several fatal foodborne outbreaks. The organism is able to survive in extreme conditions, which causes various problems for food manufacturers. When analyzing commodities for contamination, it can take up to seven days to confirm and identify an organism following the various traditional reference methods for the detection or enumeration of *Listeria* species or *Listeria monocytogenes* in food products. This extended time can lead to delays in recalls and result in widespread outbreaks. With the matrix assisted laser desorption ionization (MALDI) Biotyper method, the required time for accurate and reliable identifications and confirmations from isolated colonies can be reduced to between 30 minutes and two h.

Purpose: The purpose of this AOAC OMA Collaborative Study was to compare the candidate method to the confirmation procedures from the U.S. Food and Drug Administration (FDA), United States Department of Agriculture (USDA), and International Organization for Standardization (ISO) reference methods, as well as evaluate the candidate method as an identification method.

Methods: The multi-laboratory collaborative study involved a total of 16 collaborators. Each participant evaluated 36 blind coded isolates, which included 16 *Listeria monocytogenes* strains, 12 non-monocytogenes *Listeria* species, and eight additional gram-positive bacteria. Each strain was evaluated from four agars: tryptic soy agar with yeast extract, Oxford, Ottaviani and Agosti, and RAPID' L.mono.

Results: The MALDI Biotyper provided a correct confirmation of 100% for *Listeria* spp. and 99.5% for *L. monocytogenes,* and 97.8% of correct identification at the species level of the tested *Listeria* strains. For comparison, the reference methods provided a correct confirmation rate of 100% for *Listeria* spp. and 87.1% for *Listeria monocytogenes*, and a correct identification rate of 86.5% of the tested *Listeria* strains.

Significance: The results indicated that the alternative method produced equivalent results when compared to the confirmatory procedures specified by each reference method and demonstrated reliability as a rapid confirmation and identification method.

P3-43 Independent Evaluation of a Sturdy Polyurethane Sampling Sponge Tip for Bacterial Recovery from Non-porous Food Contact Surfaces

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Introduction: Although great strides have been made over the last half century in ensuring the food production environment is safe, recalls related to pathogenic bacteria continue to happen regularly. It is estimated that food safety recalls result in 48 million illnesses in the United States, leading to $55.5 billion spent on medical treatment. In order to combat these numbers, environmental sampling programs are becoming more important for food manufacturers. The need for environmental sampling devices that are easy to use and effective in removal of organisms from the surface without retaining the organisms within the device are crucial to reducing theses outbreaks.

Purpose: To evaluate the sturdy sampling sponge tip and the performance of three commercially available environmental sampling sponges in removal and release of pathogenic bacteria from stainless steel surfaces.

Methods: Four different types of environmental sampling devices, an oversized polyurethane sponge tip on a sturdy paddle handle , a polyurethane sampling sponge stick, a nitrocellulose sampling sponge stick, and a nitrocellulose sampling sponge, were evaluated in their ability to remove low levels (~1000 CFU/12" × 12" surface area) of 3 pathogenic foodborne bacteria (*Listeria, Salmonella*, and *E. coli*) from stainless steel surfaces. Each sponge was evaluated using a single diluent, 0.85% saline solution. After removal, the devices' ability to release the bacteria into solution was quantified using traditional plate counting methodologies.

Results: The results of the study demonstrated that the sturdy sampling sponge tip was as or more effective in the capture and release of bacteria than traditional environmental sampling sponges based on Log_{10} quantifications for each organism tested.

Significance: The data generated within the evaluation indicated that the sturdy sampling sponge tip can be an effective tool for food manufacturers in evaluating their facilities and its unique design provides an added benefit for hard-to-analyze areas.

P3-44 Application of Matrix-assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry for Rapid and Reliable Identification of Foodborne Bacteria from Chromogenics

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Introduction: Characterization of microbial isolates provides crucial information to decision makers, and helps track and monitor pathogens, spoilers, and technological strains. Since the first description of microorganisms, taxonomical schemes have been developed with evolutionary perspectives. But despite the difficulties to establish a coherent definition for bacterial species, phenotypic and biochemical tests are still widely used and recognized. Although routine testing laboratories handle next-day alternative methods to detect and enumerate foodborne bacteria in their daily routines, characterization of isolates is still based on the conventional tests, increasing the time-to-result. Complementary genomic and proteomic methods have significantly changed systematics, and are now perceived as alternatives to identify or routinely confirm isolates.

Purpose: The predominant proteomics methodologies are based on matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS). Thanks to their easy usability and readability, chromogenics have rapidly replaced standard culture media in routine testing. Does the combination of chromogenics and MALDI-TOF MS improve the accuracy, robustness, and practicability of the confirmation step after a first positive detection or enumeration screening?

Methods: Pre-collaborative testing was run by testing a minimum of 250 target and non-target strains on non-selective agars, and four chromogenics for *Cronobacter* spp., *Campylobacter* spp., *Listeria* spp., and *Salmonella* spp. screening. All the isolates were identified using MALDI-TOF MS. The data were compared to conventional identification results. The robustness of the new identification method combined with fast isolation on chromogenics was assessed in parallel by running four interlaboratory studies involving more than 10 operators from more than eight different organizations; 16 to 24 strains were tested per study.

Results: All the pathogens were correctly confirmed with MALDI-TOF MS on all tested media in both pre-collaborative and collaborative testing, demonstrating clearly the accuracy, robustness, and practicability of the workflow.

Significance: Combining chromogenics with MALDI-TOF MS considerably improved the screening and identification of foodborne pathogens by using the same workflow for all types of microorganisms, reducing the handling time and the time-to-result to only few minutes, and decreasing the resources required.

P3-45 AOAC PTM Validation of the Clear *Salmonella* Detection and Identification Kit in Select Foods and Environmental Surfaces Using Next Generation Sequencing Technology

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Introduction: Salmonella has been implicated as a main cause of foodborne illness outbreaks and detection can be time-consuming and expensive. The Clear *Salmonella* Detection and Identification Kit is a test based on PCR amplification and targeted next generation sequencing (NGS) technology to rapidly detect and identify *Salmonella* spp. in select foods and environmental surfaces. NGS offers multiple benefits, including pathogen detection and identification within a single reaction, increased sensitivity and specificity, and lower rates of false positives and false negatives.

Purpose: The purpose of this AOAC Performance Tested Method (PTM) was to compare the novel method to the U.S. Food and Drug Administration's Bacteriological Analytical Manual and the United States Department of Agriculture Food Safety and Inspection Service's Microbiology Laboratory Guidebook for five food matrices. Other PTM requirements (inclusivity and exclusivity evaluation, robustness, and stability evaluations) were also evaluated.

Methods: For the inclusivity and exclusivity testing, 100 target strains (including *Salmonella enterica* and *Salmonella bongori*) and 30 exclusivity non-target organisms were evaluated. For the method comparison, 30 unpaired samples for each matrix (five high-level replicates, 20 low-inoculation level replicates, and five uninoculated control replicates) were evaluated. Robustness and stability evaluates were conducted using low-inoculation level test portions.

Results: Statistical analysis was performed according to AOAC Appendix J, Probability of Detection (POD) statistical model. Fractional positive results for the low inoculum level, (5-15 positives out of 20 results) was achieved for each of the matrices tested, indicating no statistically significant difference was observed between the candidate and reference method. Results from additional PTM requirements (robustness, stability, inclusivity/exclusivity) indicated no adverse performance of the method.

Significance: The data from the study, within the statistical uncertainty, supported the certification of the candidate method as an AOAC Performance Tested Method in select food matrices and environmental surfaces.

P3-46 Pathogen Detection by Loop-mediated Amplification: Is Inhibition a Concern?

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Introduction: Pathogen detection in foods and environmental samples is a critical element in a food safety management system. Speed, sensitivity, and precision are of vital importance to manufacturers of perishable foods, and PCR has become an industry standard because it has these attributes. Unfortunately, many food components, particularly from spices and botanicals, inhibit the amplification cycle in PCR.

Purpose: This study set out to compare the robustness of loop-mediated isothermal amplification (LAMP) with that of PCR, as illustrated by the detection threshold of *Listeria* and *Salmonella* spiked into culture broths previously incubated with various spices.

Methods: Spices and botanicals, including dried mustard, garlic, cinnamon, paprika, basil, oregano, onion, cilantro, allspice, and cocoa powder, were incubated at high concentrations in buffered peptone water and Demi Fraser broth for 24 h at 37°C. The incubated broths were spiked in duplicate, respectively, with *Salmonella* Abaetetuba and *Listeria monocytogenes* at concentrations from 10^2 to 10^6 CFU/ml and, without further incubation, sampled for detection using LAMP on the 3M Molecular Detection System and PCR on the Hygiena BAX.

Results: Cocoa, basil, oregano, and allspice resulted in "Invalids" by PCR, suggesting inhibition of the amplification, but gave "Valid" results by LAMP, with *Listeria* spp. detected. Moreover, LAMP showed at least one log better sensitivity for *Listeria* spp. detection than PCR for garlic, mustard, paprika, cilantro, and onion, while cinnamon affected both technologies similarly. Patterns of results for *Salmonella* detection were similar to those for *Listeria* spp. Amplification inhibition may be overcome by dilution of the sample, but this extra step is cumbersome and would be needed less frequently with LAMP than PCR.

Significance: LAMP is at least as robust as PCR for detecting pathogens in foods containing spices and botanicals, and may offer higher sensitivity.

P3-47 Evaluation of Loop-mediated Isothermal Amplification Bioluminescent Technology for the Detection of *Listeria monocytogenes* and *Salmonella* in Cooked Sausage

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Introduction: Cooked sausages used to prepare hot dogs have been related to outbreaks caused by human pathogens, including *Listeria monocytogenes*. Pathogens can contaminate RTE cooked foods after the killing step. Pathogen detection based on loop-mediated isothermal amplification (LAMP) coupled with bioluminescent detection can provide a fast and accurate result for rapid release of this type of product.

Purpose: To evaluate LAMP bioluminescent assays for the detection of *Listeria monocytogenes* and *Salmonella* on cooked sausage.

Methods: Sausages were obtained from a local supermarket. Forty 25-g samples were inoculated with approximately five cells of *Salmonella* Typhimurium or *L. monocytogenes*. Twenty of the samples spiked with *Salmonella* were also inoculated with about 150 cells of *Citrobacter freundii*, and samples spiked with *L. mono*cytogenes with about 180 cells of *Listeria innocua* as competitive flora. Samples were enriched with 225 ml of buffered peptone water (ISO) for *Salmonella* and Demi Fraser broth for *L. monocytogenes* and incubated at 35°C for 24 to 30h. After enrichment, samples were analyzed by respective LAMP bioluminescent assay to detect *Salmonella* or *L. monocytogenes,* and confirmed by culture (ISO 6579 for *Salmonella* and 11290-1:2017 for *L. monocytogenes*). Three samples were used as a negative control. Samples were analyzed by two different technicians.

Results: All the inoculated samples were positive for *Salmonella* with LAMP bioluminescent assay, regardless of the presence of *Citrobacter* at higher levels. For *L. monocytogenes*, all the inoculated samples were positive with or without the presence of *L. innocua*. All the samples were further confirmed by culture methods. All negative control samples yielded a negative result. The LAMP bioluminescent assays provided next-day results, unlike the culture method, which took three to five days. The results were similar for both of the technicians.

Significance: The respective LAMP bioluminescent assays can be used as rapid methods for the detection of *Salmonella* or *L. monocytogenes* in cooked sausage products.

P3-48 Rapid Detection of *Campylobacter* in Meat Matrices and Environmental Samples Utilizing a Ready-to-Use (RTU) Enrichment Broth and Loop Mediated Isothermal Amplification (LAMP)-Bioluminescent Detection.

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Introduction: Campylobacteriosis is one of the most common causes of diarrheal illness in United States, and most of these illnesses likely occur due to eating raw or undercooked poultry. *Campylobacter* spp. are microaerophilic and require complex media for optimal growth and viability. A combination of ready-to-use (RTU) enrichment broth and rapid detection technologies can enable easier and faster detection.

Purpose: Evaluate the performance of a RTU enrichment broth and loop-mediated isothermal amplification (LAMP) bioluminescent method for the detection of *Campylobacter* spp. from a variety of poultry rinses, meat matrices, and environmental samples when compared to the United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS) Microbiology Laboratory Guidebook (MLG) 41.04 and ISO 10272-1 (2017) standard.

Methods: Inclusivity (102 *Campylobacter* isolates of *C. jejuni, C. coli*, and *C. lari*) and exclusivity (100 non-target microbes) were assessed following AOAC guidelines. Artificially and naturally contaminated samples (*n*=563) were evaluated as non-paired samples, with one portion analyzed by the LAMP bioluminescent method, one portion analyzed by the ISO method, and one portion analyzed by the MLG method, when applicable. Artificially inoculated samples (1 to 25 CFU/sample) were enriched per recommended protocols. Enrichments were analyzed using LAMP bioluminescent assay and further confirmed following MLG or ISO method. Results were analyzed using a discordant analysis.

Results: Total (100%) inclusivity and exclusivity were determined for the LAMP bioluminescent assay. Performance of the RTU enrichment media and LAMP bioluminescent assay for each of the matrices tested showed that there was no significant difference when compared to the USDA FSIS MLG 41.04 or ISO 10272-1 standard. The limit of detection of the assay was 10^3 CFU/ml for pure cultures and the lowest concentration detected was 1 to 5 CFU/sample in enrichments.

Significance: The new RTU enrichment broth and LAMP bioluminescent method offers a specific and rapid approach for the detection of *Campylobacter* spp., offering poultry producers/manufacturers and commercial laboratories a next-day result to evaluate the microbiological quality and safety of poultry and other products.

P3-49 Rapid Detection of *Salmonella* spp. in Poultry-related Matrices Using a Loop-mediated Isothermal Amplification Bioluminescent Assay

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Introduction: Salmonella is the most frequently reported cause of foodborne illness. Poultry and meat-associated products are recognized as recurrent vehicles for *Salmonella* outbreaks.

Purpose: The objective of this study was to evaluate the performance of a loop-mediated isothermal amplification (LAMP) bioluminescent assay for the detection of *Salmonella* in primary production samples, poultry rinses, and raw poultry products as compared to United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS) Microbiology Laboratory Guidebook (MLG) 4.09 and United States National Poultry Improvement Plan (NPIP) methods.

Methods: For primary production samples, boot swabs from each of two farms (*n*=30/farm) were analyzed by a paired study comparing detection of *Salmonella* by LAMP assay and NPIP methods. Chicken carcass rinsates collected from post-chill and re-hang sites (total *n*=120) were analyzed by an unpaired study using LAMP assay and MLG 4.09. For raw poultry products, 12 types of raw products (ground turkey, ground chicken, marinated tenders, partially fried chicken patties and chicken nuggets, whole bird without giblets, mechanically separated chicken, seasoned chicken breast, plain chicken breast, chicken tenders, chicken thighs, ground chicken, and chicken legs; *n*=5 samples of 325g raw product per method) were analyzed with the LAMP assay or MLG 4.09.

Results: Based on the results obtained, direct enrichment of primary production boot swabs in tetrathionate broth (NPIP protocol) is compatible with the LAMP assay. The paired comparison results were in total agreement between the two methods. For the post-chilled rinsates, sensitivity was 91.3 and 87.5%, respectively, for the LAMP assay and MLG 4.09. For the re-hanged rinsates, sensitivity was 100% for both the LAMP assay and MLG 4.09. For raw poultry products, sensitivity of the LAMP assay was 96.5 and 93.3% for the USDA MLG 4.09. For this group of samples, specificity was 96.9% for both methods.

Poster

Significance: The LAMP assay is comparable to MLG 4.09 for detection of *Salmonella* in a variety of poultry matrices.

P3-50 Comparative Study on the Detection of *Cronobacter* spp. Using Loop-Mediated Isothermal Amplification Bioluminescent Detection in a Variety of Dairy Food Matrices

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Introduction: Cronobacter spp. are known to cause neonatal bacterial meningitis and necrotizing enterocolitis. Traditional cultural methods require up to four to six days to obtain results. A new detection assay based on loop-mediated isothermal amplification (LAMP) bioluminescent detection enables faster (next-day results) and easier detection.

Purpose: The purpose of the study was to compare the performance of a LAMP bioluminescent assay against the ISO/TS 22964:2006 reference method for detection of *Cronobacter sakazakii* in milk and milk products.

Methods: A total of 21 samples comprised of a variety of dairy matrices (powder infant formula stages 1, 2 and 3, special formulations for premature babies, anti-reflux formulations with large amount of gum, demineralized whey, egg powder, and environmental samples) were artificially inoculated with 1 to 5 CFU of *C. sakazakii*/25 g of sample and enriched in 225 ml of buffered peptone water ISO at 37°C for 24 h. The enriched samples were analyzed by the LAMP bioluminescent method and further confirmed following the ISO 22964-2006 standard.

Results: In this study, all artificially inoculated samples were detected by the LAMP bioluminescent method and confirmed positive by the reference methods. The sensitivity and specificity were 100% for the LAMP bioluminescent method. The non-inoculated samples were detected as negative by the LAMP bioluminescent method and confirmed to be free of the pathogen via culture method. The LAMP bioluminescent method provided next-day results for detection of *Cronobacter* spp. in dairy matrices.

Significance: Results demonstrate the suitability of the LAMP bioluminescent method for rapid analysis of dairy food matrices for *Cronobacter* spp., offering dairy manufacturers next-day results to evaluate the microbiological quality of their products.

P3-51 Performance Evaluation of Loop-mediated Isothermal Amplification Bioluminescent Assay for Rapid Detection of *Salmonella* spp. and *Listeria monocytogenes* in Quinoa

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Introduction: Rapid detection of *Salmonella* and *Listeria monocytogenes* is critical in order to guarantee food safety and to protect food company brands. These pathogen contaminations can result in public health problems and economic losses.

Purpose: To verify the specificity and sensitivity of *Salmonella* or *L. monocytogenes* loop-mediated isothermal amplification (LAMP) bioluminescent assay for detection of respective targets in several varieties of quinoa.

Methods: A variety of quinoa samples (white, red, black, three-colored, and processed) were steam sterilized, divided into 25-g samples, and processed in the following ways: 1) uninoculated (*n*=4); 2) 5 CFU of *Salmonella* Poona (*n*=25; *n*=5 of each variety); 3) 5 CFU each of *Salmonella*, *Citrobacter freundii*, and *Proteus mirabilis* (*n*=25; 5 of each variety); 4) 5 CFU of *C. freundii* (*n*=10; 2 of each variety); 5) 5 CFU of *P. mirabilis* (*n*=10; 2 of each variety); 6) 5 CFU of *L. innocua* (*n*=25; 5 of each variety); 7) 5 CFU of *L. monocytogenes* (*n*=25; 5 of each variety); or 8) 5 CFU each of *L. monocytogenes* and *L. innocua* (*n*=25, 5 of each variety). The samples were enriched for 24 h at 37°C (buffered peptone water [ISO] for *Salmonella* and Demi Fraser broth for *L. monocytogenes*) and analyzed by the LAMP bioluminescent method. All samples were further culture-confirmed following ISO 6579-1:2017 (*Salmonella*) and ISO 11290-1:2017 (*L. monocytogenes*).

Results: All the different varieties of quinoa inoculated with either *Salmonella* or *L. monocytogenes*, with or without interfering organisms, were detected by the respective LAMP bioluminescent assay. The non-target bacteria were not detected by the LAMP bioluminescent assay. In addition, all the samples were culture-confirmed and the respective LAMP bioluminescent assay results were in complete agreement with the culture confirmation.

Significance: The LAMP bioluminescent molecular method enabled reliable, rapid, and automated detection of *Salmonella* or *L. monocytogenes* in different varieties of quinoa.

P3-52 Design of a Novel Loop-mediated Isothermal Amplification Assay for Detecting *Salmonella* Typhimurium

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Introduction: Loop-mediated isothermal amplification (LAMP) has been widely investigated for the detection of microbial pathogens in many fields due to its advantages of specificity, sensitivity, speed, accuracy, simplicity, and low cost.

Purpose: The objective of this study was to develop a new LAMP assay for the detection of *Salmonella* serovar Typhimurium.

Methods: The primers were designed by using PrimerExplorer V4 software, based on *Salmonella* Typhimurium LT2 chromosome, complete genome: STM3845 (National Center for Biotechnology Information Reference Sequence NC_003197.1). Six sets of primers ranked top by the software were selected and evaluated for their effectiveness in detecting *Salmonella* Typhimurium using isothermal master mix, with two strains of *Salmonella* Typhimurium (Sal 0723, Sal 0728), three strains of *Salmonella* Enteritidis isolates (SE12, 18579, CDC_2010K_1441), and two strains of *Salmonella* Heidelberg (607310-1, 579082-8). The ratio of outer and inner primers and the amount of DNA template per reaction for the assay were optimized for this set of primers.

Results: Results demonstrated that the following set of primers was determined to be effective in detecting *Salmonella* Typhimurium: F3 5'-TCTCCTTTTCGTGTGTGG-3', B3 5'- GATGAAATACTGGCTATATCATCT-3', FIP 5'- GCATTTTTTGCTGTGTAAGTGAGTACGATGTACGTGCACCAAT-3', BIP 5'-CTTCACGAACATTTCATTCTAGCTGCAAACACCAGAAGGTCCG-3'. The newly designed assay can differentiate *Salmonella* Typhimurium from *Salmonella* Enteritidis, *Salmonella* Heidelberg, other *Salmonella* serotypes, and some non-*Salmonella* bacteria of pure cultures. Its effectiveness in detecting *Salmonella* Typhimurium in food products is being investigated.

Significance: This new LAMP method could be another quick molecular tool for detecting *Salmonella* Typhimurium in food products.

P3-53 Co-Extraction and Quantification of DNA from Enteric Pathogens in Surface Water Samples from Watersheds in California

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Introduction: Pathogen contamination of surface water is a health hazard in agricultural environments primarily due to the potential for crop contamination. Furthermore, pathogen levels in these environments are often unreported due to difficulty with culture of the pathogen. The pathogens are often present in surface water but require resuscitation, making quantification difficult. Frequently, this leads to the use of quantitative PCR targeted to genes unique to the pathogens. However, multiple pathogen types are commonly in the same water sample, both gram-positive and gram-negative, leading to problems with DNA extraction.

Purpose: With Shiga toxin-producing *Escherichia coli* (STEC), *Salmonella enterica*, and *Listeria monocytogenes* as targets, a method was developed to co-extract all three and quantify the level of each using a new technique: droplet digital PCR (ddPCR).

Methods: Multiplexed target genes in STEC were virulence genes, Shiga toxin 2 (*stx*2), and hemolysin (*ehx*). Likewise, multiplexed targets in *Listeria* and *Salmonella* were the virulence genes listeriolysin (*hly*) and invasion protein A (*inv*A). Thirty-six Moore swabs from various watersheds were processed using microbiological techniques for each of the pathogens and duplicate swabs were quantified for the target genes by ddPCR.

Results: Significant correlation was found between culture and ddPCR results for all the target genes, indicating detection primarily of culturable cells by ddPCR. Average virulence gene levels were 923, 23k, 69, and 152 copies per swab for *stx*2, *ehx*, *hly* and *inv*A, respectively. Additionally, *stx*2 and *ehx* levels were highly correlated ($P<10^{-8}$, R=0.8) with generic *E. coli* most probable number levels in the duplicate samples.

Significance: Digital droplet PCR was highly effective for surface water analysis. Indirect quantification with ddPCR will improve understanding of prevalence of the pathogens and may reduce risks associated with contaminated surface water.

P3-54 Evaluation of a Shorter Protocol of the Pall Genedisc® Shiga Toxin-producing *Escherichia coli* Top 7 Test System for Same Day Release of Raw Ground Beef Samples

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Introduction: For release of raw ground beef products, manufacturers have to guarantee absence of Shiga toxin-producing *E. coli* (STEC) in 25 g of sample. In order to limit cost linked to storage, the time to result (TTR) of the method for STEC detection should be less than one work day shot, i.e., eight hours.

Purpose: Evaluate the performance of a new protocol with a very limited enrichment time combined with the PALL GeneDisc STEC Top7 method on raw ground beef samples.

Methods: The study included 116 raw ground beef samples previously identified as positive for a Top7 serogroup. To decrease the enrichment time, the test portion was increased from 50 µl to 5 or 10 ml. Bacteria were concentrated by centrifugation before lysis by sonication and heating. Resulting crude DNA extracts were analyzed using the STEC Top7 GeneDisc Plate.

Results: Seventy-eight samples were positive for O26 (76) or O111 (2). Presumptive positive samples were obtained after 3-, 4-, and 5-h enrichment times for 31, 50, and 19% of samples, respectively. The 38 negative samples after 5-h enrichment were also negative after 8-h enrichment. The 10-ml test portion enabled recovery of 18 positive samples at a shorter enrichment time compared to the 5-ml test portion. However, this largest test portion did not allow shortening the enrichment time to fewer than 5 h.

Significance: This study showed that the Pall GeneDisc STEC Top7 method including an enrichment time as short as 5 h and a test portion of 5 ml was able to guarantee absence of STEC in 25 g of raw ground beef. Applied to the beef industry, this method enables manufacturers to significantly decrease the storage time of fresh products before release.

P3-55 Performance Assessment of the Thermo Scientific Rapidfinder *Salmonella* spp., *Salmonella* Typhimurium, and *Salmonella* Enteritidis Flex Kit with Poultry House Primary Production Samples

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Introduction: *Salmonella* infection from foodborne cases in the United States presents a considerable economic burden at $2.8 billion per year. Approximately half of these cases are linked to poultry, and the most frequently occurring serotypes (Typhimurium and Enteritidis) account for ~70% of cases. Preventing *Salmonella* from entering the foodchain is a high priority for producers; a rapid and reliable test may have a sizable positive impact on both the cost for producers and incidence of illness.

Purpose: To evaluate the performance of the RapidFinder Salmonella Flex Kit for detection of *Salmonella* species and identification of *Salmonella* serovars Typhimurium and Enteritidis from poultry house primary production samples.

Methods: Primary production samples (faeces, litter, boot socks and swabs) were sourced from poultry houses and spiked with 1 to 15 CFU/sample-*Salmonella*. Samples were enriched in tetrathionate broth at 37°C then subcultured to BPW for a total incubation period of ≤24 h. Enrichments were processed with automated immunomagnetic separation (IMS), including cell lysis, on the Applied Biosystems KingFisher Flex Magnetic Particle Processor before testing with the RapidFinder Salmonella Flex Kit. Samples were confirmed using culture media according to methodology detailed in the National Poultry Improvement Plan (NPIP, 2014) and ISO 6579:2017.

Results: Boot socks (n=15), litter (n=7) and faeces (n=6) achieved 100% agreement with the reference methods. Swabs (n=5) achieved three true positives with the PCR test; the remaining two samples were undetected. Comparatively, the NPIP method also detected 3/5 positives from spiked samples.

Significance: A two-stage enrichment through tetrathionate broth and BPW allows for the successful recovery of *Salmonella* from primary production samples in ≤24 h. The RapidFinder Salmonella Flex Kit is able to detect *Salmonella* species and identify serovars Typhimurium and Enteritidis from primary production samples in ≤27 h with comparable results to the NPIP and ISO methods for *Salmonella* detection.

P3-56 Identification of *Campylobacter jejuni* and *Campylobacter coli* Isolates Recovered from Poultry and Environmental Samples by Matrix-assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry and rRNA Sequence Analysis

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Introduction: Campylobacter species are considered the leading cause of diarrheal illnesses worldwide. The incidence of human campylobacteriosis has increased significantly over recent decades, and the increase has been linked with the advancement made in *Campylobacter* detection and identification methods. Of these, *Campylobacter jejuni* and *Campylobacter coli* are the two major species that can colonize poultry and cause outbreaks of foodborne disease.

Purpose: This surveillance study was carried out to rapidly identify the *Campylobacter* isolates recovered from poultry and environmental samples by performing rRNA sequencing and matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis.

Methods: A total of 27 isolates of *Campylobacter*-like organisms were recovered from poultry and environmental samples and cultured on selective blood agars for DNA isolation and MALDI-TOF MS analysis. For each isolate, one to two colonies were directly spotted on VITEK-MS system for species identification. Genomic DNA was extracted from overnight bacterial cultures using the QIAGEN DNeasy Blood and Tissue DNA Extraction Kit. Afterwards, two-directional Sanger sequencing was performed to confirm species identification characterizing the regions of rRNA (16S, 23S, Universal) and *gyr*A loci on an ABI 3500XL Genetic Analyzer.

Results: The VITEK-MS system identified all 27 *Campylobacter*-like organisms (13 isolates as *Campylobacter jejuni* and 14 isolates as *Campylobacter coli*) to species level. Species identification attained by the VITEK-MS system matched completely with the generated rRNA and *gyr*A sequence characterization data.

Significance: MALDI-TOF MS and rRNA sequence analysis can identify *Campylobacter jejuni* and *Campylobacter coli* isolates known to cause foodborne illness.

P3-57 Comparison of an Automated Most Probable Number Method with Direct Colony Count Methods for the Enumeration of Total Viable Count, Total Coliforms, *Bacillus cereus*, *Staphylococcus aureus*, and Yeast and Mold in Various Processed Food Products

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Introduction: An instrument has been developed to automate the most probable number (MPN) technique and reduce the effort required to estimate some bacterial populations. We compared the automated MPN technique to colony count protocols (Korea food code) for estimating the total viable count (TVC) of aerobic microorganisms, total coliforms, *Bacillus cereus* and *Staphylococcus aureus*, and yeast/mold populations on various processed food products.

Purpose: The purpose of the study was to compare automated MPN method with colony count method and determine whether the automated MPN technique was applicable to various processed food products.

Methods: Food samples artificially inoculated with *Escherichia coli* ATCC8739*, S. aureus* ATCC25923*, B. cereus* ATCC21769, *Zygosaccharomyces* spp., and *Aspergillus* spp. (40 meat products, 16 seafood products, eight various noodles, 20 sauces, and 30 rice products) were tested. *Zygosaccharomyces* spp. and *Aspergillus* spp. were used from laboratory isolates. The samples were artificially contaminated to 5−6 log CFU/g and duplicates were analyzed.

Results: The results showed good agreement with both methods. The individual differences for all results of TVC and total coliforms were less than 0.5 log CFU/g and demonstrated a good agreement between both methods. The gaps in log ranges were approximately 0.04 to 0.37 log CFU/g. Analysis of the 114 pairs of results indicated that there was no statistically significant difference between the two methods using the paired *t* test ($P>0.05$).

Significance: The automated MPN method was statistically equivalent to the reference methods, while saving effort and time per analysis. Therefore, the automated MPN method would contribute to cost-reduction for quality control and storage in the food industry.

P3-58 Isolation, Characterization, and Immunological Reaction of *Proteus mirabilis* Isolates from Broilers

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Introduction: *Proteus mirabilis*, which is ubiquitous in the environment, is an opportunistic human pathogen that causes urinary tract infections. Recently, this bacterium has been isolated from many food-producing animals, including poultry and its products. Moreover, reports have shown *P. mirabilis* can also cause foodborne illness in humans.

Purpose: While routinely monitoring broilers for fecal excretion of two important foodborne pathogens (*Salmonella* and *Campylobacter*), it was observed that one group of the broilers excreted *Proteus* in their fecal droppings. Our interest in food safety of poultry products prompted us to characterize the molecular, biochemical, immunological, and antimicrobial characteristics of the *Proteus* isolates.

Methods: Cecal contents and fecal droppings were collected and treated according to the standard protocol for isolation. Speciation based on biochemical reactions was carried out using a Biolog Microbial ID System kit, and the antimicrobial activity of the isolates were determined using a Phenotype MicroArray kit. Western blot was used to determine the immune status of broilers against *P. mirabilis*. A total of 10 *P. mirabilis* isolates were selected for further characterization.

Results: The selected isolates could grow at pH 6.0 and in 1% NaCl. Additionally, the isolates were resistant to sodium lactate, troleandomycin, rifamycin SV, and vancomycin, but were sensitive to nalidixic acid, cefotaxime, and novobiocin. Moreover, the CTX, ACC, VEB, CMY-1, BIC, NDM, *qnrB* and *qnrD* genes were detected by PCR amplification in all isolates. Sera from broilers harboring this bacterium reacted to the *P. mirabilis* soluble proteins, but not from litter- and age-matched *P. mirabilis*-negative and specific pathogen-free chickens, indicating that this bacterium could colonize chickens with a humoral immune response against *P. mirabilis*.

Significance: This study may provide a rationale for further monitoring *P. mirabilis* in poultry production to determine whether *P. mirabilis* could pose a potential threat to public health.

Poster

P3-59 Development of a Loop-mediated Isothermal Amplification Method for Rapid *Campylobacter jejuni* Detection

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Introduction: *Campylobacter jejuni* is the leading foodborne pathogen that causes human bacterial gastroenteritis worldwide. Poultry products are regarded as a major source for human infection. Early, rapid detection of this microorganism in poultry products is necessary for contamination control and reduction of economic losses. The loop-mediated isothermal amplification assay (LAMP) that rapidly and specifically amplifies DNA at an isothermal temperature may be an alternative for *C. jejuni* detection in poultry products.

Purpose: The purpose was to evaluate LAMP for rapid detection of *C. jejuni*.

Methods: A set of four primers were designed specifically to recognize the *C. jejuni* potassium transport ATPaseA (*kta*) gene. Genomic DNA was prepared by a genomic DNA isolation kit. Amplification was performed at 65°C for 1 h. The amplicons were analyzed by agarose gel electrophoresis, and the gels were stained with ethidium bromide.

Results: The results show the ladder-like patterns of the amplicons specific to the *kta* gene. The LAMP reaction conditions were optimized. The detection limit of this assay was approximately 1 pg/reaction.

Significance: Based on our preliminary results, we concluded that this assay is rapid, sensitive, and specific for identification of *C. jejuni* and does not require sophisticated equipment. Further evaluation of LAMP on *C. jejuni* detection in complex food matrices is needed.

P3-60 Method Verification of Dehydrated Film Media for Quantification of Microbial Quality Indicators in Egg Products

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Introduction: Although egg contents are meant to be sterile, they can become contaminated. Contamination of egg white and egg yolk through contact with spoiled shells can occurring during egg breaking in the manufacture of egg products, thus affecting their shelf life. Quantification of microbial indicators, including total aerobic count (TAC), yeast and molds (YM), *Escherichia coli*, and *Staphylococcus aureus,* are relevant to assessing microbiological quality of egg products. Egg components may have inherent antimicrobial components that impact microbial recovery on agar plates; thus, verification of microbiological method performance is important.

Purpose: To verify the performance of various dehydrated film media for the enumeration of microbial indicators in egg-based products.

Methods: ATCC strains of *E. coli*, *S. aureus*, *Enterobacter amnigenus*, *Candida albican*s, and *Geotrichum candidum* were spiked in two 11-g replicate portions of six egg products, including liquid and dried whole egg, whites, and yolk (n=54 samples). Known concentrations of microorganisms were prepared from an overnight culture to achieve 10^4 to 10^5 CFU/g for quality indicators, 10^2 CFU/g of *S. aureus*, and 10^5 to 10^6 CFU/g of YM. Quantification of inoculum in diluent was used as a control. Quantification of TAC, *E. coli*, *S. aureus*, and YM were determined before and after spiking 3M Petrifilm Plates following manufacturer's instructions.

Results: Quantification of TAC, *E. coli*, and *S. aureus* on dehydrated film media was not significantly different from the control sample ($P>0.05$), indicating that there is no matrix interference that affected recovery. Quantification of YM on dehydrated media selective for YM in dried whites required 72 h to recover the spiked microorganisms, compared to the other egg products in which recovery was achieved after 48 h of incubation.

Significance: The use of 3M Petrifilm Plates is suitable to determine microbial indicators and *S. aureus* in egg products.

P3-61 Loop-mediated Isothermal Amplification Bioluminescent Assay for Rapid Detection of *Cronobacter* spp. in Powdered Infant Formula

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Introduction: Cronobacter is considered an emerging pathogen that often affects newborns and the elderly, with highest incidence and severity occurring in pre-term infants. Clinical symptoms are mainly meningitis, septicemia, or necrotizing enterocolitis in infants. The disease is associated with the consumption of hydrated milk as a carrier of the pathogen and the eventual contamination of utensils and equipment as reservoirs. *Cronobacter* is not yet regulated in the Chilean Food Sanitary Regulation (RSA), despite the recommendation of the World Health Organization. It is important to find quick and efficient methods to detect this pathogen.

Purpose: The aim of this work was to evaluate the 3M Molecular Detection Assay 2 – *Cronobacter* (MDA) based on loop-mediated isothermal amplification (LAMP) for rapid detection using pure culture and powdered milk.

Methods: Limit of detection (LOD) of the LAMP assay was determined using different concentrations of *Cronobacter sakazakii* (ATCC 29004). Powdered infant formula (PIF) samples (n=8) were spiked with either *C. sakazakii* (100 or 1,000 CFU/25g) alone or with a non-target organism (*Salmonella enterica*, ATCC 13076; 100 or 10^6 CFU/25g). The samples were enriched in buffered peptone water (ISO) for 24 h at 37°C and analyzed by the LAMP assay. The enriched samples were also culture-confirmed.

Results: The LOD of MDA was about 10^2 CFU/ml in pure culture. The milk powder did not interfere with the assay. All spiked enriched samples with or without *Salmonella* were positive for *Cronobacter* by the LAMP assay. In one of the samples (Stage1 PIF) spiked with 10^6 CFU of *Salmonella*, there was no detection of *Cronobacter*, possibly due to lack of growth of *Cronobacter.* All results were culture-confirmed and correlated with the LAMP assay.

Significance: The 3M Molecular Detection Assay 2- *Cronobacter* enabled rapid detection of *Cronobacter* in PIF with adequate sensitivity and specificity.

P3-62 Comparison of Methods for the Enumeration of Lactic Acid Bacteria in Ready-to-Eat Meat and Sauce Matrices

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Introduction: Lactic acid bacteria are microorganisms which have been known to benefit the human digestive system as probiotics, in addition to being an essential component for production of fermented and cultured foods. However, they can also be key spoilage organisms, causing off-flavors, discoloration, slime production, and bloating.

Purpose: The objective of this study was to evaluate the performance of the 3M Petrifilm Lactic Acid Bacteria Count Plate, a dehydrated film medium, for the recovery of lactic acid bacteria in a variety of types of RTE meat and sauces as compared to the traditional De Man, Rogosa and Sharpe (MRS) agar method and a secondary commercial method for lactic acid bacteria.

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Methods: A total of 20 food matrices were screened for the presence of lactic acid bacteria using the dehydrated film medium. If very low or no lactic acid bacteria were present, foods were artificially inoculated with equal numbers of *Lactobacillus plantarum* ATCC 8014 and *Enterococcus faecalis* ATCC 7080 and then tested using each method. For naturally contaminated matrices, three dilution levels were plated for each method. Presumptive colonies were chosen for confirmation from each set of duplicate plates counted, for both the MRS agar and dehydrated film medium.

Results: The overall results were comparable and not statistically different when comparing the MRS agar reference method to the dehydrated film medium (P=0.085) and the secondary commercial method (P=0.373). Two samples were naturally contaminated with yeast and were removed from the paired *t* test analysis. Counts for these samples were significantly higher on the MRS agar as compared to the dehydrated film medium, which inhibited the yeast growth by design.

Significance: The 3M Petrifilm Lactic Acid Bacteria Count Plate was comparable to the MRS agar method and the commercial method for the detection of lactic acid bacteria in a variety of RTE meats and sauces.

P3-63 Comparison of Sensitivity by Three Methods for Counting Coliforms and *Escherichia coli* in Cheese

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Introduction: Currently, one of the microorganisms that cause foodborne diseases is *Escherichia coli,* which is considered an indicator of hygiene in the food industry. The isolation of this bacteria in foods gives a high degree of certainty of contamination of fecal origin. It is convenient to check the presence of this microorganism, and whenever possible, the study must be completed with the specific analysis assessing the risks of the presence of false positives and false negatives.

Purpose: The purpose of this study is to evaluate the sensitivity of three methods to determine total coliforms and *E. coli* in cheese.

Methods: The rapid count in petrifilm plates, conventional method (NMP), lauryl sulfate triptose broth, bright green broth and *E.coli* broth, and red bile violet (RBV) agar (35°C for 24 to 48 h). Recoveries were performed with the reference strain *E. coli* ATCC 8739. Statistical analysis of linear regression was used to determine the correlation between the methods.

Results: In the conventional method (NMP), false negative and false positive results were presented. In the plate count method with RBV agar, it was determined that this type of microorganisms tend to be stressed and the cells remain undetected. In the method of counting in petrifilm, plate sensitivity and efficiency were determined for the coliforms and *E. coli* count. The correlation coefficient between the conventional method (NMP) and the method of counting in petrifilm plate was 0.94, the plate count method with RBV agar and the method of counting in petrifilm was 0.73. The conventional method (NMP) and the plate count method with RBV agar was 0.70.

Significance: These data suggest that the method of rapid counting in petrifilm plates for coliforms and *E. coli* showed a greater sensitivity to determine this type of microorganisms.

P3-64 Improved Detection and Isolation of *Listeria monocytogenes* from Environmental Samples to Support Outbreak Investigations in New York State

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Introduction: Listeria monocytogenes causes approximately 1,600 illnesses per year in the United States, ranking 3rd in the country in total number of deaths caused by foodborne pathogens. Environmental sampling is often helpful in identifying the source of *Listeria* infections.

Purpose: The purpose of this study was to compare enrichment methods to detect and isolate *L. monocytogenes* from environmental sources.

Methods: The current method used at the Wadsworth Center is a 48-h incubation at 30°C in University of Vermont enrichment (UVM) broth. To determine if detection and isolation was improved with a secondary enrichment, an additional incubation in Fraser broth or buffered listeria enrichment broth (BLEB) for 48 h at 37°C was incorporated into testing during two outbreaks in New York State. In total, 73 environmental sponges were enriched, extracted, and tested using a laboratory developed real-time PCR for *L. monocytogenes.* PCR-positive samples were cultured on chromogenic media for isolation. Pulsed-field gel electrophoresis (PFGE) and whole-genome sequencing (WGS) were performed on the isolates to assess relatedness.

Results: Fourteen of the 73 samples tested from two outbreak investigations were positive for *L. monocytogenes* DNA by real-time PCR (six from outbreak A and eight from outbreak B). Detection rates were highest following secondary enrichment in Fraser broth. Isolation was achieved in six samples (five from outbreak A and one from outbreak B). PFGE results from the environmental isolates were determined to be indistinguishable from the outbreak-related patient isolates in both investigations. Furthermore, WGS results showed that all isolates were likely to be related, having only between zero and six alleles different.

Significance: These results demonstrate that an added secondary enrichment in Fraser or BLEB medium was beneficial for the detection and isolation for *L. monocytogenes* from environmental samples. Ongoing studies will further evaluate incubation times to determine if longer incubation times may contribute to more successful recovery and isolation of *L. monocytogenes*.

P3-65 Effect of Shipping Time, Temperature, and Transport Media on Recovery of *Listeria monocytogenes* from Environmental Swabs

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Introduction: Environmental sampling is an important tool for monitoring pathogens in food production environments. Sampling is often completed using swabs rehydrated with a transport medium to neutralize sanitizers and ensure recovery after transport to a lab. Shipment is recommended at 4°C for up to 48 h; however, the effect of sub-optimal temperatures and extended times is not fully understood.

Purpose: To assess the effect of standard (4°C) and abuse (15°C) shipping temperatures and extended storage time (up to 72 h) on recovery of *Listeria monocytogenes* from swabs rehydrated with four transport media with and without food.

Methods: Sanitized surfaces were simulated using stainless steel coupons inoculated with *L. monocytogenes* at ~10^6 CFU per 4-in by 4-in area and dried for 3 h, followed by a dip in 10 ppm sodium hypochlorite and additional drying for 1 h. Coupons were sampled with swabs rehydrated with Dey-Engley neutralizing broth (D/E), neutralizing buffer (NB), Butterfield's phosphate buffer (BPB), or Letheen broth (LB) and stored for 0, 24, 48, and 72 h at either 4 or 15°C prior to quantitation and enrichment. To simulate swabbing on surfaces contaminated with a food matrix, melted ice cream or raw milk cheese whey was added to swabs before storage. *L. monocytogenes* in swabs were quantitated on modified Oxford (MOX) agar and enriched with University of Vermont medium and Fraser broth, followed by MOX confirmation.

Results: Without a food matrix, all transport media allowed detection of *L. monocytogenes* in swabs stored at 4 and 15°C, with *L. monocytogenes* outgrowth seen at 15°C. Swabs with ice cream at 4 and 15°C showed reduced detection from all media, except LB. After 72 h at 4°C, the transport media having the highest % positives were LB>NB=BPB>D/E. In the presence of the cheese whey, *L. monocytogenes* recovered better in NB and LB than D/E and BPB at 4°C after 48 and 72 h, and was positive in all media at 15°C after 24 h.

Significance: Shipping time, temperature, and transport media used for environmental swabs can greatly affect *L. monocytogenes* detection.

P3-66 Improve Workflow Flexibility with up to 72-Hour Preenrichment Hold at 2 to 8°C with RapidChek *Listeria N*extDay Pur-Blue DUO Samplers for Environmental *Listeria* Testing

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Introduction: RapidChek *Listeria* NextDay Pur-Blue DUO Samplers (DUO) are ideal for the two most common environment sampling methods: surface sponging (12-in by 12-in area) or swabbing (for small, hard-to-reach areas). Users have expressed interested in being able to hold samplers at 2 to 8°C prior to enriching in order to allow greater workflow flexibility due to work hours, holidays, or shipping samples off-site for testing.

Purpose: To validate the DUO for detection of *Listeria* in environmental samples held for up to 72 h both preenrichment (holding sponges both in and out of media) and post-enrichment.

Methods: Four sample hold times were evaluated in the study (0, 24, 48, and 72 h) at 2 to 8°C, both pre- and postenrichment. Four sets of DUO were spiked with inoculum cocktail (n=10/hold time) at 4 CFU/sample *Listeria monocytogenes* and 10^5 hot dog background. Two uninoculated samples per hold time were treated as negative controls and spiked with only 10^5 hot dog background. At the appropriate time points, samples were enriched for 24 h and evaluated with the test strips. All samples were confirmed by plating on selective agars.

Results: At all hold time points, DUO demonstrated 100% specificity and 100% sensitivity when inoculated at 4 cells/sampler. There were no false negative results observed in the spiked samplers and no false positive results observed in non-inoculated negative control samplers at any pre- or postenrichment time point. There was no significant difference realized in the number of positives from those held from 0 to 72 h preenrichment or those tested from 0 to 72 h post-enrichment.

Significance: RapidChek *Listeria* NextDay Pur-Blue DUO Samplers offer a rapid and reliable tool for screening environmental samples for *Listeria*, along with greater workflow flexibility.

P3-67 Detection of Low Levels of *Salmonella* and *Escherichia coli O157* in Compost Using the RapidChek Select *Salmonella* and RapidChek *E. coli O157* (Including H7) Test Methods

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Introduction: Composting is an attractive alternative to landfills for recycling waste organic matter. As composting becomes more popular, the presence of pathogenic bacteria like *Salmonella* and *Escherichia coli O157* is of concern in compost used on crops grown for human consumption. Of the four methods of composting (hot, cold, sheet, and trench), only hot composting is capable of killing pathogens. As the metabolic rate of microbes increases during this process of converting organic material into compost, heat is generated. Maintaining a temperature of 130°F (55°C) for more than three to four days favors the destruction of pathogens such as *Salmonella* and *E. coli O157*. While properly produced hot compost should be pathogen-free, any compost used for food chain purposes needs to be tested to confirm.

Purpose: To validate the RapidChek SELECT *Salmonella* and RapidChek *E. coli O157* (including H7) methods for the detection of *Salmonella* and *E. coli O157* (including H7) in compost.

Methods: Based on Delaware Department of Natural Resources and Environmental Control guidelines, both *Salmonella* and *E.coli O157* require a set of five non-spiked and 20 low-level spiked samples to be tested at a total enrichment time of 22 h. The 5-g samples were enriched in 45 ml of primary media, transferred to secondary media, enriched, and evaluated with the test strips. All samples were confirmed using immunomagnetic beads prior to plating on selective agars.

Results: Both test kits demonstrated 100% specificity and 100% sensitivity in compost when inoculated at 3 to 6 cells per sample. There were no false negatives or false positives observed with either test kit.

Significance: RapidChek offers rapid and reliable tools for screening compost for *Salmonella* and *E. coli O157* (including H7).

P3-68 Robustness Study of a Hermetically Sealed and Permanently Locked Detection Tube for Pathogen Assays in a Food Production Environment

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Introduction: On-site testing for food and environmental pathogens enables early and focused remediation, reduces cost, minimizes risk, and shortens the time to product release for shipment. Handling samples with potentially harmful bacterial pathogens in proximity to food production is a concern. CERTUS's innovative hermetically sealed and locked detection tube provides the solution, protecting the environment and operator from accidental contamination. The sample is placed in the tube and the cap is locked; enrichment and detection proceed simultaneously without the need for further sample manipulation.

Purpose: Validate the robustness of a tube and cap assembly that is hermetically sealed and permanently locked to withstand pressure build-up from fermentation.

Methods: In Phase 1 of the study, pressure build-up due to CO_2 release from sugar fermentation by CO_2-generating organisms was measured directly over a 72-h period. Into selective media, CO_2-generating log phase organisms were inoculated individually or combined at levels of 10^3 to 10^4 CFU. In Phase 2, modified Fraser broth medium was inoculated with a mixed culture of *Listeria monocytogenes* 4a, *Listeria welshimeri*, and *Listeria innocua* at levels of 10^3 to 10^4 CFU. In Phase 3, the ability of the novel detection tube to withstand and surpass the pressure limits identified in Phases 1 and 2 while on board the incubator/detection system was evaluated.

Results: Direct pressure measurement followed typical growth kinetics of the organisms tested and is consistent with previously reported results. Pressure testing the detection tube demonstrated pressure tolerance above the maximum possible build-up, and no leakage was detected.

Significance: A hermetically sealed and locked detection tube enables on-site enrichment and detection of food pathogens from any sample, allowing established facility remediation responses to be initiated that minimize product shipment delays whilst avoiding environmental and operator exposure. This methodology also allows for presumptive positive live samples to be sent for 3rd party confirmation.

Poster

P3-69 Bio-contained, Real-time Detection of Growing Environmental *Listeria* in the Presence of a Large Foam Collection Swab

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Introduction: On-site pathogen detection at food production facilities is now possible due to technology advances that allow for faster recognition and remediation. Immunoassays are an acknowledged method for sensitive, specific, and rapid food pathogen testing. CERTUS has incorporated a Raman-based immunoassay into their automated detection system, which is made safe by performing the enrichment and measurement inside a detection tube that is never opened after enrichment begins.

Purpose: This study aims to characterize a novel detection format (surface-enhanced Raman scattering [SERS] immunoassay) developed for the detection of environmental *Listeria* species in the presence of a large foam collection swab, pre-wetted with a neutralizing buffer and with no sample manipulation post-collection.

Methods: Environmental surface samples are collected on large foam swabs with letheen broth and inserted into tubes containing reagents and selective media. Tubes are closed and inserted into the instrument, which incubates and interrogates the samples every 10 min. The homogeneous immunoassay consists of SERS nanoparticles and magnetic microparticles conjugated to *Listeria* species-specific antibodies. Inclusivity and exclusivity were assessed with a panel of 11 *Listeria* species and 11 non-*Listeria* species.

Results: Spiked *Listeria* samples exhibited increases in SERS signal as the *Listeria* organisms grew in the media. Presumptive positive samples are detected at the *Listeria* species detection threshold, typically 10^5 to 10^6 CFU/ml levels. Spiked non-*Listeria* strains did not exhibit increases in SERS signals, even if growth was not fully suppressed by the selective media. All *Listeria* and non-*Listeria* species were correctly detected and excluded, respectively, by the assay. *Listeria* species dried on stainless steel coupons at ~10^2 CFU levels were detected in <24 h.

Significance: This new pathogen detection method combines real-time monitoring, selective enrichment, and sensitive/specific detection in a single tube, giving food producers a safe, simple, and accurate method of detecting environmental pathogens on-site.

P3-70 Application of Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry for the Monitoring of *Staphylococcus* Strain Isolated from Foods in Korea

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Introduction: Staphylococcus species have ubiquitous habitat in wide range of foods and in the niches of human living areas. Recently, coagulase-negative staphylococci (CoNS) have emerged as major nosocomial pathogens similar to *S. epidermidis*, *S. haemolyticus*, and *S. saprophyticus*, while *S. aureus* has been regarded as a major foodborne pathogen. Therefore, the ability to diagnose staphylococci at the species level has become critical in food safety and clinical microbiology.

Purpose: To evaluate potential application of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) systems with *Staphylococcus* species isolated from food stuffs and fermented foods, to investigate the population of *Staphylococcus* species isolated from Korean foods, and to note issues in the application of MALDI-TOF MS systems in the food industry.

Methods: A MALDI-TOF MS platform (Bruker Daltonic) was evaluated for the identification of *Staphylococcus*-type strains (*n*=19) and isolates (*n*=108) from various foods, including traditional fermented foods, as well as the impact of sample preparation methods (DT, EDT, EX) and culture-time of bacterial colonies. Additionally, the spectrums of isolated *Staphylococcus* strains were analyzed using principal component analysis and dendrogram analysis.

Results: The MALDI-TOF MS system revealed high accuracy for the identification of 19 *Staphylococcus*-type strains. The MALDI-TOF MS system revealed highest performance with the EX method (89%) for species-level identification (EDT, approximately 78%; DT, less than 64%) of 24-h cultured colonies. The analyzed spectra using MALDI-TOF MS and main spectra profile-based dendrogram showed that the isolated *Staphylococcus* strains were characterized by species level according to food source.

Significance: This study will give valuable information on the application of MALDI-TOF MS for rapid monitoring of microbial population and pathogenic bacteria in the food industry.

P3-71 Isolation of *Bifidobacterium* Strain Characterizing the Utilization of Resistant Starch

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Introduction: Resistant starch (RS), a type of dietary fiber, has been studied for its functional ability as a substrate for the large bowel microflora in the gastrointestinal (GI) tract of humans and its beneficial physiological effects on humans, responding to consumers' demand for high-quality food products. To our knowledge, few studies have reported screening of lactic acid bacteria for potential usability of RS and most studies on fermentation of RS by lactic acid bacteria have used equivocal starch source of RS (30 to 60%), including hydrolysable starch.

Purpose: We intend to develop and characterize a novel probiotic source of potential lactic acid bacteria to maximize the functional role and application of RS as prebiotics in the food industry.

Methods: High RS-usable lactic acid bacteria were isolated and screened from human feces using purified RS as a carbon substrate. These screened isolates were identified and their properties were characterized for further industrial application of probiotics, enhancing the potential ability of RS as a prebiotics in humans.

Results: Two isolates were identified as *Bifidobacterium adolescentis* by morphological, physiological, biochemical properties and designated *B. adolescentis* JCS2 and JCS16. The RS-utilization ability of these isolates was demonstrated by rapidly decreased pH with RS-containing modified De Man, Rogosa, and Sharpe medium and by pinhole traces left on the surface of RS particles after 24-h culture. Interestingly, *B. adolescentis* JSC2 was shown to have negative activity of β-glucuronidase, which implies it is harmless for humans and has a possible probiotic application in the food industry.

Significance: We expect that the isolate *Bifidobacterium adolescentis* JCS2 could be applied to food industry as a functional probiotic, enhancing RS utilization for promotion of human health.

P3-72 Performance Assessment of the 3M Petrifilm Lactic Acid Bacteria Count Plate According to ISO 16140-2:2016 Standard in Food Products and Environmental Samples: Method Comparison and Interlaboratory Studies

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Introduction: Lactic acid bacteria (LAB) are non-sporeforming, gram-positive cocci or rods which produce lactic acid during carbohydrate fermentation. The 3M Petrifilm Lactic Acid Bacteria Count Plate is a self-contained, sample-ready culture medium system designed to enumerate LAB; nutrients, selective agents, and oxygen scavenger compounds create an ideal environment for LAB recovery from food and beverages.

Purpose: Method comparison study and interlaboratory study were conducted to compare this new alternative method to the ISO 15214 (1998) method according to the ISO 16140-2 (2016) standard for NF Validation approval.

Methods: Different matrices were tested: meat, dairy and seafood products, composite foods, meal components, and environmental samples. The alternative plates were inoculated with 1 ml of successive dilutions in peptone salt and incubated for 45 h at 30±1°C. Red colonies with gas (heterofermentative) or without gas (homofermentative) were enumerated. The possibility to store the inoculated plates for 1 week at -18°C was evaluated. The method comparison study investigated the relative trueness, accuracy profile, inclusivity, and exclusivity. For the interlaboratory study, canned ham was inoculated with *Lactobacillus sakei* and analyzed by 10 different laboratories throughout Europe using both methods.

Results: Among the 97 samples analyzed by both methods, 49% were naturally contaminated. Depending on the tested food categories, the mean difference between alternative method and reference method counts ranged between -0.05 and 0.18 log CFU/g. No significant change was observed after storage at -18°C. For accuracy profile, the lower and upper β-ETI were comprised within the acceptability limits. The alternative method was as specific as the reference method but showed higher selectivity. The interlaboratory study was conducted in appropriate conditions and demonstrated that both methods provide equivalent data.

Significance: The alternative method is reliable to enumerate LAB and offers more practicability to the user than the reference method.

P3-73 Performance Assessment of the GENE-up *Cronobacter* spp. According to ISO 16140-2 (2016) Standard in Infant Formula with and without Probiotics

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Introduction: Cronobacter species form a group of gram-negative bacteria which may cause lethal illness in infants. The GENE-UP *Cronobacter* spp. is designed to detect *Cronobacter* spp. in infant preparations by real-time PCR.

Purpose: An independent study was conducted to compare this new alternative method to the ISO 22964:2017 (reference method) according to the ISO 16140-2 (2016) standard for NF validation approval.

Methods: Infant formula samples (25 g) were 1:10 diluted in buffered peptone water (BPW) and incubated at 37°C for 18 to 26 h. Samples containing probiotics were supplemented with novobiocin (10 mg/l) prior to incubation. After lysis (bead-beating on 20 µl of enriched sample), real-time PCR was performed. The possibility to store enrichment broth and lysates for 72 h at 5±3°C was also evaluated. The study compared the sensitivity, relative limit of detection (RLOD), inclusivity, and exclusivity.

Results: Overall, 128 samples were analyzed by both methods. Regardless of the presence of probiotics in the tested sample, good performance was observed, as the sensitivity of the alternative method (81.0%) was higher than that of the reference method (73.0%).The relative trueness was calculated at 77.3% and the false positive ratio of the reference method was 3.1%. The RLODs calculated for infant cereals and infant formula containing probiotics were 1.227 and 1.854, respectively, suggesting that both methods have a similar limit of detection. The 50 tested target strains were detected, and no cross-reaction was observed with the 31 tested non-target strains.

Significance: The alternative method is reliable for the detection of *Cronobacter* spp., and the negative results were available two days earlier than the reference method.

P3-74 Performance Assessment of the 3M Molecular Detection Assay 2 – *Cronobacter* According to ISO 16140-2 (2016) Standard in Infant Formula, Infant Cereals, Raw Materials and Environmental Samples

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Introduction: Cronobacter species form a group of gram-negative bacteria which may cause lethal illness in infants. The 3M Molecular Detection Assay 2 - *Cronobacter* is designed to detect *Cronobacter* spp. in food by means of loop-mediated isothermal amplification of specific DNA target sequences and detection by bioluminescence.

Purpose: An independent study was conducted to compare this new alternative method to the ISO 22964:2017 (reference method) according to the ISO 16140-2 (2016) standard for NF validation approval.

Methods: Different enrichment protocols were tested: food and environmental samples were 1:10 diluted and incubated at 37±1°C for 18 to 24 h in buffered peptone water (BPW; for 10 g samples) or pre-warmed BPW (for 300 g samples). Samples of 300 g each containing probiotics were supplemented with vancomycin (10 mg/L) and incubated during 22 to 24 h. After lysis (15±1 min at 100±1°C), DNA amplification was performed. The study compared the sensitivity, relative limit of detection (RLOD), inclusivity, and exclusivity.

Results: Overall, 314 samples were analyzed using both methods. Depending on the enrichment protocol, the sensitivity ranged between 80.0 and 96.0% for the alternative method and between 66.7 and 100% for the reference method. The relative trueness ranged between 77.4 and 97.9%, and the false positive ratio for the alternative method ranged between 1.1 and 3.3%. Depending on the tested matrix, the RLOD ranged between 0.255 and 2.317, suggesting that both methods have a similar level of detection. The 50 tested target strains were detected, and no cross-reaction was observed with the 30 tested non-target strains.

Significance: The alternative method is reliable for the detection of *Cronobacter* spp., and the negative results are available two days earlier than the reference method.

P3-75 Evaluation of the Biomérieux GENE-up Real-time PCR Assay for the Detection of *Listeria* species in a Variety of Environmental Surfaces

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Introduction: The GENE-UP *Listeria* species assay is a fluorescence resonance energy transfer (FRET)-based real-time PCR test used for rapid and specific detection of *Listeria* species in foods and environmental surfaces.

Purpose: The performance of the real time *Listeria* species PCR assay (alternative method) was compared to the Canadian culture reference method MFHPB-30 and evaluated according to Health Canada Microbiological Methods Committee (MMC) guidelines for the relative validation of qualitative microbiological methods for testing environmental surface samples from food production environments (2017).

Methods: Forty unpaired stainless steel, plastic, rubber, ceramic, and sealed concrete surface areas measuring 100 cm^2 were inoculated with *L. monocytogenes*, *L. welshimeri, L. ivanovii*, *L. innocua*, and *L. seeligeri,* respectively, at a level (L_1) likely to yield fractional positive (25 to 75%). Five unpaired surface areas (L_2) inoculated at 10×L_1 and five un-inoculated surface sample areas were also tested. All surface samples were co-inoculated with *Staphylococcus aureus* (interference organism) and *Enterococcus faecalis* (background organism). The inoculated surfaces were dried for 18 to 24 h at 22°C. All acclimated surfaces were sampled with a variety of bacterial carriers and wetting agents and held for 24 h at 4°C, followed by enrichment in proprietary *Listeria* enrichment broth (LPT) at 35°C and the MFHPB method enrichment media at 30°C. Samples were tested by the alternative method at 20 h of incubation. All analytical outcomes were biochemically confirmed by the reference method.

Results: Collectively, from the analysis of 250 samples, the alternative method achieved an overall relative sensitivity of 100%, relative specificity of 97.3%, a false positive rate of 2.7%, a false negative rate of 0%, and a test efficacy of 99.2%, which exceeds the MMC criteria.

Significance: The GENE-UP *Listeria* species real-time PCR assay is a suitable method for detecting *Listeria* spp. in a variety of environmental surfaces after only 20 hours of incubation, thereby significantly reducing presumptive reporting times over the reference method.

P3-76 Evaluation of Sampling Devices to Identify an Environmental Swabbing Protocol to Detect Genetically Modified Organisms on Stainless Steel Surfaces

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Introduction: Public attention to genetically modified (GM) foods in the United States is rising, and this increases the interest by food companies to label their products with such information. Testing for genetically modified organisms (GMO) primarily involves food ingredients and final products; however, determining the presence of GMO in the production environment or food preparation space has not been evaluated.

Purpose: The purpose of this study is to identify an appropriate sampling material and sampling protocol that can be used to swab for GMO-containing residue from stainless steel surfaces.

Methods: Twenty stainless steel food containers were inoculated with GTS 40-3-2 soybean and MON89788 soybean powders at 100% and 1% GM levels. At each level, ten polyurethane sponges and ten polyester cloths were used to swab separate containers. After swabbing, two sampling protocols were evaluated (wet vs. dry), with five of each material type following each protocol. DNA extraction and real-time PCR was performed for each sampling device.

Results: The wet sampling protocol picked up more powder from the containers compared to the dry approach. Powder on the polyurethane sponge was easily released into solution after hand massaging the sample bag whereas powder adhered to the polyester cloth. For 100% MON89788 soybean, PCR results showed that P-FMV and CTP2-cp4epsps were detected in 100% of the twenty samples regardless of sampling device and protocol. For 1% GTS 40-3-2 soybean, P-35S was detected from 100% of twenty samples while T-NOS was detected from 60% of twenty samples following the dry protocol and 100% of samples processed by the wet protocol.

Significance: These results indicate that DNA from GMO powder in the environment can be collected, extracted, and detected. The combination of polyurethane sponges following a wet sampling protocol can be an effective tool to monitor the environment for GMO.

P3-77 Validation of the RapidChek *Listeria monocytogenes* Test System for the Detection of *Listeria monocytogenes* in Foods and on Environmental Surfaces

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Introduction: Routine testing of food processing environments and ready-to-eat foods that may support the growth of *Listeria* is essential for the prevention of illness and in order to avoid costly recalls. To reduce the cost and time of industry testing, a cost-effective, reliable, and rapid 44 to 48 h lateral flow-based method was developed for the detection of *Listeria monocytogenes* in several food types and environmental surfaces.

Purpose: The purpose of this study was to compare the lateral flow-based method to the United States Department of Agriculture Microbiology Laboratory Guidebook, U.S. Food and Drug Administration Bacteriological Analytical Manual, and AOAC Official Methods of Analysis reference methods.

Methods: Food and environmental samples were inoculated with *Listeria monocytogenes*. For each matrix and method, 20 low-level inoculated, five high-level inoculated, and five negative control samples were tested. Low inoculation levels ranged from 0.6 to 1.6 CFU per analytical unit (25 g or 25 ml). The candidate method samples were enriched at 30°C for 44 to 48 h, then tested with the lateral flow strip. The candidate method was confirmed culturally, according to the respective reference method. The reference method samples were enriched and tested according to the respective manual.

Results: A total of 140 low-level inoculated samples were tested by the candidate method and the respective cultural reference method. The alternative method gave 88 presumptive positive results and the reference method gave 63. All negative control samples were negative for *Listeria monocytogenes*. The candidate method exhibited a sensitivity of 98.9% and a specificity of 98.1%. Probability of detection analysis showed no significant differences between the test method and the reference method.

Significance: The RapidChek *Listeria monocytogenes* method provides the end user with a rapid and reliable tool for monitoring and controlling *Listeria monocytogenes* in food and in the food processing environment, ultimately minimizing the risk of *Listeria monocytogenes*-contaminated food products entering into commerce.

P3-78 Colorimetric Detection of *Cronobacter sakazakii* in Artificially Contaminated Powdered Infant Formula Using Microfluidic Paper-based Analytical Devices

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Introduction: Disease caused by the opportunistic pathogen *Cronobacter sakazakii* is rare, but can be life-threatening in infants, who often contract it via ingestion of contaminated powdered infant formula (PIF). The disease is frequently manifested with sepsis or meningitis and has an estimated mortality rate of 40%. There is a need for rapid detection methods for this pathogen in PIF, motivating efforts to develop and validate rapid diagnostics, including U.S. Food and Drug Administration official methods involving accelerated culture-based (chromogenic agar) or molecular (PCR) detection.

Purpose: µPADs (paper-based analytical devices) are inexpensive, portable, and easy-to-use microfluidic devices that would be beneficial as a rapid detection platform for *C. sakazakii* in PIF. Our purpose was to develop and optimize µPAD-based rapid diagnostics for *C. sakazakii* in PIF, relying on colorimetric detection of enzymatic activity.

Methods: µPADs were impregnated with optimized concentrations of 5-bromo-4-chloro-3-indolyl-α-D-glucopyranoside and 4-nitrophenyl α-D-glucopyranoside, substrates hydrolyzed by α-glucosidase (produced by *C. sakazakii*, but not closely related bacteria) on 5-mm-diameter wax paper spot arrays. PIF (25-g portions, *n*=30) were artificially inoculated with an average of 7.4×101, 7.4×102, 7.4×103, or 7.4×106 CFU/g of *C. sakazakii*, with PIF spiked with 5.0×104 CFU/g of a four-strain cocktail of closely related *Enterobacteriaceae* (including *Cronobacter muytjensii*) or non-spiked PIF serving as negative controls. Enrichment was performed in 225 ml buffered peptone water (24 h, 37°C) tested at 0, 6, 8, 18, and 24 h, followed by visual confirmation or ImageJ analysis.

Results: Visual confirmation of the presence of yellow or indigo color indicated α-glucosidase activity and was further quantitated using ImageJ. Both visual confirmations and change in greyscale intensity ($P<0.05$) allowed for unambiguous detection of *C. sakazakii* from spiked PIF at 7.4×101 CFU/g after 18 h and 7.4×102 CFU/g after 8 h enrichment, respectively.

Significance: We developed a portable, inexpensive, and rapid µPAD-based diagnostic for detection of *C. sakazakii*-contaminated PIF.

P3-79 Detection of *Listeria* spp. from Environmental Surfaces without Enrichment

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Introduction: A molecular test was developed for the detection of *Listeria* spp. from environmental surfaces without enrichment. A sample is collected using a swab, and the entire contents of the swab are taken through lysis steps and tested by the assay. This method is based on nicking enzyme isothermal amplification reaction technology preceded by the reverse transcription of 23s ribosomal target RNA. Amplified target sequences are detected in real time using fluorescent molecular beacon probes.

Purpose: ANSR *Listeria* Right Now (LRN) assay performance was evaluated for rapid detection of *Listeria* spp. without an enrichment step from stainless steel, plastic, ceramic tile, sealed concrete, and rubber and compared to culture-based enrichment method.

Methods: A low level of *Listeria* (along with a consortium of three background flora organisms, in some instances with residual sanitizers) were inoculated on environmental surfaces. For each surface, 20 replicate surfaces were inoculated to produce fractional positive levels, along with five high-level and five uninoculated samples. Statistical analysis was conducted on the test and reference method data using probability of detection analysis models.

Results: The internal study data and an independent study by NSF International showed, for detection of the target organism in a semi-paired analysis, LRN test is statistically as sensitive as an enrichment-based culture method. Internal studies determined the limit of detection of the test to be as low at 4 CFU, with a 95% confidence level.

Significance: No enrichment with an easy-to-use system means you can perform vectoring, re-cleaning, and *Listeria* environmental testing without concern for growing pathogens. The novel utility of the enrichment-free ANSR LRN test is that environmental sampling and testing can be completed in less than one hour, allowing personnel in food service, processing, or production settings to take immediate corrective action if *Listeria* spp. are found in the environment.

P3-80 Rapid Detection of *Salmonella* in Infant Formula and Infant Cereals Compared to ISO 6579

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Introduction: Salmonella is a pathogen of concern in infant nutrition, especially due to a recent outbreak of *Salmonella* at Lactalis in 2017. An extension for AFNOR certification (ADRIA Développement, Expert Lab) for Assurance GDS *Salmonella* Tq (GDS) was performed for detection of *Salmonella* in the food category of infant formula and infant cereals. Milk powder as an ingredient was also tested. In total, this data included 182 samples for the sensitivity study, and relative limits of detection (RLOD) on milk powder and infant formula with probiotics. Finally, inclusivity was run on 100 *Salmonella* strains enriched in brilliant green water (BGW), the primary enrichment media for milk powder.

Purpose: To evaluate the next-day detection of *Salmonella* by Assurance GDS *Salmonella* Tq method in infant formula and infant cereals as compared against the ISO reference method.

Methods: Lyophilized cultures of *Salmonella* were inoculated into foods and stabilized at room temperature for a minimum of 2 weeks. *Salmonella* were principally inoculated at less than 3 CFU/sample for the sensitivity study. *Salmonella* were diluted in bulk uninoculated foods to achieve partial recovery for RLOD studies. Samples were enriched 1:10 in media for 18 h (20 h for milk powder) and analyzed by GDS. For inclusivity, *Salmonella* were inoculated between 10 to 100 cells/225 ml and incubated at 37°C for 20 h. Milk powder was added to BGW as nutrient for inclusivity.

Results: For sensitivity study, the observed values for positive and negative deviations were successfully below the acceptability limit (AL) required per category. The RLOD for milk powder was 1.09 and infant formula was 0.88, both below the AL of 2.5. Of the 100 *Salmonella* inclusivity strains tested, 99 were detected and one strain was not detected.

Significance: Assurance GDS *Salmonella* Tq provides a reliable next-day method for detection of *Salmonella* in infant formula and infant cereals, including milk powders.

P3-81 Comparative Validation Study to Demonstrate the Equivalence of an Alternate Next-day Enrichment Protocol for VIP Gold for *Salmonella* Method to Culture Methods for the Detection of *Salmonella* in Selected Foods and Environmental Surfaces

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Introduction: Salmonella is a significant pathogen and the causative factor of many foodborne illnesses and related hospitalizations. BioControl Systems, Inc. has developed a protocol that allows next-day detection of *Salmonella* in foods and environmental surfaces using the VIP Gold for *Salmonella* (VIP) lateral flow device.

Purpose: To validate the equivalence of an alternate next-day enrichment method to detect *Salmonella* in various foods and environmental surfaces in comparison to aa reference method using a proprietary enrichment media (mEHEC, BioControl Systems).

Methods: Various 25-g food samples were seeded with low levels (1 to 5 CFU/sample) of *Salmonella* and stabilized. Various surfaces were inoculated with *Salmonella* and allowed to dry overnight. The samples were enriched using two methods. Foods with low microbial background (roast beef and deli turkey) and environmental surfaces (steel, concrete, and plastic) were incubated in mEHEC broth for 20 to 24 h at 42°C. Foods with high microbial background (raw spinach, almonds, raw pasta, and chicken rinsate) were incubated in mEHEC containing novobiocin for 20 h, then transferred to 10 ml of tryptic soy broth containing novobiocin for an additional 6 h. Both 25-g and composite (325 to 375 g) samples were run. The samples were tested using the VIP. In all cases, the reference protocol (United States Department of Agriculture Microbiology Laboratory Guidebook or U.S. Food and Drug Administration Bacteriological Analytical Method) was run for comparison. Inclusivity/exclusivity studies were also run.

Results: The comparative testing between the alternate next-day protocol for VIP method and the reference method yielded statistically equivalent recovery data for each food and environmental surface tested. The VIP method detected all 140 strains of *Salmonella*, representing all serogroups, and was negative for all 50 non-*Salmonella* tested.

Significance: Based on the results of the method comparison studies and inclusivity/exclusivity studies, the VIP Gold for *Salmonella* method is a viable next-day protocol for detection of *Salmonella* in foods. This data was also used for the certification of VIP as an AOAC Performance Tested Method.

P3-82 Performance Evaluation of a Real-time PCR for the Simultaneous Detection of *Salmonella* and STECs in Co-enriched and Wet Pooled Green Leafy Produce

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Introduction: In the United States, the production and consumption of green leafy produce has been steadily rising. The most recent attribution estimates from Centers for Disease Control and Prevention (2013) indicated leafy produce to be responsible for the highest amount of foodborne illnesses and among the top five causes of foodborne hospitalizations and deaths. Between 1973 and 2012, *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC) species were the top bacterial pathogens responsible for outbreaks caused by leafy vegetables. Most commercial foodborne pathogen detection systems tend to use separate, and often complex, workflows to provide *Salmonella* and STEC detection. Given the perishable nature of leafy produce and the recent testing trends moving towards 375-g sample size, it is critical for a test to be rapid and maintain a high specificity and sensitivity.

Purpose: The purpose of this study was to evaluate co-detection of *Salmonella* and STEC using real-time PCR GENE-UP based *Salmonella*, *E. coli* O157:H7, and enterohemorrhagic *E. coli* (EHEC1/2) assays from the same enrichment broth (buffered peptone water) for green leafy matrices (lettuce and spinach, 375 g) among non-pooled and wet-pooled (pool size, *n*=4, 5, 6) samples.

Methods: The AOAC Performance Tested Method validation study design was followed. Results for the assay were analyzed by probability of detection (POD) analysis. Each matrix was spiked with fractional and high combined-microbe spike, followed by equilibration at 4°C before testing. Wet-pooling was done postenrichment by combining equal volumes of enrichments after 10 and 22 h incubation at 42°C. All results were culture confirmed (U.S. Food and Drug Administration Bacteriological Analytical Manual).

Results: No statistically significant differences were observed between *Salmonella*, *E. coli,* EHEC1/2, and the reference methods (dPOD: 0.0 to 0.80 with 95% confidence interval). Testing the wet-pooled samples provided accurate detection, even with the worst case scenario (1 positive pooled with *n*=3, 4, or 5 negative).

Significance: These data demonstrate that GENE-UP can be used for co-detection of *Salmonella, E. coli* O157:H7, and non-O157 STECs using a common workflow, thereby reducing the time and labor needed.

P3-83 Performance Evaluation of a Real-time PCR for the Detection of *Cronobacter* spp. in Powdered Infant Formula

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Introduction: The ubiquitous nature *Cronobacter* spp., as well as its resistance adaptive attributes like desiccation and osmotic stress resistance, make it a persistent cause of concern for powdered infant formula (PIF) manufacturing facilities. Therefore, despite all the mitigation steps employed at these facilities, testing the final product remains an effective strategy to detect *Cronobacter*-contaminated PIF. The GENE-UP *Cronobacter* spp. assay (CRO) is a real-time PCR assay that utilizes fluorescence resonance energy transfer (FRET) hybridization chemistry to provide highly sensitive multi-target detection in the same reaction vessel from a crude DNA preparation.

Purpose: The purpose of this study was to comparatively evaluate the performance of CRO for the detection of *Cronobacter sakazakii* in 25 g of PIF.

Methods: The AOAC Performance Tested Method validation study design was used. Overall, 30 unpaired samples were tested, five replicates were inoculated at a high inoculation level, 20 were inoculated at a low inoculation level, and they were all evaluated along with five uninoculated control replicates. After sample enrichment in buffered peptone water, test portions were evaluated by both CRO and the reference method (ISO 22964:2017). All CRO presumptive results were confirmed using the procedures outlined in the ISO 22964:2017 and bioMérieux's alternative confirmation procedure. Probability of detection analysis with a 95% confidence interval was performed for statistical evaluation of the results.

Results: CRO demonstrated no significant differences from the reference method. Results were ($dPOD_{cp}$: 0.0; LCL:-0.43; UCL: 0.43) for high and uninoculated, and ($dPOD_{cp}$: 0.05; LCL: -0.23; UCL: 0.32) for low samples.

Significance: These data support the high sensitivity and use of CRO for the detection of *Cronobacter* spp. in 25 g of PIF. Combining this with a convenient workflow, including simple sample preparation, user-friendly assay format, instrumentation and software interface, makes CRO a viable alternative for *Cronobacter* spp. detection in PIF.

P3-84 Key Role of Enrichment Broth for the Detection of Sublethally Injured *Listeria* in Environmental Samples

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Introduction: Nowadays, an effective environmental monitoring program is becoming an increasingly relevant proactive food safety strategy for controlling *Listeria monocytogenes* in food processing facilities. The efficacy of using various swabbing devices, as well as the importance of transport and storage conditions for reliability of environmental testing, have been clearly demonstrated in numerous reports, while the contribution of enrichment broth to the detection of *Listeria* in environmental samples remains underestimated.

Purpose: This study evaluated the impact of selective enrichment broths on the recovery and growth of sublethally stressed *Listeria* in environmental samples to be then detected by different rapid methods.

Methods: Efficacy of Actero Listeria Enrichment Media in promoting the repair and enrichment of sublethally injured *Listeria* was compared with that of different primary enrichment and single-step enrichment media. Lag phase duration and growth rate values of bacterial populations were evaluated by using PRECOG software. An overall analysis of the matrix study data was completed using the results generated through multiple validation studies performed during 2012 to 2015.

Results: Culturing *L. monocytogenes* alone or in the presence of a high number of competing bacteria in the proprietary broth resulted in the shortest lag phase and the highest growth rate in comparison to other broths tested. Effectively controlling a broad spectre of non-target bacteria, the proprietary broth showed strong capacity to promote the growth of fastidious species such as *Listeria ivanovii* and *Listeria grayi.* Overall, the results obtained using 880 environmental samples collected from artificially contaminated stainless steel, plastic, ceramic, sealed concrete, and rubber had indicated a significantly better recovery (28.6% increase, $P<0.05$) of *Listeria* injured by desiccation using the proprietary broth, rather than University of Vermont medium.

Significance: Actero Listeria has a strong ability to provide an ideal growing environment for sublethally injured *Listeria* in environmental samples to enable accelerated, reliable, and faster detection.

P3-85 Analyzing Food Integrity Using Paramagnetic Particles

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Introduction: Consumer protection and preventing fraudulent or deceptive practices such as food adulteration are important and challenging issues facing the food industry.

Purpose: We report on the utility of paramagnetic particle chemistry to purify amplifiable DNA used in PCR-based testing for genetically modified organism (GMO) sequences and ingredient authentication. We tested a variety of plant and food samples for GMO and ground beef and pork sausage for species authentication using the Maxwell RSC PureFood GMO and Authentication Kit.

Methods: We analyzed the DNA from the food samples using Maxwell RSC PureFood GMO and Authentication Kit, the DNeasy mericon, and r-Biopharm SureFood for yield, purity and amplifiability. DNA was extracted from 2-g samples. DNA eluates were amplified using the TaqMan GMO Maize 35S detection kit to identify the percent GMO in the samples. For authentication testing, ground beef was spiked with pork sausage. DNA was extracted from 2-g samples, and DNA eluates were amplified using the RapidFinder Pork ID kit to identify swine DNA.

Results: All DNA purified from the food samples using the three different kits were amplifiable using the GoTaq quantitative PCR system with either meat or plant universal primers and showed no PCR inhibition. For GMO testing, both isolation methods extracted DNA from the 2-g maize samples. The 35S GMO event was detected down to 0.01% in the PureFood samples and down to 0.1% in the DNeasy mericon samples. DNA was amplifiable with the swine-specific kit from all samples except the 100% beef sample. Based on the Ct cut-off from positive control, swine DNA was identified down to 0.01% in the pork sample.

Significance: These studies together demonstrate the utility of the Maxwell RSC for automated purification of food DNA upstream of amplification-based GMO and authentication testing.

P3-86 Validation of a Lateral Flow Device for the Detection of Ricin in Foods

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Introduction: Ricin is a potent cytotoxin commonly found in the castor bean plant, *Ricinus communis.* There is no known antidote or antitoxin currently available for treatment of ricin toxicity. Due to the commonality of the plant, its ease of dissemination, and historical nefarious use, it is imperative to validate a reliable screening method for the detection of ricin toxin in foods for biodefense purposes.

Purpose: This study's objective was to provide a lateral flow screening method, combined with a single optimized extraction for preliminary and confirmatory detection of ricin toxin in foods.

Methods: Matrix limit of detection and validation studies were conducted in hot dogs, ground beef, liquid eggs, cooked sausage, ham, and dried meat by six Food Emergency Response Network (FERN) laboratories. Samples were fortified with ricin toxin at levels of 0, 2.5, and 5 µg/g and cold stressed overnight. Six brands of each matrix were analyzed at each fortification. Phosphate buffered saline containing Tween and nonfat dry milk was added in a 1:5 preparation, followed by a period of inversion, rest, and centrifugation. A 150-µl aliquot of the liquid eluate was removed and analyzed using commercially available lateral flow devices (LFD) and read at both 15- and 25-minute intervals. A 100-µl aliquot was removed and analyzed using a commercially available enzyme-linked immunosorbent assay for result confirmation.

Results: The limit of detection exhibited some degree of variance (100 ng/g to 2.5 µg/g) between each matrix type and brand evaluated. Each matrix analyzed demonstrated 100% (12 of 12) sensitivity at both low and high fortifications of ricin and 100% (6 of 6) specificity.

Significance: The data from this study suggest that the extraction procedure, combined with the LFD detection assay, may offer a suitable screening method for the detection of ricin in foods. This study has been submitted for review as an official FERN screening method and offers a single extraction for both the preliminary and confirmatory tests.

Poster

P3-87 Inactivation of *Salmonella* on Fresh Produce with a Water-assisted Ultraviolet System in Combination with Chlorine and Peroxyacetic Acid

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❖ ***Developing Scientist Competitor***

Introduction: Fresh produce has been linked to numerous outbreaks of illness caused by microbial pathogens. Recently, we developed a small scaled water-assisted ultraviolet (WUV) system, which used agitated water to wash fresh produce during UV treatment, and demonstrated its potential for fresh produce decontamination.

Purpose: A large scale WUV system was built. The ability of WUV alone and in combination with chlorine or peroxyacetic acid (PAA) to decontaminate fresh produce and prevent cross contamination through wash water was evaluated using wash water with extremely high organic load and turbidity.

Methods: Fresh produce (baby spinach, iceberg lettuce, grape tomato, blueberry, and baby-cut carrot) were dip-inoculated in *Salmonella* cocktail to final levels of 4.5 to 7.5 log CFU/g. They were then treated by WUV (~25 mW/cm^2) alone and WUV in combination with chlorine (10 ppm free chlorine) or PAA (80 ppm) using turbid wash water (turbidity>60 NTU; chemical oxygen demand~1800 ppm).

Results: The combination of PAA and WUV had higher *Salmonella* reductions on all fresh produce items than chlorine or PAA wash and for lettuce, the difference in *Salmonella* reduction between the combined WUV and PAA treatment and chlorine wash was significant (P<0.05). The combined treatments, WUV with PAA or chlorine, were able to keep residual *Salmonella* in wash water below the detection limit (2 CFU/ml) for almost all the replicates completed and were in general better than PAA alone. *Salmonella* inactivation effect by single and combined WUV treatments was correlated with surface structures of produce. Single WUV treatment achieved reductions of 1.29, 1.73, 3.47, 1.57, and 2.16 log for spinach, lettuce, tomato, blueberry, and carrot, respectively.

Significance: The combined WUV and PAA treatment could be a better alternative to chlorine or PAA wash for fresh produce decontamination.

P3-88 Detection of Viable but Non-culturable State of Enteric Bacterial Pathogens in Fresh Produce

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❖ ***Developing Scientist Competitor***

Introduction: Viable but non-culturable (VBNC) cells are a state of cells that maintain a low metabolic and respiratory rate but fail to grow on laboratory media. These cells have been shown to continuously produce toxins and maintain their virulence following resuscitation. The risks implied by VBNC pathogen cells are usually underestimated because they cannot be detected by conventional plating assays.

Purpose: This study is to develop a novel isothermal technique using loop-mediated isothermal amplification (LAMP) coupled with intercalating dye to detect VBNC and viable cells on fresh produce in a rapid and cost-effective way.

Methods: Primers for LAMP were designed to target *agfA, wzy*, and *wzx* genes of *Salmonella* spp. and Shiga toxin-producing *Escherichia coli* (STEC). The assay was incorporated with the intercalating dye of propidium monoazide (PMA) to exclude false positive results because it can preferentially bind to dead cell DNA, which makes it unable to be amplified. The conditions for PMA-LAMP were optimized and PMA quantitative PCR (qPCR) assay was performed as the parallel test. The VBNC cell counts were calculated by using the viable cell counts determined by LAMP or qPCR assay, minus the culturable cell counts determined by the plate assay.

Results: Our developed assay was 100% specific to the targeted microorganisms, as no false negative or positive results were observed by using PMA-LAMP assay. The detection limit of the PMA-LAMP assay was comparable to PMA-qPCR assay ranging from 10^3 to 10^4 CFU/ml in pure culture and 10^4 to 10^5 CFU/g for spiked lettuce samples. In general, PMA-LAMP and PMA-qPCR can achieve a good sensitivity and linearity to detect viable and VBNC cells in fresh produce.

Significance: The research will contribute to the development of a rapid and sensitive PMA-LAMP method which will have the ability to detect pathogens that evade detection by methods currently used by the majority of quality control laboratories.

P3-89 Using Whole Genome Sequencing for Detection of *Bacillus cereus* Toxin Genes in Food

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Introduction: Bacillus cereus is among the top 10 pathogens associated with foodborne illness in the United States; it causes diarrheal disease (ingestion and production of hemolysin BL [Hbl], nonhemolytic enterotoxin [Nhe], or cytotoxin [CytK] in the gut) or emetic disease (ingestion of preformed cereulide [Ces]). Currently, toxin detection is only available for one component of each tri-part toxin (HblC and NheB) or by mass spectrometry (Ces).

Purpose: Presently, there is no primary screening method for detection of toxin genes before detection using a commercialized kit or mass spectrometry method. Development of a genomic sequencing method and pipeline for initial screening of toxin-producing genes would provide fast primary detection for potential toxins and other virulence factors.

Methods: A sensitive whole genome sequencing method and analysis pipeline using BTyper, a computational tool that classifies and characterizes virulence potential of suspected strains, was developed. DNA was extracted and sequenced from *B. cereus* culture or spiked food (rice, gravy, whey powder, pancake mix, and infant formula), and BTyper was used to analyze sequence data.

Results: Our results show that BTyper can be used to detect toxin-producing strains of *B. cereus* in culture and in six different food types. BTyper analysis confirmed a toxin profile of 44 out of 50 inclusivity strains and 30 out of 30 exclusivity strains previously studied by PCR and examined for toxin production by commercialized protein assays.

Significance: Detection of *B. cereus* toxins is paramount to ensuring food safety; however, toxin detection from food is laborious and time-consuming, and some toxins are not directly detectable from food. Development of this method for detection of toxin genes is a powerful primary screening method and allows for the acquisition of more in-depth data about the suspected strain.

Poster

P3-90 Comparison between Real-time PCR and Enzyme-linked Immunosorbent Assay for the Detection and Quantitation of Crustacean Allergens

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Introduction: In 2004, the United States Congress passed the Food Allergen Labeling and Consumer Protection Act (FALCPA), requiring that foods containing any of the eight major food allergens be properly and accurately labeled. Furthermore, it states that crustaceans must be labeled by specific type. Current allergen detection methods include enzyme-linked immunosorbent assays (ELISA), which detect allergen proteins, and PCR-based methods, which detect the allergen DNA. While ELISA methods can be used to detect crustacean allergen protein, PCR-based detection methods are required to both detect and differentiate between crustacean types.

Purpose: This work aims to compare commercial ELISA kits and PCR-based assays for the detection and quantitation of crustacean allergens with respect to performance in complex food matrices.

Methods: Each type of crustacean tissue (shrimp, crab, and lobster) was spiked into fish sauce and Manhattan clam chowder separately in the range of 125 to 4,000 ppm. The spikes were split into two aliquots: one analyzed by a commercial ELISA kit according to manufacturer's instructions and the other by previously validated quantitative PCR (qPCR) assays.

Results: Crustacean allergen was detected in every spike for both the ELISA kit and the qPCR assay. Quantitation values for the qPCR assays were in the range of 14 to 10,105 ppm (weight/weight). Quantitation values for the ELISA kits ranged from 45 to 68,540 ng/ml of total crustacean protein. The results were, however, consistent between spiking experiments.

Significance: The enforcement of FALCPA requires highly sensitive detection methods. This work shows that these two methods can detect and quantitate crustacean allergens at low concentrations and that the qPCR method can serve as a confirmatory method, as well as identifying crustacean type, following positive ELISA kit results.

P3-91 Relative Effectiveness of Lactose Broth and Selected Buffered Preenrichment Media for the Detection of *Salmonella* in Artificially Contaminated Whole Almonds and Creamy Peanut Butter

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Introduction: Buffered preenrichments are more effective than lactose broth (LB) for the detection of *Salmonella* in high microbial foods (P<0.05). LB is the default preenrichment medium for foods, unless otherwise specified, in the U.S. Food and Drug Administration's Bacteriological Analytical Manual (BAM) *Salmonella* culture method. It is unknown if LB is also less effective than buffered media for analysis of low-microbial/low-moisture foods (LMF).

Purpose: To determine the relative effectiveness of LB default preenrichment medium and three buffered preenrichment media (universal preenrichment broth [UPB], buffered peptone water [BPW], and modified buffered peptone water [mBPW]) to detect *Salmonella* in artificially contaminated LMF (P<0.05).

Methods: Twenty artificially contaminated test portions were analyzed for each preenrichment medium evaluated in experimental trials of peanut butter and whole almonds. Statistical differences among the preenrichments were determined with chi-square two-tailed F-test (P<0.05).

Results: For peanut butter, the recovery of *Salmonella* Mbandaka with LB was statistically equivalent (P>0.05) to the buffered preenrichments: LB (seven positive test portions), UPB (eight positives), BPW (10 positives), and mBPW (11 positives). For almonds, the recovery of *Salmonella* Enteritidis with LB was statistically equivalent (P>0.05) to the buffered preenrichments: LB (14 positives), UPB (16 positives), BPW (16 positives), and mBPW (12 positives).

Significance: LB preenrichments are as effective as buffered preenrichments for analysis of creamy peanut butter and whole almonds with the BAM *Salmonella* culture method, so any of these buffered enrichments can be used for analysis of creamy peanut butter and whole almonds.

P3-92 A Comparison of Two Chromogenic Agars for *Vibrio* Growth

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Introduction: Chromogenic agar is used to differentiate pathogens isolated from food and environmental samples. *Vibrio vulnificus* and *Vibrio parahaemolyticus*, the leading bacterial causes of mortality and morbidity, respectively, among shellfish consumers in the United States can be isolated and differentiated from other commonly occurring vibrios on chromogenic agars.

Purpose: This study compares two chromogenic agars, formulated to differentiate *V. vulnificus* and *V. parahaemolyticus* from one another and other vibrios, used for the isolation of *V. vulnificus* and *V. parahaemolyticus*.

Methods: Six species of vibrio were used in this study: *V. vulnificus* (10)*, V. parahaemolyticus* (20)*, V. alginolyticus* (3)*, V. fluvialis* (6)*, V. mimicus* (5), and *V. cholerae* (9). All strains were grown overnight in tryptic soy broth (TSB) at 35°C at 200 rpm. Cultures were diluted into fresh TSB and incubated at 35°C at 200 rpm until an optical density at 600 nM was between 3.50 and 6.50. After serial dilution, the cultures were spread plated (100 µL) onto triplicate plates of tryptic soy agar (TSA) and both chromogenic agars and incubated 18 to 24 h at 35°C. Colony counts to determine recovery from the two chromogenic agars were compared to TSA, a non-inhibitory agar.

Results: The mean recovery for all vibrios on TSA and the chromogenic agars was 8.59, 8.39, and 8.52 log CFU/m, respectively. The mean recovery for *V. vulnificus* on TSA and the chromogenic agars was 8.85, 8.50, and 8.69 log CFU/mL, respectively. The mean recovery for *V. parahaemolyticus* on TSA and the two chromogenic agars was 8.43, 8.38, and 8.37 log CFU/mL, respectively. No statistically significant difference (P<0.05; analysis of variance) between the chromogenic agars nor between those agars and TSA was observed.

Significance: The chromogenic agars did not significantly inhibit growth. Added to their ability to differentiate growth among species, these agars may prove to be a beneficial media for the isolation of *V. vulnificus* and *V. parahaemolyticus* from food and environmental samples.

P3-93 A Label-free Quartz Crystal Microbalance Sensor for Rapid Detection of Avian Influenza Virus Based on Polydopamine Surface-imprinted Recognition Polymer

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Introduction: Avian influenza is a significant animal and human health problem worldwide. The avian influenza virus (AIV) can survive on contaminated raw poultry meat and can be passed around through contaminated food products. The development of a rapid, specific, and sensitive method to detect AIV is needed to help control its spread.

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Purpose: The objective of the present study was to develop a quartz crystal microbalance (QCM) biosensor for rapid detection of AIV in poultry using an improved recognition element: polydopamine surface imprinted recognition polymer (PDA-SIRP).

Methods: In a typical PDA-SIRP film synthesis, a solution of dopamine (3.33 mg/ml) and AIV (25.6 HAU [hemagglutinin Unit]) were mixed in Tris-HCl (pH=8.0). Then, a QCM crystal was immersed in the solution overnight to allow the self-polymerization of dopamine and AIV on the gold surface of the crystal. Finally, the virus template was removed by acetic acid (5%) and sodium dodecyl sulfate (10%). The developed PDA-SIRP could selectively recognize and capture target virus, resulting in a decrease of the resonant frequency (Hz), which was recorded in real-time by QCM.

Results: The selective recognizing and rebinding of target virus was observed as a frequency decrease quantified by the QCM sensor. The QCM result showed a good linear relationship in the target virus range between 2^0 and 2^5 HAU, with a correlation coefficient of 0.98. The total detection time from sampling to final results was less than one hour. When compared to the QCM immunosensor for detection of AIV, the developed PDA-SIRP-based QCM biosensor not only extended life time, but also enhanced the detection sensitivity.

Significance: The outcome of this study will provide a simple and inexpensive method to fabricate the PDA-SIRP, which can be an alternative to recognition biomolecules, such as antibodies or enzymes, for use in harsh environments and in-field applications.

P3-94 Application of Surface Plasmon Resonance Biosensor for Detection of *Salmonella* Typhimurium in Leafy Vegetables

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❖ ***Developing Scientist Competitor***

Introduction: *Salmonella*-contaminated leafy vegetables are responsible for 17 outbreaks, 762 illnesses, and 26 hospitalizations every year in United States. Although different methods are available to detect *Salmonella* in foods in a timely manner, surface plasmon resonance (SPR) has the benefit of real-time, label-free, and rapid detection with higher sensitivity and specificity.

Purpose: The purpose of this study was to develop a SPR method in conjugation with immunomagnetic separation (IMS) for detection of low levels of *Salmonella* in leafy vegetables.

Methods: Samples of romaine lettuce were inoculated with *Salmonella* Typhimurium ATCC-13311 at low levels (10^1 to 10^2 CFU/g) and incubated overnight in buffered peptone water at 37°C. The numbers of naturally occurring bacteria and the inoculated *Salmonella* in the samples before and after incubation were determined by aerobic plate count and XLT-4 agars. Flagellin antigen was captured by the antibody immobilized on magnetic beads and then eluted by glycine-HCl, pH 3.0. Flagellin antigen was neutralized and injected onto the SPR sensor surface immobilized with a flagellin-specific monoclonal antibody. SPR signals resulting from the binding of flagellin on the sensor surface were observed and quantified.

Results: The IMS-isolated samples yielded an average detection signal of 22.9±5.5 uRIU, whereas the average detection signal for non-isolated samples was 4.1±0.4 uRIU. The biosensor detection sensitivity was 2.4×10^5 CFU/ml after enrichment. The lowest detection limit in inoculated samples before enrichment was 80 CFU/g. Four other bacteria (*Enterobacter cloacae*, *Pseudomonas fluorescens*, *Serratia* spp., and *Aeromonas salmonicida*) were isolated from the vegetable samples after enrichment, but none of them were detected by the SPR when analyzed individually.

Significance: Our results suggest that SPR can be used for faster detection of *Salmonella* Typhimurium in leafy vegetables with higher specificity and sensitivity. Further studies will be conducted to improve this developed method for detection of other serotypes.

P3-95 Detection of RNase Treated and Untreated Enteric Viruses in Shellfish Concentrates

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Introduction: Norovirus (NoV) is the leading cause of foodborne illness, and hepatitis A virus (HAV) is prevalent. Viral propagation determines intact, live virus concentration from contaminated food products, while reverse transcription quantitative PCR (RT-qPCR) detects all RNA, making it difficult to differentiate intact viral particles from those with damaged capsids or free RNA.

Purpose: The objective of this study was to use ribonucleases (RNases) for the removal of free RNA from viral inoculates to ensure the detection of intact virus particles in shellfish.

Methods: Viral spikes of NoV (0.3 to 300 genomic copies/g), HAV (30 to 3,000 genomic copies/g), and murine norovirus (MNV) (1,000 to 10,000 genomic copies/g) were treated with RNase-A (RnA, 17.5 U/µL) and RNase-H (RnH, 2 U/µL). Treated spikes were inactivated using Superasin prior to inoculation. Concentration, extraction, and detection were performed using ultracentrifugation, Qiagen RNeasy Mini kit, and RT-qPCR assays, respectively.

Results: Following treatment of inoculates prior to spiking shellfish, a 1-log reduction in the initial titer between untreated (Unt) MNV and RnA MNV was determined. There was no difference in Unt MNV and RnH MNV. There was no difference between HAV treatments. A 2-log reduction between Unt and RnA/RnH was determined in NoV GI. A 1-log reduction between Unt and RnA was determined in NoV GII. A 2-log reduction between Unt and RnH was determined in NoV GII. After RT-qPCR of the shellfish extracts, there were no significant differences in detection among all the treatments: MNV (3.63±0.64), HAV (1.64±0.48), GI (0.37±0.68), and GII (2.46±0.48).

Significance: Based on RT-qPCR results, while treating MNV, NoV, and HAV inoculates with RNase A or H prior to spiking shellfish yielded an initial reduction in RNA, there were no significant changes in the amount of RNA recovered. This study demonstrated that this shellfish protocol likely yields intact virus with the minimal presence of damaged viral particles and free RNA.

P3-96 Lytic, Tailed *Bacillus cereus*-specific Phage Suggests Its Novel Employment in a Ferromagnetoelastic Biosensor as Biorecognition Element

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❖ ***Developing Scientist Competitor***

Introduction: Bacillus cereus is one of the major food-poisoning pathogensm specifically because of its ubiquitous existence and ability to form enterotoxins and heat-resistant endospores. Our research group has constructed an on-site applicable ferromagnetoelastic (FME) biosensor for *Salmonella* detection by using a lysogenic, filamentous E2 phage. The employment of lytic, tailed *B. cereus*-specific phage was challenging due to its influence on the sensitivity and reliability of the FME biosensor.

Purpose: The purpose of this study was to investigate the optimum immobilization method of tailed *B. cereus*-specific phage and the effects of incubation time and temperature on the lytic property of the *B. cereus*-specific phage prior to its use on the FME biosensor.

Methods: B. cereus-specific phage was immobilized at various concentrations through direct adsorption or a combination of 11-MUA and EDC/NHS. The effects of time and temperature on the lytic properties of the phage were investigated through the exposure of *B. cereus* to *B. cereus*-specific phage (multiplicity of infection=0.0001 and 1) for various incubation times (0, 15, 30, 45, and 60 min) at 22°C and at various temperatures (4, 15, 22, and 37°C) for 30 and 60 min.

Results: Phage density on the FME sensor increased and corresponded with phage concentration for both immobilization methods. The density of phages immobilized with the combination of 11-MUA and EDC/NHS was significantly greater than those immobilized with the direct adsorption method at every concentration ($P<0.05$). There were no significant differences observed in the survival rate of *B. cereus* within 30 min, which was in contrast to the significant decreases observed at 45 and 60 min ($P<0.05$). On the contrary, temperature exerted no significant effects on the survival rate across the entire temperature range.

Significance: This study investigated the feasibility of the lytic, tailed *B. cereus*-specific phage for use in a FME biosensor as a novel recognition element.

P3-97 Identification of Foodborne Pathogens in Shellfish Samples Using a New Generation Microarray Assay

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Introduction: There is a paucity of methods for the rapid and simultaneous detection of multiple pathogens in food and water. We posited that our new generation custom DNA microarray designed for broad coverage of microbial pathogens may offer an approach for analytical investigation of samples containing multiple enteric microbes/viruses.

Purpose: Shellfish are well-known bioaccumulators of diverse microbes and are among the food products that most often cause viral illnesses. We investigated whether our new generation custom DNA microarray could detect multiple pathogens contained within a single sample.

Methods: The Affymetrix microarray was designed with the perfect match/mismatch probe sets for individual genes of common foodborne viruses, their surrogates, bacteria, phages, and parasites. Viral RNA from hepatitis A virus (HAV) HM175/18f infected-culture supernatants, *in vitro* transcripts from norovirus (NoV) MD145, and *Cyclospora cayetanensis* DNA from purified oocytes were used for evaluation of array performance. To provide experimental material, oysters were placed close to an untreated wastewater outfall for 2 to 4 weeks. RNA was extracted from shellfish digestive glands according to ISO 15216-1:2017. To increase sensitivity of multi-pathogen detection in shellfish samples, complementary DNA derived from shellfish RNA was amplified using a commercial kit. Microarray hybridization was performed following the GeneAtlas Manual.

Results: Specific identification was achieved for NoV/MD145, HAV/18f, and *C. cayetanensis*, suggesting the array performs well as a detection tool for tested organisms. Data analysis using the MAS5 algorithm revealed the presence of multiple pathogens such as NoV, HAV, coxsackievirus, rotavirus, hepatitis E virus, Aichivirus, enterovirus, sapovirus, and *Escherichia coli* within shellfish samples. Quantitative reverse transcription PCR and next-generation sequencing were used in selected samples for confirmation of array-based identification.

Significance: We demonstrated the application of microarray toward multi-pathogen identification within naturally contaminated food samples. We will continue the research of this technology with the aim of future application in U.S. Food and Drug Administration surveillance and outbreaks.

P3-98 Advanced Mapping of Pesticides on Biological Samples Using Surface-enhanced Raman Spectroscopy

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❖ ***Developing Scientist Competitor***

Introduction: Pesticides are widely applied to prevent loss of agricultural production, but extensive exposure can cause health problems. The determination of pesticide residues on crops, as well as dermal exposure to pesticides, is important in order to evaluate the health risks of pesticides. The development of a simple and effective analytical method for detection of pesticides on biological samples is necessary; surface-enhanced Raman spectroscopy (SERS) is a powerful analytical tool that enables direct identification of analytes in close proximity to plasmonic nanostructures.

Purpose: The purpose of this study was to develop an advanced SERS technique for mapping pesticides on biological samples, including tomato leaves and mouse skin, as well as to evaluate the transmission of pesticides from tomato fruits to mouse skin.

Methods: Advanced SERS mapping techniques were developed by first fabrication of an effective SERS substrate (gold mirror). The gold mirror was prepared with commercially available citrate-coated gold nanoparticles (250 mg/L) via a water-solvent interface assembly with 100 µL mediating solvents (acetonitrile:hexane, 1:1). After adding the gold mirror on the positions where pesticides were presented on biological samples, SERS mapping images can be easily obtained with a confocal Raman instrument.

Results: The gold mirror exhibited higher signal reproducibility and sensitivity for SERS mapping various concentrations of ferbam pesticide (100, 10, and 1 mg/L) on biological samples compared with commercial gold nanoparticles. After ferbam (20 µL, 10 mg/L) transmission from tomato fruits to mouse skin for 5 sec, SERS mapping results showed the amount of ferbam on the surface of the tomatoes decreased, while the amount of ferbam on the surface of the mouse skin increased.

Significance: This study provides an advanced SERS mapping approach for effectively imaging pesticides with an innovative gold mirror. The information obtained is significant to evaluate health risks of pesticide exposure on biological samples.

P3-99 Amplifying Weak Surface Enhanced Raman Scattering of Organochlorine Pesticides through a Facile Rolling Approach

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❖ ***Developing Scientist Competitor***

Introduction: Surface-enhanced Raman scattering (SERS) has been developed as a robust pesticides analytical tool because it is superior to other techniques in terms of sensitivity, selectivity, speed, reproducibility, portability, and cost. Meanwhile, SERS has not been adopted in the detection of organochloride pesticides due to their extremely weak SERS sensitivity.

Purpose: Herein, a pretreatment method (rolling) is developed to amplify the SERS signal of chlordane (an organochloride pesticide).

Methods: A sample of chlordane (2.5 µl, 0.01 to 10 ppm) was initially mixed with same volume of gold nanoparticles, then was pipetted onto Parafilm for 30 sec until approximately half volume of mixture was evaporated. Subsequently, another 2.5 µl chlordane stock was added to the mixture and then

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pipetted for 30 sec to continuously saturate the gold nanoparticles. The process will be repeated for 15 times and then transferred and dried on the gold slides; the time of repeating will be determined based on the appearance of observable chlordane signature SERS spectra.

Results: The limit of detection (LOD) of chlordane was only 10 ppm without any pretreatment. However, with the rolling step, 1 ppm (10-fold enhancement) chlordane showed signature spectra after six rolling repetitions and signals of chlordane at the concentration of 0.1 ppm (100-fold enhancement) appeared with 13 rolling repetitions. The limit of detection was even increased to 0.01 ppm (1,000-fold enhancement) after additional rolling repetitions. The results suggested that rolling times shared a positive correlation with both SERS intensity and the LOD extension, which is hypothetically due to the continuous loading of chlordane to gold nanoparticles until saturation.

Significance: The rolling method provides a very simple but innovative approach to amplify the signal of organochloride pesticides with weak SERS sensitivity.

P3-100 Simultaneous Detection of Major Food Allergens Using Fluorescent Multiplex Array

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Introduction: Quantification of food allergens is increasingly important for dose assessments of food preparations used in oral immunotherapy (OIT), food allergy prevention, and monitoring safety in the food industry.

Purpose: Our aim was to develop and validate a multiplex immunoassay capable of simultaneously measuring major food allergens from peanut, cow's milk, shellfish, egg, cashew, hazelnut, and soy.

Methods: The multiplex array was developed on the Luminex xMAP system. Microspheres coupled to specific monoclonal antibodies were used for allergen capture. Biotinylated specific monoclonal or polyclonal antibodies were used for detection. Reference standards were formulated from natural or recombinant allergens, with purity established by mass spectrometry. A full method validation was performed to determine parameters of linearity, range, limits of quantification and detection, accuracy, and precision of the multiplex food immunoassay.

Results: Full method validations were completed for peanut (Ara h 3, Ara h 6), cow's milk (Bos d 5) and shrimp tropomyosin, with egg (Gal d 2), cow's milk (Bos d 8, casein) cashew (Ana o 3), hazelnut (Cor a 9), and soy (Gly m 5) in final development stages. The standard curves for all analytes allow for quantification over a large dynamic range. The lower limits of detection (LLOD) were as low as 0.02 and 0.06ng/ml. Intra- and interassay accuracy and precision results for three samples assayed in triplicate on four occasions passed acceptance criteria within the range of 70 to 130% recovery and a coefficient of variation of ≤15%.

Significance: A quantitative, accurate, and precise multiplex immunoassay was validated for the simultaneous detection of major food allergens. The multiplex array provides a sensitive and efficient tool for measuring specific food allergens, as opposed to generic food source proteins, with potential application for risk assessment in the food industry.

P3-101 Comparing Quantitative MPN and PCR *Vibrio parahaemolyticus* Methods in Oyster Samples: A Six-year Study

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Introduction: Since 2006, three recorded *Vibrio parahaemolyticus* outbreaks across 13 states have resulted in approximately 284 reported cases of illness. Because *V. parahaemolyticus* is a major foodborne pathogen, it is imperative that laboratories nationwide are able to detect and quantify this pathogen, especially in seafood samples.

Purpose: This study quantitatively analyzes variability and compares performance for *V. parahaemolyticus* enumeration methods in oyster samples based on U.S. Food and Drug Administration (FDA) proficiency tests (PT) spanning from 2012 to 2017.

Methods: Eight blind replicate samples consisting of puréed, canned oysters were inoculated with a cocktail of *Vibrio* species annually for the FDA Shellfish PT. Inoculation procedures, mixing methods, and homogeneity/stability testing were carried out per ISO 17043 and 13528. Laboratories participating in the PT typically test samples for *V. parahaemolyticus* using most probable number (MPN) or PCR methods. Statistical analyses were performed using PT results from more than 600 sets of results for *V. parahaemolyticus* quantification. These analyses using Shellfish PT data from 2012 to 2017 included calculating mean bias and matrix standard deviation to compare MPN and PCR methods. To consider these methods equivalent, the absolute value of mean bias and matrix standard deviation must be less than 0.1 (log10).

Results: Mean bias of PCR was 0.069 (log10) based on the analyses of 1,280 data points, which indicates there are no large deviations within the population of samples analyzed. Matrix standard deviation was 0.104 (log10) and shows some risk for sample-specific bias. These methods could be considered equivalent with a relaxed threshold for matrix standard deviation.

Significance: This study shows how large-scale PT studies in food matrices of concern can be used to generate data that can be used to evaluate method performance over extended periods of time. The results highlight that microbiological methods, even those used in regulatory labs, have unique considerations and should be periodically evaluated.

P3-102 Heat-Killing *Vibrio parahaemolyticus* Improves Its Immunoreactivity with a Commercial Antibody

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❖ ***Developing Scientist Competitor***

Introduction: Vibrio spp. are ubiquitous in marine and estuarine environments. *Vibrio parahaemolyticus* is an important indicator organism for seafood quality. Thus detection of contaminating *V. parahaemolyticus* is valuable for seafood producers and public health agencies to prevent foodborne illnesses.

Purpose: The objective of this study is to optimize the immunologic reactivity of a polycolonal antibody against *V. parahaemolyticus.*

Methods: V. parahaemolyticus culture samples were incubated with a commercially available *Vibrio* antibody that was adhered to 3-µm polystyrene microspheres. The samples were then evaluated to detect cell-microsphere aggregates. Aggregate formation was compared between heat-treated and non-heat-treated samples. Common foodborne pathogens were used to test the specificity of the assay.

Results: The aggregate formation was significantly improved when using the heat-treated samples. Microscopy suggests that static incubation facilitates the formation of larger and more stable aggregates. With the optimized protocol, 20 of 21 heat-treated *Vibrio* strains formed aggregates during 20-h test period. The assay was able to exclude 14 of 15 non-*Vibrio* strains. Minor cross-reactivity was observed between other *Vibrio* species.

Significance: This study demonstrates the sensitivity of the immunoassay was significantly improved by heat treatment of *Vibrio* cell cultures.

P3-103 Specific Detection of *Listeria monocytogenes* at a Concentration of 10 Cells in 100 ml of Leafy Green Environmental Swab Eluate without Incubation

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Introduction: Due to intensely short shelf lives and an increasing consumer demand for food testing, the food processing industry is in great need of sensitive same-shift methods capable of pathogen detection in complex food matrixes. Our platform sensors utilize a field effect enzymatic biosensor (FEED sensor) to rapidly and specifically detect pathogens. There were 196 recalls associated with *Listeria* in 2016 alone.

Purpose: The purpose of this study was to demonstrate the sensitivity and specificity of a rapid assay capable of *L. monocytogenes* detection from leafy greens environmental swab eluate in under two hours.

Methods: FEED sensors functionalized with anti-*Listeria*-tag antibodies were used to detect the presence of *L. monocytogenes* (ATCC 43251) at different concentrations in blinded food processing environmental swab eluate solutions. Swabs were provided by a collaborator in the leafy greens processing industry and eluted into 100 ml of buffer.

Results: There were statistically significant differences (P<0.05, n=25) in analytical signal between sensors treated with eluate spiked with *L. monocytogenes* and between those treated with un-spiked eluate. Preliminary results show the lowest amount of *L. monocytogenes* detectable was 10 cells in 100 ml eluate, giving an analytical signal of 10±2 nA. Total time from spiking to result output was under 2 h.

Significance: It is demonstrated, for the first time, that the presence of extremely low amounts of *L. monocytogenes* in complex matrixes representative of real world samples may be detected in under two hours. Our sensor can be readily used with little training at the point of testing to accurately allow for same-shift identification of contamination from complex samples.

P3-104 Combatting *Cryptosporidium* in Raw Milk

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Introduction: From 2009 to 2014, 16 foodborne cryptosporidiosis outbreaks were reported to the National Outbreak Reporting System; of these, four were associated with raw milk. The Centers for Disease Control and Prevention (CDC) and state public health laboratories have had difficulty using traditional processing techniques to test for *Cryptosporidium* in raw milk samples during outbreak investigations.

Purpose: The purpose of this study was to improve and optimize *Cryptosporidium* processing and detection in the complex matrix of raw milk.

Methods: For recent outbreak investigations, raw milk samples were combined with an equal volume of a Tween 20 solution and centrifuged at 1,000×g. A portion of the pellet was subjected to nucleic acid extraction for real-time PCR testing and the remaining pellet was subjected to immunomagnetic separation followed by fluorescent antibody microscopy (IMS-FA). The cream layer was recovered and analyzed separately. Additional methods were investigated and optimized, including ultrafiltration and continuous flow centrifugation (CFC). Ultrafiltration concentrates a raw milk sample by recirculation through a dialysis filter with pore size of ~30 kDa. Continuous flow centrifugation allows a steady stream of sample to be pumped into an enclosed centrifuge chamber while supernatant is continuously pumped out, thereby resulting in an increasingly concentrated sample within the chamber.

Results: Recovery of *Cryptosporidium* from raw milk using the centrifugation-based method was <10%. Recovery of *Cryptosporidium* from complex water matrices was 48% by ultrafiltration and 54% by CFC. Evaluation of the ultrafiltration and CFC recovery methods in a raw milk matrix are expected to show a similar trend.

Significance: An improved processing method for raw milk will allow CDC, and ultimately state public health laboratories, to process raw milk samples and other complex aqueous food matrices to detect *Cryptosporidium* and molecularly link environmental samples to clinical isolates during outbreaks. Additionally, the methods to process milk samples could be transferrable to other foodborne pathogens, such as *Campylobacter*.

P3-105 Predictive Model for Growth of *Bacillus cereus* during Cooling of Cooked Rice

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Introduction: Bacillus cereus is frequently implicated in foodborne outbreaks associated with the consumption of cooked rice. The main contributing factors leading to outbreaks is rice cooked in large quantities and subsequently, inadequately chilled or stored at room temperatures for a prolonged period of time prior to consumption.

Purpose: To develop a dynamic predictive model for *B. cereus* in cooked rice and to validate the model using exponential and two-step temperature profiles.

Methods: *Bacillus cereus* growth was quantified in cooked rice inoculated with approximately 2 log CFU/g of heat-shocked (80°C/10 min) spores at several isothermal conditions (between 10 and 49°C). *B. cereus* populations were determined by plating on mannitol egg yolk polymyxin agar and incubating at 30°C for 24 h. Data were fitted into primary growth models, namely Baranyi, Huang, modified Gompertz, and logistic models. Growth rates generated by each primary model were fitted with the modified Ratkowsky secondary model with respect to temperature.

Results: All four primary models were well-fitted by the modified Ratkowsky model (R^2 values from 0.90 to 0.99). Based on the goodness of fit secondary model statistics (R^2, SSE, RMSE), the Baranyi model performed the best. Acceptable prediction zone (APZ) analysis was performed for validation of the Baranyi model predictions during exponential single rate and two-step cooling temperature profiles. For single rate cooling, 23 of the 24 predictions fell within the APZ (-1.0 to 0.5 log CFU/g). For two-step cooling, 26 of the 28 predictions fell within the APZ.

Significance: The developed dynamic model can be used to predict potential *B. cereus* growth from spores in cooked rice during chilling and thus with the disposition of product subject to cooling deviations.

P3-106 The Semi-quantitative Rapid Detection Method of *Bacillus cereus* for Fresh-cut Lettuce and Baby Leafy Vegetables

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❖ ***Developing Scientist Competitor***

Introduction: Bacillus cereus is a major foodborne pathogen that can contaminate fresh-cut vegetables. Fresh-cut vegetables are generally consumed without any cooking, and thus, microbiological safety is important. However, current quantitative tests for *B. cereus* are so slow that they produce results after fresh-cut vegetables are consumed.

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Purpose: This study developed a semi-quantitative rapid detection method for *B. cereus* in fresh-cut lettuce and baby leafy vegetables.

Methods: B. cereus was inoculated on the lettuce (20 g) and baby leafy vegetables (10 g) at 1, 2, 3, 4, and 5 log CFU/g. The attached cell concentrations were enumerated on mannitol egg yolk polymyxin agar. Eighty (lettuce) and 90 ml (baby leafy vegetables) of tryptic soy broth with 0.15% polymyxin B were placed into sample bags and incubated at 42°C for 0, 1, 2, 3, 4, 5, 6, and 7 h for enrichment. One-milliliter aliquots of the enrichment from each inoculation level and inoculation time were used for DNA extraction, and PCR analysis was then performed with commercial *B. cereus* primers.

Results: The detection limit of the PCR analysis with primers for *B. cereus* was 4 to 5 log CFU/ml. In fresh-cut lettuce, 1 log CFU/g of *B. cereus* inoculated samples showed *B. cereus* positive result from PCR analysis after 7 h of enrichment, and 2, 3, 4, and 5 log CFU/g inoculated samples were positive after 6, 5, 4, 3 h of enrichment, respectively. For baby leafy vegetables, 1 log CFU/g inoculated samples showed positive result after 6 h of enrichment, and 2, 3, 4, and 5 log CFU/g inoculated samples were positive after 5, 4, 3, 2 h of enrichment, respectively.

Significance: The results indicated developing a semi-quantitative rapid detection method for *B. cereus* in fresh-cut vegetables can be used to rapidly quantify *B. cereus* cell counts.

P3-107 Using Reliability Analysis to Assess the Utility of Non-pathogenic Surrogates

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Introduction: The validity of microbial surrogates is critical for process validations. Although there are guidelines for surrogate-based process validations and reports of specific surrogate suitability for certain products and processes, there is little information about how to rigorously and quantitatively assess whether a surrogate is appropriate for a particular product or process.

Purpose: Use reliability analysis to establish the validity of a non-pathogenic surrogate (*Enterococcus faecium* NRRL B-2354) for *Salmonella* in low-moisture foods.

Methods: Two different inactivation data sets for the surrogate and pathogen were obtained. The data consisted of thermal inactivation curves for both bacteria on multiple low-moisture foods (e.g., walnuts and almond meal) at different process temperatures, air velocities, and process humidities (~120 data points). A linear model was fit to the corresponding log reductions of both organisms. Estimations of the log reduction of the pathogen from 4-log reduction of the surrogate were performed, implementing a Monte Carlo simulation (n=1,000). Performance of the surrogate (% of log reductions of pathogen >4) and the log reduction of the surrogate necessary to achieve >4-log reductions of the pathogen 90% of the time were estimated.

Results: The linear fit of the two paired log reduction data sets yielded good fits, with RMSE=1.14 and 0.45, slope=0.49 and 1.2, and intercept=1.6 and 0.14 for walnuts and almond meal, respectively. The reliability analysis shows that when on average 4-log reductions of the surrogate are observed, 4-log reductions of the pathogen are expected 37 and 83% of the time, respectively. The level of inactivation necessary to achieve a reliability of 90% was 7.8- and 3.9-log reductions of the surrogate.

Significance: These results show that reliability analysis is a useful tool in assessing performance of surrogates for process validation, which is a critical, but rarely documented, step that should be included in interpretation of process validation results.

P3-108 Measuring and Modeling the Influence of Relative Humidity and Buffer Type on the Survival of *Enterobacter aerogenes*

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Introduction: The survival of microorganisms on food contact surfaces is an important part of understanding cross-contamination. Relative humidity (RH) and matrix type both appear to play a role. Primary and secondary models for describing microbial behavior under these circumstances are limited.

Purpose: The purpose of this study was to measure and develop primary models for the effects of buffer and RH on the survival of the non-pathogenic surrogate *Enterobacter aerogenes* (B199A).

Methods: Stainless steel tiles were inoculated with a range of *E. aerogenes* concentrations in 1% peptone, 0.1% phosphate buffered saline (PBS), or distilled water, dried and placed in desiccators containing saturated salt solutions at ~15, 50, 100% RH at 21°C and sampled at intervals from 4 h to 21 days. Survival modeling was conducted using DMfit and GinaFit software.

Results: E. aerogenes survival at 15 and 50% RH could be modeled using Biphasic, Weibull, or Baranyi and Roberts models with R squared values between 0.68 and 0.98. Survival at 100% RH was more difficult to model due to the complex shape of the survival curve. Final concentrations of *E. aerogenes* were 1.3, 2.7, and 6.5 in peptone, 1.2, 1.5, and 6.8 in PBS after 21 days, and 1.6, 1.6, and 2.9 log CFU/surface in distilled water after 1 week in 15, 50, and 100% RH, respectively, after inoculation with ~7 log CFU. *E. aerogenes* displayed either injury, recovery, then decline, or growth at 100% with final concentrations of 6.1, 6.5, and 6.2 in peptone and 4.1, 2.9, and 6.8 log CFU/surface in PBS at starting concentrations of ~2, 4, and 6 log CFU/surface respectively.

Significance: These results show the complex interactions between relative humidity and matrix on survival of microorganisms. More research is needed to develop secondary models for survival on surfaces at different environmental RH and buffer matrices.

P3-109 Growth and No Growth Boundary of *Clostridium perfringens* in Cooked Beef

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Introduction: Clostridium perfringens is a spore-forming pathogen that can cause acute gastroenteritis. It is often found in cooked meat and poultry products. Rapid growth of *C. perfringens* may occur if cooked meats are not properly cooled after cooking, producing an enterotoxin that causes food poisoning.

Purpose: This study was conducted to determine the growth/no growth boundary in cooked beef by evaluating the effect of sodium tripolyphosphate (STPP), sodium lactate (NaL), and sodium chloride (NaCl) on the germination and outgrowth of *C. perfringens* spores in meat products.

Methods: The growth/no growth study was conducted in Shahidi-Ferguson Perfringens agar mixed with STPP (0 to 2500 ppm), NaL (0 to 4%), and NaCl (0 to 4%) in microplates. The growth/no growth was determined by comparing the turbidity measurements at 600 nm before and after overnight anaerobic incubation at 46°C. The dichotomous responses were analyzed by logistic regression to develop a model for estimating the growth probability of *C. perfringens*. The model was used to set the threshold of growth (probability > 0.1 or 0.2) of *C. perfringens* in ground beef.

Results: The growth/no growth boundary model was validated using inoculated ground beef mixed with different combinations of STPP, NaL, and NaCl. The growth/no growth of *C. perfringens* was observed after 24 h of incubation under optimum temperature, and the outcomes were compared

with the model. For a threshold of growth of 0.2, the accuracy of the growth and no growth predictions was 95.7%, with 4.3% over-prediction of growth events (fail-safe).

Significance: This study showed that proper combinations of STPP, NaL, and NaCl could be used to control the growth of *C. perfringens* from spores in cooked beef under the optimum temperature. The model developed in this study can be used by the food industry to formulate meat and poultry products to prevent the growth of *C. perfringens* during cooling.

P3-110 An Agent-based Model for Norovirus Contamination of Berries by Infected Farm Workers

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❖ ***Developing Scientist Competitor***

Introduction: Norovirus (NoV) is a highly contagious gastrointestinal illness that causes the rapid onset of vomiting, diarrhea, and fever. Fresh and frozen berries have been linked to viral foodborne disease outbreaks around the world, but little is known about how berries become contaminated or the levels of pathogens found on berries once contaminated.

Purpose: The objective of this study was to simulate NoV prevalence and contamination on individual strawberries after harvest by infected fruit pickers. The effect of handwashing as an intervention was used to determine its effectiveness in decreasing NoV prevalence and concentrations on strawberries.

Methods: The model was developed using the agent-based features of AnyLogic software. An agent-based model allows the simulation of the level of "agents" that can operate independently. In this simulation, the agents are individual workers and individual berries. The model simulates the transmission from hands of infected pickers to berries. The pickers' health status was allowed to change through the simulation from susceptible, exposed, infectious, shedding, recovered, to immune. The model predicts prevalence and concentration on about 1 million strawberries picked on a single farm over a 1-week period by 100 pickers. Handwashing compliance and effectiveness were varied during the simulation to assess their effect on NoV prevalence and concentration on berries. Every simulation started with a single infected worker.

Results: The number of infected workers at the end of the week ranged from 0 to 34. The fraction of contaminated berries harvested ranged from 0.15 to 16.38%. If a berry was contaminated, the concentration of NoV had a mean log of 3.40±1.27. As handwashing compliance improved by 50%, the contamination of berries declined by 10.78%.

Significance: Our results show the importance and limitations of worker health and hygienic interventions, such as handwashing, and how they impact the safety of hand-harvested berries.

P3-111 Quantitative Risk Assessment of *Salmonella* spp. for Yellow Broiler Supply Chain in China

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Introduction: Foodborne illness due to *Salmonella* spp. is a major public health problem, and poultry products are the most common outbreak sources throughout the world. Yellow broiler is one of the most popular poultry species for consumers in China. A few risk assessment models for yellow broilers have been studied, but they mainly focus on the stage of retail to consumption instead of whole supply chain.

Purpose: The aim of this study was to develop a quantitative risk assessment model (QRAM) of *Salmonella* spp. for the yellow broiler supply chain in order to estimate the infection risk for consumers and determine the critical control points (CCPs) for preventing *Salmonella* spp. contamination in poultry supply chain.

Methods: The pathway of the broiler supply chain was modeled as a series of modules, including the initial contamination on the farm, bacterial growth/inactivation and cross-contamination in slaughter house, bacterial survival/growth during storage, transportation, and at the retail market, thermal inactivation during cooking, and dose response after consumption. Both experimental data of microbial tests and the results of predictive models for *Salmonella* spp. were used to establish model inputs. The QRAM was constructed in an Excel spreadsheet and simulated using @Risk.

Results: The QRAM predicted one case of salmonellosis per 10,000,000 consumers. Sensitivity analysis showed that scalding (Spearman rank coefficient 0.95) and chilling (0.61) in the slaughter house were the CCPs for preventing the contamination level and prevalence of *Salmonella* spp., respectively. The use of chlorinated water at the chlorine concentration above 20 ppm could minimize the bacterial cross-contamination during chilling.

Significance: The QRAM has high potential for use in risk management to determine strategies to control *Salmonella* spp. contamination in the yellow broiler supply chain.

P3-112 Predictive Modeling Using a Monte Carlo Simulation to Estimate the Probability of Bacterial Spore Survival

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Introduction: Conventional kinetic models describing increases and/or decreases in bacterial numbers do not account for variability and uncertainty. In addition, conventional decimal reduction time (D-value)-based calculations do not appropriately estimate the survival probabilities of bacterial populations because of model extrapolation. In contrast, stochastic models describe bacterial death with probability distributions, taking individual cell heterogeneity into account. Accurate estimation of survival probability is required for the appropriate design of minimal food processing.

Purpose: This study aimed to develop a stochastic model with Monte Carlo simulation to estimate the survival probability of bacterial spores during heating.

Methods: Bacillus simplex spores were prepared and adjusted to 1×10^n CFU/10 µL (where n=1, 2, 3, 4, and 5) by 10-fold culture dilution. The inocula were dispensed into a 96-well microplate in 60 replicates (10 µL/well) and heated to 94°C in a thermal cycler for arbitrary durations. Subsequently, the survival of bacterial spores in each well was assessed by plating on nutrient agar plates and incubation at 30°C for 2 days. We concurrently examined

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kinetic changes in the spore survival numbers under the same temperature conditions; the kinetics were described by the Weibull function. Changes in the survival probability during heating were estimated with Monte Carlo simulations using the fitted kinetic parameters.

Results: The survival probability of *B. simplex* during heating showed a sigmoidal decrease over time, regardless of the initial inoculum level. In addition, the survival kinetics of *B. simplex* spores were successfully described by the Weibull function. Using the estimated Weibullian parameters, Monte Carlo simulations of spore inactivation were conducted in 60 replicates. The results successfully corresponded with observed changes in survival probability.

Significance: A stochastic model with Monte Carlo simulation for changes in the survival probability of bacterial spores during heating was successfully developed.

P3-113 Exposure Assessment of *Salmonella* in Street-vended Grilled Chicken Intestines

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Introduction: Street foods are considered an indispensable part of the food chain, having both positive features of improving food security and economic stability and negative aspects of potentially poor quality and compromised safety. Grilled chicken intestines, locally known as *isaw*, are one of the best-selling street foods in the Philippines, but they are also considered potentially hazardous based on previously reported contamination with pathogens, including *Salmonella*.

Purpose: The purpose of the study is to demonstrate the exposure pathway of *Salmonella* from raw chicken intestines up to consumption of street-vended grilled chicken intestines.

Methods: An exposure assessment model with a probabilistic framework was developed with inputs that include prevalence and initial concentration of *Salmonella* in raw chicken intestines, growth and inactivation kinetics of the pathogen in pre-cooked offal, consumption patterns among locals of Los Baños, Laguna, Philippines, and other pertinent details related to preparation and vending operation of street-vended grilled chicken intestines.

Results: Simulation of the developed exposure assessment model by the Monte Carlo approach predicted a mean *Salmonella* concentration of 12.68 most probable number per serving of street-vended grilled chicken intestines. Result of sensitivity analysis by Spearman Rank correlation also indicates that time and temperature during cooking steps were the factors most correlated to the concentration of the pathogen at the point of consumption.

Significance: Results of the study suggest that the likelihood and level of *Salmonella* per serving of street-vended grilled chicken intestines is high, and the most promising strategy to eliminate potential consumer exposure to the pathogen is by control of time and temperature in the cooking steps of the given street food product.

P3-114 Reinterpretation of the Mathematical Description of Variability in Bacterial Inactivation: A Stochastic Formulation and Its Application to the Time-to-Inactivation of Bacterial Populations

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Introduction: Conventional bacterial inactivation models describe changes in the average behaviour of bacterial survivors. However, because of individual cell heterogeneity, conventional kinetic models do not demonstrate precise estimation of the death probability of bacterial populations.

Purpose: We aimed to describe the variability in time required for a specific reduction in the number of bacterial cells and the death probability of bacterial populations via mathematical analysis.

Methods: Our target model was linear using the Weibull kinetic model. Variation of the death timing of individual cells was calculated using an exponential distribution. Then, we aggregated the death timing of the individual cells to describe the variability in time required for a specific reduction in the number of cells as a convolution of the exponential distribution. Furthermore, we compared the death probability calculated from a decimal reduction time (*D*-value) model with that provided by a stochastic formula developed in the present study.

Results: The time-to-inactivation and death probability of bacterial populations were successfully estimated. As the number of survivors decreased, the variance in the time required for a certain level of cell inactivation increased. Furthermore, the death probability of the bacterial population has been proposed as an alternative index to extrapolate the *D*-values of initial cell numbers of 1 to 10^5 cells. In the case of a linear survival kinetic model, the death probability of the bacterial population exhibited a linear relationship between inactivation time and target initial cell number. Estimations using a conventional *D*-value model and our stochastic model demonstrated similar results. In contrast, the death probability of the bacterial population calculated with the Weibull kinetic model exhibited some curvature between inactivation time and target initial cell number.

Significance: For death probability estimation, the *D*-value calculation is valid only for linear kinetic models. Stochastic mathematical models provide realistic and probabilistic estimations of bacterial inactivation.

P3-115 A Method for Estimating the Pathogenic Microbial Risk Level Using Bayesian Inference

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Introduction: Microbial risk assessments are designed to quantitatively predict the probability that specific foodborne diseases, such as *Escherichia coli* infections, listeriosis, and salmonellosis, will occur owing to the presence of causative pathogenic agents in one or more food products.

Purpose: We developed a novel method for microbial risk assessment (MRA) in which risk is estimated using levels of contamination immediately before consumption, Bayesian inference, and a dose–response model.

Methods: We first estimated contamination levels using maximum likelihood estimation from previously obtained experimental data. These contamination levels were classified into appropriate intervals, and the probability of consuming a foodborne pathogen in each interval was determined using Bayesian inference. This probability was applied to the dose–response model to estimate the possibility of foodborne illness for each interval. Finally, the sum of the probabilities for each interval was determined as the risk level for the total population. An example, ready-to-eat sandwiches and pathogenic *Staphylococcus aureus*, was considered.

Results: The estimated level of contamination for *S. aureus* in sandwiches was –2.89±1.79 log CFU/g. The estimated consumption level for *S. aureus* via sandwich intake was –0.77±1.81 log CFU/serving/day. The probability of consuming *S. aureus* using Bayesian inference was estimated to be 3.80×10^{-3}. Then, the estimated risk level and foodborne disease cases caused by *S. aureus* by sandwich ingestion per day in South Korea were $2.35\times10^{-7}\pm5.09\times10^{-8}$ and 12.07±2.61, ranging from 7.86 (5^{th} percentile) to 16.47 (95^{th} percentile), respectively.

Significance: Although the risk estimate was performed under assumed conditions, the method provides a better understanding of risk than previous risk level estimation methods. Therefore, the present approach to microbial risk estimation using Bayesian inference is expected to increase the applicability of MRA for accurate risk characterization.

P3-116 Development of Wireless Time-temperature Monitoring Sensors to Identify Temperature-abuse Conditions in Products That Support Growth of *Listeria monocytogenes*

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❖ ***Developing Scientist Competitor***

Introduction: A recent interagency risk assessment by the U.S. Food and Drug Administration and the United States Department of Agriculture concluded that temperature control of retail deli products is one of the most influential parameters that can control *Listeria monocytogenes* growth and reduce risk per serving. Proper temperature control is not always maintained from distribution to retail sale, creating an opportunity for *L. monocytogenes* growth in contaminated product before reaching the consumer.

Purpose: The purpose of this study was to develop a proof-of-concept wireless time-temperature monitoring (TTM) sensor mounted in flexible packaging substrate (e.g., polyolefin used for pre-packaged deli meats or logs) capable of transmitting detailed time-temperature histories of foods during distribution and storage. The remote sensor was designed and used in combination with dynamic *L. monocytogenes* temperature growth models created to predict pathogen levels over time.

Methods: The TTM was constructed on a printed circuit board (PCB) and works by measuring capacitance change due to temperature variation. Changes were recorded through a capacitance-to-digital module and then processed, timed, and communicated with a Nordic semiconductor nRF52 module. *L. monocytogenes* 10403S growth in sliced turkey breast was modeled from dynamic temperatures profiles measured by the TTM cycling between 3 and 12°C for 300 h, of which 160 to 173 h were >4°C.

Results: The TTM provided time-temperature data in real time for more than two weeks and stored data during interrupted connections with the base station. The sensing elements were connected to the PCB through a flexible ribbon cable ideal for the integration of the sensor to different food packages. *Listeria monocytogenes* strain 10403S average growth rate was 0.02 log CFU/h after 166±6 h of exposure to temperatures >4°C.

Significance: The TTM can be used to identify temperature-abused foods that may subsequently have an increased risk of listeriosis per serving. This technology may prevent higher risk foods from entering commerce, thus reducing the burden of disease.

P3-117 Isolation of *Bacillus cereus* from Soft Soybean Curd and Developing a Dynamic Model to Describe Its Kinetic Behavior

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❖ ***Developing Scientist Competitor***

Introduction: Bacillus cereus spores may contaminate foods. The spores then germinate in appropriate growth conditions and cause foodborne outbreaks. In recent years, the isolation of *B. cereus* from soft soybean curd has been frequently reported.

Purpose: This study isolated *B. cereus* from soft soybean curd and developed dynamic models to describe the kinetic behavior of *B. cereus* in soft soybean curd.

Methods: Thirty-nine soft soybean curd samples from retail markets were plated on mannitol yolk polymyxin agar. Presumptive *B. cereus* colonies were identified by 16s rRNA analysis, and diarrheal or emetic type *B. cereus* were determined by PCR with *groEL* and *ces* gene primers. The isolated *B. cereus* were inoculated on soft soybean curd and enumerated during storage at 10 to 30°C. The Baranyi model (primary model) was then fitted to the *B. cereus* cell counts data to calculate death or growth rate (log CFU/g/h) and lag phase duration (LPD; h). A polynomial equation (secondary model) was fitted to the kinetic parameters. A dynamic model was subsequently developed with primary and secondary models. Root mean square error (RMSE) was calculated to evaluate the model performance.

Results: Of 39 soft soybean curd samples, two samples (5.1%) were contaminated with *B. cereus*, and six diarrheal type strains were isolated from soft soybean curd. At 10 to 15°C, *B. cereus* cell counts decreased from -0.04 to -0.02 log CFU/g/h, but the cell counts increased at 25 and 30°C. LPDs decreased from 51.4 to 1.4 h as temperature increased. The developed secondary model was appropriate to describe the effect of temperature on the kinetic parameters with 0.909 to 0.953 of R^2. The performance of the developed dynamic model was appropriate at changing temperature with 0.216 of RMSE.

Significance: The results indicate that diarrheal type *B. cereus* is a contaminant in soft soybean curd, and the developed dynamic model should be useful in describing the kinetic behavior of *B. cereus*.

P3-118 Growth and Survival of Pathogenic *Escherichia coli* in Jellied Mung Bean during Storage

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Introduction: Numerous foodborne illness outbreaks have occurred by pathogenic *Escherichia coli* through consumption of raw vegetables. Jellied mung bean is often served with various vegetables in catering settings; thus, there is a high likelihood of *E. coli* outbreak through consumption of jellied mung bean.

Purpose: This study developed a dynamic model to describe the kinetic behavior of *E. coli* in jellied mung bean at changing temperatures, simulating the catering setting.

Methods: Jellied mung bean samples were inoculated with a five-strain mixture of *E. coli* at 4 log CFU/g, followed by storage at 10°C (8 days), 15°C (7 days), 25°C (7 days), and 30°C (4 days). During storage, *E. coli* cell counts were enumerated by plating samples on 3M Petrifilm. The Baranyi model was then fitted to *E. coli* cell counts to calculate lag phage duration (LPD; h) and growth rate (log CFU/g/h). The kinetic parameters were further analyzed by a polynomial equation. For validating the developed model, root mean square error (RMSE), bias (B) factor, and accuracy (A) factor were calculated. The dynamic model was subsequently developed under changing temperatures.

Results: E. coli cell counts in jellied mung bean were not changed during storage at 10 to 15°C, but *E. coli* cell counts increased at 25 and 30°C. As storage temperature increased, LPD decreased ($P<0.05$), but growth rates increased ($P<0.05$). Secondary models were appropriate to describe the effect

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of storage temperature on the kinetic parameters with 0.897 to 0.945 of R^2. The prediction of developed models was appropriate with 0.308 of RMSE, 0.98 of B factor and 1.05 of A factor. The prediction of the dynamic model was also appropriate.

Significance: These results indicate that the developed dynamic models are useful in describing the fates of *E. coli* in jellied mung bean during storage.

P3-119 Growth of *Escherichia coli* on Diced Melon for Catering Service

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Introduction: Fruits and vegetables may allow the rapid growth of foodborne pathogens. Melon in particular has appropriate pH and water content for bacterial growth. Thus, diced melon has been associated with enteric infections, including *Escherichia coli* and other foodborne pathogens.

Purpose: The objective of this study was to develop a dynamic model to predict the growth of *E. coli* in melon during storage, simulating catering service.

Methods: A five-strain mixture of pathogenic *E. coli* was inoculated in fresh diced melon (25 g) at 4 log CFU/g. The inoculated melon samples were then stored at 4 to 25°C. *E. coli* cell counts were enumerated by plating samples on 3M Petrifilm during storage. The Baranyi model (primary model) was fitted to the *E. coli* cell counts data to calculate kinetic parameters such as lag phage duration (LPD; h) and growth rate (GR; log CFU/g/h). A polynomial equation (secondary model) was then fitted to LPD and GR values as a function of storage temperature, followed by calculating root mean square error (RMSE), bias (*B*) factor, and accuracy (*A*) factor for evaluating the model performance. A dynamic model was subsequently developed in accordance with primary and secondary models.

Results: E. coli growth was not observed at 4°C, but their growth was observed between 10 and 25°C. LPDs were decreased ($P<0.05$) and GRs were increased ($P<0.05$) as temperature increased. The developed secondary models were appropriate to describe the effect of storage temperature on the kinetic parameters with 0.903 of R^2 for *ln* LPDs and 0.991 of R^2 for square root of GRs. The performances of developed models were appropriate with 0.337 of RMSE, 1.02 of *B* factor, and 1.06 of *A* factor. The *E. coli* growth prediction of the dynamic model was also appropriate.

Significance: These results indicate that the developed dynamic model predicted the *E. coli* growth appropriately in melon under changing temperature.

P3-120 Modeling the Survival of *Salmonella* on Fresh Cucumbers under Different Storage Temperatures and Relative Humidity

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❖ ***Developing Scientist Competitor***

Introduction: There have been several multistate outbreaks of salmonellosis associated with fresh cucumbers recently in the United States. Environmental factors affect the survival of *Salmonella* in cucumbers and other fresh produce, but no validated models currently exist which describe the fate of *Salmonella* on whole fresh cucumbers.

Purpose: This study developed a mathematical model that predicts the survival of *Salmonella* on whole cucumbers at different temperatures and relative humidities.

Methods: Fresh cucumbers were spot inoculated with a four-strain cocktail of *Salmonella enterica*. Inoculated cucumbers were dried for two hours and placed in desiccators containing saturated salt (lithium chloride, potassium carbonate, and potassium sulfate) used to create controlled RH environments (~15, 50, 100% RH) at 7, 14, and 21°C. Samples were enumerated at appropriate time intervals ranging from 0 to 240 h. Predictive models were developed using the Baranyi and Roberts model as a primary model and estimated kinetic parameters were fitted into a polynomial equation by regression analysis.

Results: The R^2 values for the primary models ranged from 0.61 to 0.99. *Salmonella* on whole cucumbers showed better survival at higher temperature and relative humidity, with the greatest decline in *Salmonella* populations observed at 15% RH. The maximum death rates depended on RH and ranged from -0.009 to -0.116 log CFU/cucumber/h. Secondary models for maximum death rate and the degree of decline for *Salmonella* were linear and had high R^2 values (>0.98). Root mean square error values obtained for the maximum death rate (0.009) and the degree of decline (0.250) models showed that the models obtained are suitable for modeling the survival of *Salmonella* on the fresh cucumbers.

Significance: The models in this study will be useful for future microbial risk assessments and predictions of *Salmonella* behavior in the cucumber distribution chain.

P3-121 Quantitative Microbial Risk Assessment of *Bacillus cereus* in Packaged Tofu

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Introduction: Tofu is considered part of a healthy nutritious diet. Due to its functional and health benefits, consumption of tofu has increased worldwide. However, microorganisms can grow well in tofu due to the high protein and water content, as well as its neutral pH. Therefore, the microbiological safety of tofu needs to be confirmed.

Purpose: The objective of study was to conduct a quantitative microbial risk assessment of *Bacillus cereus* from consumption of tofu in Korea.

Methods: The initial contamination levels of *B. cereus* in packaged tofu (n=85) were monitored. To predict the change of *B.cereus* populations as a function of time and temperature from the markets to home during transportation, a primary model of *B.cereus* on tofu was developed as a function of temperature (4, 9, 11, 17, 30, 40, and 45°C). The daily consumption amount and frequency of tofu were investigated among 1,000 people in the eight largest provinces in Korea. The simulation model was developed and the probability of foodborne illness due to *B. cereus* by the consumption of tofu was estimated with @Risk.

Results: B. cereus was detected in 11 (13%) tofu samples out of 85 and the average contamination level of -2.24 log CFU/g was estimated using the Riskbeta distribution. Growth of *B. cereus* on tofu was observed at temperatures higher than 9°C. The daily consumption amount and frequency of tofu were 89.42 g and 13.62% per person, respectively. A dose response model of *B. cereus* wasn't developed and the minimum infectious dose (10^5 cells) was substituted. The mean value of probability of illness by *B.cereus* of tofu was 1.0×10^{-4} per person per day.

Significance: When tofu is contaminated with *B. cereus*, it becomes a somewhat higher risk of causing foodborne illness. Thus, care should be taken not to introduce *B. cereus* in the manufacture of tofu, and it is important to keep retail tofu at a temperature lower than 9°C, a temperature at which bacteria doesn't grow.

P3-122 Predictive Model of *Clostridium perfringens* Growth in Egg Products

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Introduction: Mathematical predictive models, a major part in exposure assessment, are useful methods for microbiological risk assessment in industry for quality control and determination of shelf life. This has led to the development of growth prediction models of *Staphylococcus aureus* and *Clostridium perfringens*, known in Korea as a main cause of foodborne diseases in egg products.

Purpose: The objective of this study was to develop mathematical models to predict the kinetic behavior of *S. aureus* and *C. perfringens* in egg products in South Korea.

Methods: Purchased egg products (grilled and liquid egg) were cut into 10-g portions. Bacterial cells were inoculated into samples to obtain 3 log CFU/g, and the samples were stored at 4, 10, 15, 20, 30 and 37°C. The number of *S. aureus* and *C. perfringens* cells were enumerated on BP (baird parker) and TSC (tryptose sulfite cycloserine) agar plates, and these results were used to develop a primary model (Baranyi) to calculate parameters (lag phase duration [LPD] and maximum specific growth rate [μmax]). Parameters were further analyzed with a secondary model as a function of storage temperature. To evaluate the accuracy of the model prediction, root mean square error (RMSE) was calculated by comparing the predicted data with observed data.

Results: LPD and μmax were calculated using a Baranyi model based on the growth at the each temperature, except 4°C where no growth was observed. The calculated parameters were then used to develop an equation for predicting temperature-dependent changes using secondary models (polynomial model for LPD and square root model for μmax). With the minimum growth temperature of 4°C (*S. aureus*) and 10°C for (*C. perfringens*), RMSEs (root mean square error) analyzed to validate the developed models in grilled eggs were 0.16 and 0.26, respectively for *S. aureus* and *C. perfringens* while, in liquid eggs, analyzed RMSEs were 0.25 and 0.3, respectively for *S. aureus* and *C. perfringens*, suggesting the models properly represent the actual growth of *S. aureus* and *C. perfringens* in egg products.

Significance: This result suggests that the developed models should be useful in describing the kinetic behavior of *S. aureus* and *C. perfringens* in egg products.

P3-123 Quantitative Microbial Risk Assessment of *Bacillus cereus* in Fermented Pastes

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Introduction: Fermented pastes, including soybean, red pepper, and *Cheonggukjang* (fast fermented bean paste) are Korean traditional foods. Recently, a problem with *Bacillus cereus* contamination has been noticed in fermented pastes.

Purpose: This study aimed at assessing the risk of *B. cereus* in fermented pastes.

Methods: Prevalence data of *B. cereus* were collected from soybean, red pepper, or mixed fermented pastes in domestic markets. Temperature and time data for distribution and storage were also collected. For the worst case scenario, predictive models for *B. cereus* were developed in soybean pastes, which allowed more growth of the pathogen than others. Consumption data were also collected for soybean pastes, which is the most consumed fermented paste. Because there were no dose-response models for *B. cereus*, the infectious dose was used in hazard characterization. With all collected data, a simulation model was prepared and the risk of *B. cereus* was calculated using @Risk.

Results: Estimated initial contamination level was 2.47 log CFU/g. The developed predictive model showed that *B. cereus* cell counts were decreased at all temperatures (4, 15, 25, and 35°C) during storage. For this reason, estimated *B. cereus* contamination levels were decreased from 2.47 to 1.51 log CFU/g. Taken together all the data and the consumption data, the probability of *B. cereus* foodborne illness by fermented pastes consumption was 0.

Significance: This result suggests that the risk of *B. cereus* in fermented pastes is very low.

P3-124 Hand Hygiene Interventions to Reduce Norovirus Contamination of Ready-to-Eat Fresh Produce during Produce Harvesting and Packing on Farms

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❖ ***Developing Scientist Competitor***

Introduction: The high attribution of foodborne norovirus outbreaks to produce necessitates prevention strategies at pathogen contamination points along the fresh produce supply chain.

Purpose: To evaluate the impact of human behavioral interventions on norovirus contamination levels in fresh produce items during production and packing.

Methods: A stochastic quantitative microbial risk assessment (QMRA) model was developed in R using a two-dimensional Monte Carlo package and 10,000 iterations. Sensitivity analysis and scenario testing were performed on model parameters for which inputs and probability distributions were derived from the literature and our human challenge studies: norovirus concentrations on worker hands; hand-to-produce transfer rates; hand washing and glove usage probabilities; and hand washing efficacy. Consumer risk estimates were generated using daily serving recommendations from the United States Department of Agriculture.

Results: With harvest following restroom use by a norovirus-infected worker, complete hand washing compliance reduced the virus contamination level on produce by 84% (SD 8.4%) relative to the absence of hand washing. Similarly, complete glove use, compared to no glove use, reduced contamination levels of produce by 77.3% (SD 14.2%). Relative to no hand washing (3.26 log virions/cm^2 on produce), improving hand washing efficacy by two-fold, paired with complete hand washing (1.26 log virions/cm^2 on produce), reduced produce contamination by 99% (SD 5.7%). Norovirus contamination on produce decreased by 1.78 log from the first produce item harvested (3.09 log virions/cm^2) to the 10th serially harvested item (1.31 log virions/cm^2). With complete glove use and hand washing, consuming the first produce item resulted in an infection risk of 0.44 (1.82 log virions/cm^2), while consuming the 10th produce item decreased the infection risk to 0.01 (<1.1 virions/cm^2).

Significance: Glove use combined with hand hygiene and sequential reduction in virus transfer through serial harvesting reduced norovirus produce contamination to below an infectious dose (18 genomic equivalents) and should minimize consumer foodborne disease risk.

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P3-125 Risk Assessment of *Clostridium perfringens* in Korean Traditional Soy Sauce

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❖ ***Developing Scientist Competitor***

Introduction: Soy sauce has long been the primary sauce consumed in Korea. In recent years, consumers have come to prefer traditional soy sauce rather over the modernized version, but the manufacturing process for traditional soy sauce may increase *Clostridium perfringens* contamination.

Purpose: The objective of this study was to assess the risk of *C. perfringens* in Korean traditional soy sauce.

Methods: ***C. perfringens*** were monitored in traditional soy sauce collected from markets. Temperature and time data for distribution and display were collected. A predictive model was developed with the Weibull model (primary model) fitted to *C. perfringens* cell counts from 7 to approximately 35°C to calculate *Delta* (time required for first decimal reduction) and *p* (shape of curves), and the parameters were analyzed with a secondary model (exponential and quadratic model). Consumption patterns for soy sauce were surveyed, and a dose-response model was also searched. With all collected data, a simulation model was prepared and the probability of *C. perfringens* outbreak was calculated by simulation with @Risk.

Results: A total of 629 samples were analyzed, and *C. perfringens* cell counts in all samples were below detection limit (# log CFU/ml). Thus, initial contamination level was estimated to be -3.94 log CFU/g by probabilistic model. *Delta* values decreased by 137.2 to 3.7 h as the storage temperature increased, and the developed secondary models were appropriate with 0.944 of R^2. Exponential model (Risk=1-exp[-r× dose] [r=1.82× 10^{-11}]) was selected as appropriate dose-response model. Subsequently, the probability of foodborne illness by *C. perfringens* per person per day with consumption of soy sauce was 1.54×10^{-14}.

Significance: This result indicates that the risk of *C. perfringens* in Korean traditional soy sauce is low.

P3-126 Quantification of Statistical Power for Surrogate-based Lethality Validation Studies

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Introduction: Pathogen surrogates are commonly used to validate preventative controls. Identification of an appropriate surrogate is critical for surrogate-based validations, but the ultimate goal is to evaluate the efficacy of the preventative control. However, very little has been reported on best methods for data interpretation in process validation studies.

Purpose: The goal of this study was to quantify the impact of experimental design on the potential for a surrogate-based validation study to discriminate between effective and ineffective preventative controls.

Methods: Multiple sampling plans were evaluated for the likelihood (statistical power) of correctly reporting effective (95% of possible samples above target lethality) or non-effective preventative controls. Using a Monte Carlo-based Bayesian approach, pre- and post-treatment samples were randomly generated and tested (using confidence and prediction intervals, α=0.05) to determine if target lethality was achieved, and the process was repeated 1,000 times. The percentage of results correctly classifying treatment effectiveness approximated the statistical power of the plan. Variables included the sampling design (≥3 samples before/after treatment, ≥2 replications), sampling error, achievable lethality, and replication error.

Results: With true mean lethality ≤ target lethality, all sampling plans achieved ≥99% likelihood of correctly indicating an ineffective treatment. Using the minimal sampling design on a barely-effective treatment (e.g., lower 95% prediction of achieved lethality was only 0.2 greater than the target), only 66% of the experiments indicated the treatment as effective. With the same barely-effective treatment, collecting 10 samples pre- and post-treatment with five replications increased the probability of correctly indicating the treatment as effective to 88%.

Significance: Statistical power of sampling designs depended on preventative control effectiveness; largely effective or ineffective treatments required fewer samples than borderline effective treatments. The prediction interval was the most sensitive metric to evaluate treatment sufficiency, making it an important measurement that should be utilized for validation experiments.

P3-127 Estimating the Effect of Retailer's Handling Practices and Socioeconomic Disparities on Food Safety Indicators at the Time of Purchase

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Introduction: Socioeconomic disparities not only can result in differential access to food but also in procurement of food of lower quality and safety.

Purpose: To assess the existence of differential handling practices at retailers within socioeconomically different neighborhoods and to compare the effects of handling practices based on the potential microbial concentration at the time of purchase.

Methods: We surveyed all food stores (*n*=333) in four socioeconomically different neighborhoods at Buenos Aires, Argentina. The surface temperature of two chilled products (custard-like dessert and sliced ham) was recorded at the time of purchase using an infrared thermometer. The expiration date of the samples was also recorded, when available, and was used to calculate the residence time of the samples in the stores. The frequency distribution functions for temperature and residence time of the data collected in combination with the growth parameters of two pathogens associated to outbreaks in the selected products were introduced into a stochastic model to estimate the probability of microbial concentration at the time of purchase.

Results: A total of 20% or less of the custard samples (*n*=196) and 15% or less of the ham samples (*n*=392) were stored at the recommended storage temperatures (≤5°C). The mean temperature for custard and ham were 8.5 and 9.3°C, respectively. The samples' temperature and residence time frequency distributions were described using asymmetrical distribution functions. While the parameters of the temperature distribution functions were similar for all neighborhoods, those of the residence time denoted significantly slower product rotation in stores located in low-income neighborhoods (mean residence time of 10 and 20 days, respectively, for high- and low-income neighborhoods). The outputs of the stochastic model consistently estimated higher probabilities of purchasing products with higher microbial levels at retailers within low-income neighborhoods.

Significance: The information obtained can provide valuable inputs for risk assessment modeling and can inform intervention strategies for underserved populations.

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P3-128 Using Food Safety and Inspection Service Data and a Prevalence-based Model to Modernize Hog Slaughter Inspection

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Introduction: The United States Department of Agriculture's Food Safety and Inspection Service (FSIS) has developed a prevalence-based model for conducting quantitative risk assessments examining the effects of changes in its inspection system on illnesses. This model does not use a dose-response relationship, instead relying on prevalence when the numbers of *Salmonella* are uniform across samples.

Purpose: The risk assessment was used to inform FSIS's proposed modernization of its hog slaughter inspection system.

Methods: This model was confirmed by quantifying *Salmonella* for each sample using most probable number analysis. FSIS has applied this prevalence-based model to evaluate its hog slaughter inspection system. The two-stage analysis initially used a multivariate distribution for a production volume-weighted logistic multiple regression model; the resultant regression coefficients were used for the model's subsequent Monte Carlo stage, which simulated daily establishment-level prevalence estimates based on individual establishment FSIS sampling data for *Salmonella* contamination and inspection procedures performed on each sample day. Modified inspection procedure approaches were simulated by applying individual Pert functions with parameters set to fit the expected modifications — that is, increased frequency of some types of inspection procedures as compared to the frequency observed under traditional inspection systems. The estimated decrease in prevalence from baseline was represented by first calculating the ratio of the simulated prevalence distributions over the observed baseline distributions. These two prevalence distributions are structurally correlated with one another, thus limiting one of the model's multiple sources of uncertainty.

Results: The number of illnesses remaining after implementing the new inspection system was then found by multiplying the prevalence ratio by the annual market hog-attributable human *Salmonella* illness distribution. The expected number of illnesses avoided due to the new inspection system was found by subtracting the resulting distribution from the original illness distribution.

Significance: FSIS used the results of this risk assessment to inform the modernization of its hog slaughter inspection system.

P3-129 Mathematical Models to Describe the Kinetic Behavior of *Staphylococcus aureus* in Meat Jerky

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Introduction: Meat jerky is dried and salted for the long period of storage and therefore has very low water activity. *Staphylococcus aureus* has been the cause of major foodborne illness outbreaks. It can survive even in high salt concentrations up to 10 to 15%. Therefore, *S. aureus* may survive in meat jerky.

Purpose: The objective of this study was to develop mathematical models to predict the kinetic behavior of *S. aureus* in seasoned meat jerky.

Methods: Purchased seasoned meat jerky was cut into 10-g portions. One-tenth milliliter of *S. aureus* ATCC 13565 was inoculated into the samples to obtain 3 log CFU/g, and the samples were stored aerobically at 10, 20, 25, 30, and 35°C. *S. aureus* cell counts were enumerated on Baird Parker agar. To develop a primary model, the Weibull model was fitted to the cell counts data to calculate *Delta* (required time for the first decimal reduction) and *p* (shape of curves). For secondary modeling, a polynomial model was fitted to *Delta* values as a function of storage temperature. To evaluate the accuracy of the model prediction, root mean square error (RMSE) was calculated by comparing the predicted data with observed data.

Results: ***S. aureus*** surviving cell counts were decreased at all storage temperatures. The *Delta* values were longer at 10 and 20°C, but shorter at higher temperature than 25°C. No differences in *p* values were observed among storage temperatures. The secondary model well-described the temperature effect on *Delta* with 0.920 of R^2 In validation, RMSE values with 0.325 suggested that the model performance was acceptable.

Significance: This result indicates that *S. aureus* survives longer at lower storage temperatures, and the developed model should be useful in describing the kinetic behavior of *S. aureus* in seasoned meat jerky.

P3-130 Efficacy of Bacteriophages as Beef Trim Intervention Treatment against Shiga Toxin-producing *Escherichia coli*

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❖ ***Developing Scientist Competitor***

Introduction: Shiga-toxigenic *Escherichia coli* (STEC), a major group of foodborne pathogens, are prevalent in cattle. It is therefore common for beef carcasses to be contaminated with STEC, despite decontamination treatments. Ground beef, produced from carcass trims, has a higher potential of STEC contamination. Furthermore, temperatures abuse (>5°C) of contaminated ground beef, during retail, could influence pathogen growth. Although conventional sanitizers, such as organic acids, are used as beef trim interventions, their efficacy at abuse temperatures, especially during longer retail storage, is questionable. It is therefore important to find alternatives that would maintain effectiveness throughout retail storage. Bacteriophages, known for enhanced activity at higher temperatures, could serve as alternatives for beef trim interventions.

Purpose: To evaluate the efficacy of bacteriophages in reducing STEC on beef trims during ground beef processing.

Methods: A total of 24 beef trims (4×4 inch) were inoculated with a cocktail (5-6 $\log_{10}$ CFU/g) of *E. coli* O157:H7 or non-O157 (O26, O45, O103, O111, O121, and O145) isolates. Following bacterial attachment, the trims were spray-washed (20 ml) with either lactic acid (5%), peracetic acid (400 ppm), sterile distilled water or STEC-specific bacteriophage cocktails (8-9 $\log_{10}$ PFU/ml), along with positive and negative controls. Treated samples were ground and stored in retail packages at 4 and 8°C. Pathogen survivors were enumerated over a 4-day period and data analyzed using one-way ANOVA ($P < 0.05$). All experiments were performed in triplicates.

Results: Overall, all the treatments showed significant reductions in STEC populations compared to the positive control. At 4°C, there were no significant differences among treatments. However, at 8°C, the most effective treatment was found to be the phage cocktail, with 0.70±0.22 log CFU/g reduction on day 4. Overall, phages showed better antimicrobial efficacy during storage of ground beef at 8°C, when compared to conventional trim wash treatments.

Significance: Bacteriophages could be used as effective beef trim interventions.

Poster

P3-131 Quantitative Microbial Risk Assessment Approach for Selecting Pathogen Control Strategies during Ground Beef Processing

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❖ ***Developing Scientist Competitor***

Introduction: *Escherichia coli* O157:H7 is well-known for its ability to cause foodborne illnesses. Ground beef has been most frequently associated with *E. coli* O157:H7 outbreaks. Its processing involves grinding of the beef trims; if they are contaminated, the possibility of bacterial contamination increases. Storage of contaminated ground beef at >4°C during retail could further increase pathogen growth. However, some ground beef processors have started using beef trim wash as an added intervention, increasing production costs significantly. It is therefore important to understand the effectiveness of any intervention prior to actual implementation. Quantitative microbial risk assessment (QMRA), which assesses the risk of foodborne illness from current production practices and predicts the effectiveness of proposed interventions, could help processors make an informed decision.

Purpose: Application of QMRA to predict the efficacy of beef trim interventions in reducing the risks of foodborne illness.

Methods: Experiments were conducted to determine the efficacy of beef trim interventions in reducing *E. coli* O157:H7, using various spray wash treatments (spray time=1 min): lactic acid (5%), peracetic acid (400 ppm), water, or bacteriophage cocktail (9 log PFU/ml). Treated samples were ground and stored in PVC-wrapped styrofoam trays for 4 days at 4 and 8°C. Pathogen survival in ground beef during storage was determined and the data modeled and incorporated in an established QMRA framework from the literature. The QMRA involved scenario analysis of beef trim interventions, retail storage, and cooking preference (rare, medium, and well-done). For each scenario, 100,000 iterations were simulated using Monte Carlo simulations via @Risk software.

Results: The QMRA predicted that at 4°C, the organic acids and phages were successful in reducing the probability of illness by 98% for a meal of rare/medium-cooked ground beef patties. At 8°C, only phage treatments reduced the risk by 98%. It was also revealed that if ground beef is well-done, beef trim interventions are not required.

Significance: Beef trim interventions are necessary to reduce the risk of foodborne illness from undercooked ground beef.

P3-132 Function Genomics Analysis of Next-Generation Sequencing Data Using Machine Learning Algorithms

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Introduction: Next-generation sequencing (NGS) technology has been widely applied in clinical and public health laboratory investigations for pathogen detection and surveillance. However, major challenges currently exist for the interpretation and analysis of NGS data. The size of NGS data can be huge, in addition to the related meta and omics data.

Purpose: The purpose of this study is to make effective use of the available NGS data in the public domain by applying big data algorithms and implementing them efficiently.

Methods: In previous studies, we have developed a framework to pursue data mining on NGS datasets by topic modeling, which is an active research field in machine learning and has been mainly used as an analytical tool to structure large textual corpora for data mining. In this study, we continued the research on applying machine learning algorithms on the functional genomic analysis of NGS data.

Results: Taking *Salmonella* serotyping as an example, an NGS data set of 323 *Salmonella* isolates was retrieved from National Center for Biotechnology Information database, and SNPs were generated using our previously developed framework. We applied the topic modeling on the SNPs to generate a corpus of SNPs. Random Forest (RF) and Supporting Vector Machine (SVM) algorithms were then applied on both the SNPs dataset and the SNPs corpus to predict the serotypes of *Salmonella.* The accuracy and specificity were calculated and compared. High prediction accuracy and specificity were obtained in both datasets, with even better performance in the SNPs corpus.

Significance: The implementation of topic modeling and other machine learning algorithms provides a new way in functional genomics analysis of NGS data to elucidate genetic information and potential biomarker identification, which is especially useful in the era of big data.

P3-133 Effect of Layer-by-Layer Antimicrobial Edible Coating for Shelf-life Extension of Shrimp (*Litopenaeus vannamei*) Stored at 4°C

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Introduction: Shrimp are easily perishable during storage and have a short shelf life. Edible coatings are efficient techniques to prevent food quality deterioration and extend shelf life.

Purpose: In the present study, the effect of layer-by-layer (LbL) electrostatic deposition coating of alginate and chitosan solutions that contain grapefruit seed extract as a natural antimicrobial agent was investigated on the shelf life of shrimp for 15 days under refrigeration.

Methods: The effects of alginate–chitosan and chitosan–alginate coatings were compared with those of alginate only, chitosan only, and non-coatings. The shrimp samples were periodically analyzed for changes in microbiological parameters (total aerobic mesophilic bacteria and total aerobic psychrophilic bacteria counts), chemical parameters (pH, total volatile basic nitrogen [TVB-N]), melanosis, and sensory characteristics.

Results: The chitosan–alginate coating had the advantages of reducing the bacterial count by 2 log CFU in combination with the antimicrobial activity of the inner chitosan layer, providing good adhesion of the outer alginate layer to the shrimp and imparting external environment barrier properties. Chitosan reduced the number of bacteria by about 2.5 log CFU over the same period. The bilayer coating reduced the off-flavor of shrimp during the storage period by preventing the odor of acetic acid that was used to dissolve the chitosan. Compared with uncoated shrimp, those that were coated with chitosan–alginate had predominantly reduced chemical spoilage, reflected in changes in TVB-N (14.74 mg/100g) and pH value (<7.2); retarded microbial spoilage (P<0.05); and increased overall sensory quality.

Significance: In conclusion, chitosan and chitosan–alginate treatments could prolong the shelf life of shrimp.

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P3-134 Shelf-life Extension of Pacific White Shrimp (*Litopenaeus vannamei*) Using Chitosan and ε-Polylysine during Cold Storage

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Introduction: Pacific white shrimp (*Litopenaeus vannamei*) are one of the most widely consumed crustaceans in many places, including Japan, Europe, and the United States. However, shrimp are highly perishable and have a short shelf life in retail distribution.

Purpose: In this study, we examined the effects of an ε-polylysine (PL) and chitosan (CH) coating on the quality of shrimp under refrigeration.

Methods: The shrimp were thawed at room temperature and coated with PL solution (2%), CH solution (1.5%), or CH + PL solution (chitosan solution with 2% ε-polylysin) and stored at 4°C for 15 days. The quality of shrimp was measured by observing changes in microbiota, pH, total volatile basic nitrogen (TVB-N), and sensory characteristics during storage.

Results: Among the coating films, the CH+PL coating most effectively inhibited the growth of mesophilic and psychrotrophic bacteria, *Pseudomonas* spp., and H_2S-producing bacteria. This coating increased the shelf life of shrimp by decreasing the amount of mesophilic and psychrotrophic bacteria, with inhibition greater than three log cycles on the ninth day of storage. In addition, the CH and CH+PL coatings effectively suppressed the formation of TVB-N compared with that in the control by 43% and 30%, respectively. The pH values of all treated samples increased slowly compared with that of the control, but no significant difference was observed. Sensory quality was similar to microbial and physicochemical properties, and the acceptability of all treated samples gradually decreased.

Significance: In conclusion, in this study, it was found that coatings of chitosan and combination with ε-polylysin may effectively prolong the shelf life of shrimp by inhibiting the growth of mesophilic and psychrotrophic bacteria, *Pseudomonas* spp., and H_2S-producing bacteria. Therefore, CH and PL coatings could be used as natural preservatives for seafood products such as shrimp in cold storage.

P3-135 Use of Lipid Nanoemulsion-doped Anti-fungal Packaging Films to Control Post-harvest Disease in Small Fruits

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❖ ***Developing Scientist Competitor***

Introduction: Postharvest losses can occur anywhere from harvesting to handling and shipping. In 2014, approximately $30 billion of fresh produce was lost in the United States food supply chain. In particular, shelf life of small fruits can be reduced by weight loss, stem scar injury, gray mold, and ripe rot. It is important, therefore, to find sustainable packing alternatives to protect produce, increase shelf life, minimize waste, and preserve resources.

Purpose: The objective of this study was to evaluate the mechanical, physical, and anti-fungal properties of packaging films loaded with essential oil (EO) nanoemulsions.

Methods: Food-grade emulsions with sub-micron droplets were used to encapsulate cinnamaldehyde (CY), eugenol (E), and thymol (T) within a refined coconut oil (carrier) in pullulan packaging systems (formulated in a previous work). Different film combinations (5 to 10% Pullulan, 1 to 2% EO, 0 to 50% water, and 0.5 and 1% gum concentrations) loaded with EO nanoemulsions were investigated. Tensile strength, moisture content and anti-fungal activity against *Rhizopus stolonifer*, *Alternaria* spp., and *Aspergillus niger* were measured and compared to control pullulan systems.

Results: A lower tensile strength (P>0.05) was measured for combinations loaded with EO nanoemulsions as compared to films without active lipid solutions: 5.7, 7.9, 6.8, and 17.1 MPa for CY, E, T, and control system, respectively. Nevertheless, the active combinations presented good elasticity and ductility. Active film moisture contents were lower as compared to film without EO (P<0.05). The control formulations showed no anti-fungal activity. Conversely, active combinations exhibited significant inhibition zones (P<0.05). Film containing CY had the biggest inhibition halos: 13.7, 15.7, and 14.5 mm were measured against *R. stolonifer*, *Alternaria*, and *A. niger*, respectively.

Significance: This study demonstrates the potential application of pullulan packaging films loaded with EO nanoemulsions as a means of controlling and reducing postharvest disease in small fruits during shipping and storage.

P3-136 Structure and Performance Investigation of Novel Barrier Coating Packaging Technologies for Microwave-assisted Thermal Sterilization

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Introduction: Aluminum foil-based materials for high-barrier packaging have been the industry standard for decades due to their excellent performance as an oxygen and water vapor barrier which provides extended shelf life to various items. Increasing consumer preference for high-quality (i.e., improved nutritional and organoleptic properties) shelf-stable foods and the food industry's need to use energy-efficient, high-throughput, and cost-effective processing technologies have led to the development of microwave-assisted thermal sterilization (MATS). MATS is an alternative food processing technology which is incompatible with foil-based packaging and therefore requires an alternative barrier material solution.

Purpose: The purpose of this study was to determine the ability of graphene and metal oxide coating technologies to achieve the required barrier performance for extended shelf life.

Methods: Coatings were deposited onto various common packaging materials including: polyester terephthalate (PET), nylon, and polypropylene (PP). Single layer graphene coatings were deposited using a PMMA-assisted wet transfer process. Aluminum oxide and titanium dioxide coatings were deposited at varying thicknesses ranging from 20 to 100 nm using atomic layer deposition (ALD). The coated materials were evaluated for oxygen and water vapor barrier performance using a MOCON Oxtran 2/21 and Permatran-W 3/33.

Results: The largest reductions in oxygen and water vapor transmission rates were observed on PET samples coated with a 25-nm layer of Al_2O_3 followed by a 25-nm layer of TiO_2. This sample had a measured oxygen transmission rate (OTR) of 0.48 cc/m^2/day (a 118-fold reduction) with a standard deviation of 0.08 cc/m^2/day. A water vapor transmission rate (WVTR) of 0.80 g/m^2/day (a 48-fold reduction) was measured with a standard deviation of 0.22 g/m^2/day. The largest reductions observed in the graphene coated samples were a 10-fold reduction of OTR on the graphene coated PET and a 2.3-fold reduction in WVTR on the graphene coated nylon.

Significance: The data suggests nanoscale metal oxide coatings deposited by ALD onto traditional flexible packaging materials have the potential to be used in MATS-compatible packaging used in food rations and other food storage applications.

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P3-137 Prevalence and Characteristics of Shiga-toxigenic *Escherichia coli* (STEC) Isolates in Raw Cow Milk from Agro-pastoral Farms in Ghana

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) is an important foodborne pathogen of public health concern in both developed and developing countries. STEC can cause severe clinical symptoms such as hemorrhagic colitis and hemolytic-uremic syndrome.

Purpose: This study determined the prevalence and characteristics of STEC isolates in raw cow milk from agro-pastoral farms in Ghana.

Methods: A total of 210 raw cow milk samples were collected from 42 agro-pastoral farms in Ghana for the isolation of *E. coli*. Suspected *E. coli* isolates were identified using Vitek II Compact system. STEC virulence genes encoding Shiga toxin (*stx*1 and *stx*2), intimin (*eae*A), and STEC autoagglutination adhesin (*saa*) were determined by PCR. The O-serotypes of STEC isolates were determined using *E. coli* antisera. Susceptibility of STEC isolates to 15 different antibiotics were determined using the microdilution method.

Results: In total, 58.1% (122 of 210) of raw milk collected from agro-pastoral farms were positive for *E. coli*. In general, 2.4% (10 of 423) of *E. coli* isolates harboured *stx* genes. Out of the 10 STEC isolates, 40% (4 of 10) harboured only *stx*1 gene, 10% (1 of 10) harboured only *stx*2 gene, and 50% (5 of 10) possessed both *stx*1 and *stx*2 genes. Additionally, all STEC isolates harboured *eae* gene, but not *saa* gene. All 10 STEC isolates belonged to 10 different serogroups, with no O157 serotype detected. STEC isolates showed phenotypic resistance to ampicillin (10 of 10; 100%), streptomycin (10 of 10; 100%), and tetracycline (8 of 10; 80%). All STEC isolates showed resistance to at least two different antibiotics.

Significance: Detection of antibiotic-resistant shiga toxin-producing *E. coli* (STEC) in raw cow milk in this report indicates the potential health risk associated with consuming milk. Thus, this report provides useful epidemiological information and emphasizes the need to develop strategies to prevent contamination of raw cow milk on Ghanaian farms to assure safety of consumers.

P3-138 Inhibitory Activity of Reduced pH on *Salmonella* Survival in Calf Milk Replacer

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Introduction: Calf milk replacer (CMR) is commonly used in the rearing of young dairy calves. Ensuring CMR is free of bacterial pathogens will help young calves grow efficiently. Reducing pH values in CMR can present a hurdle to bacterial growth.

Purpose: This study aims to assess reduced pH in CMR and the inhibitory effects it has on *Salmonella enterica* serovars Dublin, Cerro, Montevideo, and Heidelberg.

Methods: CMR of varying pH was reconstituted using sterile deionized water and put into a 15-ml round bottom tube. *Salmonella* Dublin, Cerro, and Montevideo were grown overnight in trypticase soy broth on a rotating rack at 35°C until the culture reached a cell density of approximately 10^9 CFU/ml. The bacterial cells were harvested by centrifugation, washed, diluted in phosphate buffered saline, and added as either a cocktail of strains (*Salmonella* Dublin, Cerro, and Montevideo) or a single strain (*Salmonella* Heidelberg) to CMR at approximately 10^6 CFU/ml. The CMR was incubated at 38.5°C and samples were removed at 2, 4, and 8 h and plated on XLD agar to determine CFU/ml of *Salmonella*.

Results: When inoculated into control CMR, the cocktail of *Salmonella* strains increased 3 log CFU during an 8-h incubation, reaching a final concentration of approximately 10^9 CFU/ml. *Salmonella* grew to a lesser extent (2 log) in CMR with a pH value of 5.80. In contrast, reducing the pH value of CMR to 5.2 resulted in an approximately 2-log decrease in CFU during an 8-h incubation at 38.5°C. Similar results were observed when the experiments were performed using a single strain of *Salmonella* Heidelberg.

Significance: Dairy calves that are healthy and disease-free are a key element in a safe milk supply system for the public. Controlling of pathogens that might be present in the farm environment and contaminate CMR is a potential tool to accomplish this objective.

P3-139 Reduction of Surface-contaminated *Listeria monocytogenes* on Commercial Mozzarella Cheese by Electrostatic Spraying with the Probiotics *Lactobacillus salivarius* L28 and *Enterococcus faecium* J19

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Introduction: Commercial cheese that is produced with pasteurized milk is generally a safe product for consumption; however, under certain circumstances cheese may become contaminated and support the growth of human foodborne pathogens including *Listeria monocytogenes*.

Purpose: This study investigated the use of novel lactic acid bacteria (LAB) cultures *Lactobacillus salivarius* L28 and *Enterococcus faecium* J19 electrostatically sprayed on commercial mozzarella cheese to reduce *L. monocytogenes.*

Methods: A cookie cutter with a 3-in diameter and 1-in thickness was used to cut out round wheels of mozzarella cheese. Four treatments were evaluated: control, *L. salivarius* L28, *E. faecium* J19, and a combination of both strains. For each treatment, cheese was sampled on the following time points: D0, D1, D3, D7, D14, and D30. The surface of mozzarella cheese had a final concentration of *Listeria* 5 log CFU/cm^2. Electrostatic spraying application of LAB was conducted for 30 s at a concentration of 10^8 CFU/ml for each respective treatment. Each experiment was replicated three times and statistically analyzed by means of two-way analysis of variance and post-hoc Tukey's test.

Results: An immediate reduction of 1 log CFU/cm^2 was found on D0 when comparing the *E. faecium* LAB intervention to the control. Mozzarella cheese treated with *E. faecium* J19 had a statistically significant reduction (P<0.05) of *L. monocytogenes* that started at D3 and held through to D30. On D30, there was >2 log statistically significant reduction (P<0.05) when utilizing *E. faecium* J19 as an intervention. No statistically significant reduction (P>0.05) of *L. monocytogenes* was found when comparing the *L. salivarius* L28 LAB treated mozzarella cheese to the control samples for all 30 days.

Significance: This study describes a promising intervention for the environmental control of *L. monocytogenes* contamination on cheese surfaces by the use of novel antagonistic probiotic cultures.

P3-140 Prevalence and Characteristics of Foodborne Pathogens in Farmstead Cheeses

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Introduction: Raw milk is overproduced in Korea; thus, the Korean government has recently encouraged dairy farms to produce farmstead cheeses. However, since farmstead cheese production is a recent development, there is no obvious evidence if dairy farms control food safety properly.

Purpose: This study investigated the prevalence of foodborne pathogens and characteristics of the isolates from farmstead cheeses.

Methods: Forty-five samples for eight types of cheese (Berg, colby, cottage, Gouda, mozzarella, string, Tilsiter, and quark) were purchased from dairy farmstead plants and analyzed to detect *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium perfringens*, *Salmonella*, and *Campylobacter* spp. by plating; the isolate colonies were then subsequently identified through 16S rRNA sequencing analysis. The isolates were further analyzed for genes (*B. cereus*: *hblC*, *nheA*, *cytK*, *bceT*, *entFM*, and CER; *E. coli*: LT, ST, VT1, VT2, *eaeA*, *bfpA*, *aggR*, and *ipaH*; *S. aureus*: *mecA*) related to the pathogenicity by PCR. Also, the resistances of the *B. cereus* isolates to antibiotics (ampicillin, erythromycin, oxacillin, penicillin G, tetracycline, and vancomycin) were evaluated by a disc diffusion assay.

Results: B. *cereus*, *E. coli* and *S. aureus* were isolated from 17 samples (37.8%), two samples (4.4%), and seven samples (15.6%), respectively. The other four bacteria were not detected in farmstead cheeses. All *B. cereus* isolates were the diarrheal type; there was no emetic type. Also, most *B. cereus* isolates were resistant against oxacillin and penicillin G. The two *E. coli* isolates did not have any genes related to pathogenicity. The three isolates (42.9%) of seven *S. aureus* isolates were identified as methicillin-resistant *S. aureus* by *mecA* gene.

Significance: These results indicate that the food safety of farmstead cheese in Korea is very poor, and thus, regulation and monitoring to ensure the food safety of the products should be improved.

P3-141 Survival of Foodborne Pathogens in Raw Milk Cheddar Cheese during Ripening

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Introduction: Microbial safety of raw milk cheese has been controversial. During cheese ripening, pH, lactic acid bacteria, and water activity (A_w) can affect the survival of foodborne pathogens, but there are no obvious data evaluating the microbial food safety of ripened cheese.

Purpose: The objective of this study was to investigate the survival of foodborne pathogens such as *Listeria monocytogenes* and *Escherichia coli* during ripening for 60 days in cheddar cheese.

Methods: Three *L. monocytogenes* serotypes (1/2a, 1/2b, and 4b) and five *E. coli* strains (NCCP14037, NCCP14038, NCCP14039, NCCP15661, and NCCP11142) were used in this study. Each *L. monocytogenes* serotype and a mixture of *E. coli* strains were inoculated into raw milk or pasteurized milk, and cheddar cheeses were made from them. The cheddar cheese was stored at 13°C for 60 days. During ripening, A_w and pH of cheese were measured. The cell counts of lactic acid bacteria, *L. monocytogenes*, and *E. coli* were enumerated by plating.

Results: During ripening, A_w decreased from 1.000 to 0.700, and pH values were observed at 4.50 to 5.00 in all samples at the end of ripening. The concentrations of lactic acid bacteria in cheeses were 8.0 to 10.0 log CFU/g. *L. monocytogenes* cell counts were gradually decreased in samples. Especially, *L. monocytogenes* serotypes 1/2a and 1/2b in cheeses made from pasteurized milk were decreased (P<0.05) rapidly below detection limit (0.5 log CFU/g), but not in *L. monocytogenes* serotype 4b. *E. coli* cell counts were decreased (P<0.05) in pasteurized milk cheese during ripening, but not in raw milk cheese.

Significance: These results indicate that the microbial safety of raw milk cheddar cheese should be carefully controlled or a longer ripening period should be applied.

P3-142 Fate of *Listeria monocytogenes* during 90-day Aging of Gouda Cheese Prepared from Unpasteurized Milk

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Introduction: Unpasteurized milk may be contaminated with foodborne pathogens; thus, cheeses made from unpasteurized milk must be aged for at least 60 days at a minimum temperature of 1.7°C before entering interstate commerce. The survival of *Listeria monocytogenes* in unpasteurized milk Gouda cheese during aging has not been well-characterized.

Purpose: The purpose of this study was to determine survivability of *L. monocytogenes* in Gouda cheese prepared from unpasteurized milk throughout the 60-day aging process and beyond.

Methods: Gouda cheese was produced from unpasteurized milk artificially inoculated with 1 or 3 log CFU/ml of a four-strain *L. monocytogenes* cocktail. After molding curd, brining finished wheels, and waxing, cheese was stored at 10°C for 90 d. Sampling was conducted at weekly intervals by cutting out a 5-g pie-shaped wedge, followed by re-waxing. Cheese was homogenized with buffered *Listeria* enrichment broth, plated onto Brilliance *Listeria* agar, and enriched via U.S. Food and Drug Administration Bacteriological Analytical Manual methods. Two independent trials were conducted for each inoculation level. Data were statistically analyzed via analysis of variance (P≤0.05).

Results: Prior to waxing (0 d), the *L. monocytogenes* populations were 1.96±0.43 and 3.96±0.01 log CFU/g in Gouda cheese made from milk inoculated with 1 and 3 log CFU/ml, respectively. For the lower inoculation level, the *L. monocytogenes* population was 1.10±0.22 log CFU/g at 35 d, and negative via enrichment at 42 d for trial 1; in trial 2, *L. monocytogenes* survived the 90-d aging process (final population 1.26±0.00 log CFU/g). For both trials of the higher inoculation level, *L. monocytogenes* populations were 1.78±1.48 log CFU/g at 60 d and significantly higher (than both 0 and 60 d) at 4.50±0.34 log CFU/g at 90 d.

Significance: The results of this study provide insight into the survival of *L. monocytogenes* in unpasteurized Gouda cheese during 90-d aging and may assist in risk assessment of this pathogen.

P3-143 Population Dynamics of *Escherichia coli* O157:H7 during Unpasteurized Gouda Cheese Manufacture and Aging

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Introduction: The U.S. Food and Drug Administration (FDA) Code of Federal Regulations requires cheese made with unpasteurized milk be aged at a minimum 1.7°C for at least 60 days for consumer safety. However, these cheeses still pose safety concerns. The foodborne pathogen *Escherichia coli* O157:H7 has previously been implicated in outbreaks linked to Gouda cheese made from unpasteurized milk.

Purpose: This study assessed the survival of *E. coli* O157:H7 during manufacture and 60-day aging of Gouda cheese made from unpasteurized milk.

Methods: Unpasteurized milk was inoculated with 1 log CFU/ml, four-strain, rifampin-resistant cocktail of *E. coli* O157:H7. Milk was heated to 30°C, starter culture added, ripened for 30 min, and rennet added. After 45 to 55 min, curd was cut, stirred 20 min, 1/3 whey drained, and cooked at 38°C for 25 min. After molding, curd was weight-pressed under whey and pressure-pressed overnight. A 48-h brining and 72-h drying followed. Cheese was waxed and aged at 10°C. Sampling occurred throughout manufacture and aging. Samples were homogenized with modified buffered peptone water

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with pyruvate, plated onto rifampin plate count agar, and enriched according to FDA Bacteriological Analytical Manual methods. Two independent trials were conducted. Results were analyzed by Student's *t* test, $P \leq 0.05$.

Results: During manufacture, *E. coli* O157:H7 concentrated more in curd versus whey (1.34±0.55 log CFU/g versus 0.95±0.00 log CFU/ml, respectively). The initial population after drying was 1.48±0.75 log CFU/g. The last enumerable populations were at 7 d for trial 1 (2.01±0.32 CFU/g) and 18 d for trial 2 (1.46±0.17 CFU/g), which were not significantly different. The pathogen was undetectable via enrichment after 14 and 29 d for trials 1 and 2, respectively.

Significance: The results of this study suggest that the 60-day aging requirement may be suitable to eliminate the risk of *E. coli* O157:H7 in Gouda cheese made from unpasteurized milk.

P3-144 The Effect of Modified Atmosphere Packaging Conditions on Microbial Contaminants in Queso Fresco

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❖ ***Developing Scientist Competitor***

Introduction: The growing Hispanic population and the incorporation of Hispanic cuisine into the American diet has led to significant increases in Hispanic-style cheese production and consumption in the United States. Popular varieties, including queso fresco (QF), have limited shelf life due to high moisture content and low acidity. Modified atmospheric packaging (MAP) presents a potential clean label strategy to increase the shelf life of QF and similar soft cheeses.

Purpose: The objective of this study was to determine the effects of MAP conditions on the survival and growth of spoilage organisms in QF during refrigerated storage.

Methods: Individual 25-g QF samples were placed in 75-micron high barrier pouches (nylon/ethylene vinyl alcohol/polyethylene), packaged under one of seven conditions (air, vacuum, 100% nitrogen [N_2; MAP1], 30% carbon dioxide [CO_2]/70% N_2 [MAP2], 50% CO_2/50% N_2 [MAP3], 70% CO_2/30% N_2 [MAP4], or 100% CO_2 [MAP5]), and stored at 7°C. Samples were removed weekly through 35 days of storage for enumeration of coliforms, yeasts and molds, as well as mesophilic, psychrotolerant, and lactic acid bacteria (LAB).

Results: Differences in pH were noted between MAP5 treatments and air ($P<0.001$), vacuum ($P=0.012$), MAP1 ($P=0.007$), and MAP2 ($P=0.022$) samples. Cheeses stored under 100% N_2 (MAP1) had similar bacteria counts as the air and vacuum controls, which were the least effective in minimizing growth. Throughout storage, MAP5 treatments had lower yeast and mold, as well as psychrotolerant, mesophilic, and lactic acid bacteria counts compared to air, vacuum, and MAP1 samples ($P \leq 0.005$). Treatments containing ≥50% CO_2 maintained mesophilic and psychrotolerant bacteria and yeast and mold counts within 1.4 log CFU/g of the initial population levels. Overall, LAB levels were the least affected by MAP conditions.

Significance: These results show that modified atmospheres containing CO_2 can better inhibit microbial growth in fresh, soft cheeses when compared to air, vacuum, and 100% nitrogen treatments.

P3-145 Surface Application of a Novel Glycolipid to Control *Listeria monocytogenes* on Queso Fresco

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✦ ***Undergraduate Student Award Competitor***

Introduction: Post-lethality contamination of high moisture soft cheeses with *Listeria monocytogenes* (Lm) has resulted in several recent dairy related outbreaks of listeriosis. AM-1 is a biodegradable, non-toxic, and sustainably produced glycolipid that exhibits antimicrobial properties.

Purpose: The objective of this study was to determine the effectiveness of AM-1 for the control of *Lm* in milk and Queso Fresco (QF).

Methods: UHT whole milk containing varying concentrations of AM-1 was inoculated with a cocktail of *Lm* at 4 log CFU/mL and incubated at 35°C for 4 hours, then room temperature (20 ± 2°C) for 20 hours to mimic cheesemaking temperatures. Samples were collected for Lm enumeration at 0, 4, and 24 h. Samples of QF (25g) were surface inoculated with Lm (4 log CFU/g), dipped in solutions of AM-1 at 0% (control), 5%, 10%, 15%, and 20%, allowed to dry, and then vacuum packaged. Lm counts were determined after 24 h and weekly through storage at 7°C for 35 days.

Results: Minimum inhibitory and bactericidal concentrations of 800 and 1100 ppm were identified for AM-1 in milk, respectively. Reductions in *Lm* counts on QF ranged from ~1-2.5 log CFU/g by day 1 and remained ~2 log CFU/g lower than control on day. At day 14 counts in treatments >10% were ~1 log CFU/g lower than control. Significant treatment effects were observed when comparing each glycolipid treatment to control ($P < 0.01$) but not between glycolipid treatments.

Significance: Dip application of the glycolipid AM1 (³10%) can significantly reduce *L. monocytogenes* counts on QF early in storage (£ 14 days) but growth occurs thereafter. Higher concentrations of AM1 may not be necessary to achieve similar reductions and inhibition. Therefore, AM-1 has potential as a treatment for post-lethality exposed ready-to-eat foods that do not support the growth of Lm.

P3-146 High-pressure Pasteurization for Inactivation of Rifampin-resistant *Cronobacter sakazakii* in Reconstituted Infant Formula

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❖ ***Developing Scientist Competitor***

Introduction: Infections associated with *Cronobacter sakazakii* are often fatal in infants born prematurely and those younger than two months. Two historic outbreaks of *C. sakazakii* associated with infant formula in Tennessee in 1988 and 2001, as well as a 2016 outbreak associated with a premature infant in Pennsylvania, has brought increasing attention to endeavors for decontamination of infant formula.

Purpose: Current study investigated effects of high pressure pasteurization at 4 and 50°C for inactivation of the bacterium inoculated in reconstituted infant formula.

Methods: Various times (0, 1, 4, 7, and 10 min) and two intensity levels of 310 and 380 MPa (i.e., 45K and 55K PSI) of elevated hydrostatic pressure were investigated for inactivation of four-strain mixture of rifampin-resistant *C. sakazakii* inoculated in reconstituted infant formula. Experiment was conducted at 4 and 50°C in two biologically independent repetitions as blocking factors of a randomized complete block design. Samples were enumerated on tryptic soy agar supplemented with rifampin and yeast extract. Analysis of variance was conducted followed by LSD-based mean separation using OpenEpi software.

Results: During treatments at 380 MPa at 4°C, 1.59 and >6.01 log CFU/ml of inoculated pathogen were reduced ($P<0.05$) after 1- and 10-min treatments, respectively. At 50°C and 380 MPa, corresponding reductions ($P<0.05$) were >5.00, >5.90, >5.81, and >6.00 log CFU/ml after treatments for 1, 4, 7, and 10 min, respectively. At 310 MPa, the reductions ($P<0.05$) ranged from 1.35 to 3.67 and 3.10 to >5.72 log CFU/ml for samples treated at 4 and 50°C, respectively.

Significance: More than 5-log reduction of *C. sakazakii* in reconstituted infant formula is achievable as a result of optimized high pressure pasteurization that could be utilized to assure safety of infant formula, particularly for premature newborns and those with elevated risk of *Cronobacter* infection.

P3-147 Growth of *Listeria monocytogenes* on the Surface of Camembert Cheese is Influenced by Timing of Contamination

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Introduction: Camembert and related cheese varieties have a higher moisture content, undergo de-acidification during ripening (pH 4.6 to >7), and are open-air ripened. These conditions make these cheeses highly susceptible to *Listeria monocytogenes* contamination and outgrowth. Additional information on *L. monocytogenes* behavior in these cheeses is needed to identify and evaluate potential risk mitigation strategies.

Purpose: The purpose of this study was to quantify *L. monocytogenes* growth on the surface of Camembert when inoculated at different stages of ripening.

Methods: Camembert wheels were manufactured and surface-inoculated with *L. monocytogenes* (2 log CFU/g) at various points throughout ripening: i) prior to salt addition (day 0), ii) 1 h after the salt addition (day 0), iii) following surface yeast (*Geotrichum candidum* and *Kluyveromyces marxianus*) outgrowth (day 5), and iv) following surface mold (*Penicillium candidum*) mycelium development (day 11). The cheeses were sampled through ripening and shelf life (50 d) and enumerated for *L. monocytogenes* using CHROMagar *Listeria* with incubation at 37°C for 48 h.

Results: During the first five days of ripening, *L. monocytogenes* populations experienced reductions of 0.76 and 0.47 log CFU/g when inoculated pre- and post-salting (day 0), respectively. For cheeses that were inoculated early in the ripening process (0 to 5 d), *L. monocytogenes* populations increased to >6.87 log CFU/g by day 25. Cheeses that were inoculated later in the ripening phase (day 11) did not support significant increases (0.10 log CFU/g increase) in *L. monocytogenes* at day 25.

Significance: This study demonstrates the safety risk associated with environmental contamination of Camembert cheese during ripening. Effective strategies are needed to prevent contamination and outgrowth of *L. monocytogenes* in soft cheeses.

P3-148 Comparative Recovery of *Listeria* spp. from Dairy Environmental Surfaces Using 3M and World Bioproducts Environmental Sponges and Standard Enrichment and Enumeration Methods

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Introduction: Preventing *Listeria* contamination of artisan cheese requires routine and effective environmental monitoring of product contact surfaces within the production environment. The sensitivity of environmental monitoring methods is essential when testing for the presence of *Listeria* spp. within the processing environment as a way to control the risk of cheese contamination.

Purpose: Compare the efficacy of World Bioproducts environmental swabs against 3M environmental swabs for the detection of *Listeria monocytogenes* and *Listeria innocua* on dairy processing environmental surfaces.

Methods: Four environmental surfaces (dairy brick, stainless steel, plastic, and wood; *n*=216/surface type) were inoculated with *L. innocua* (GFP), *L. monocytogenes* ATTC 19115 and 1042 at high (10^8 to 10^9 CFU/ml) and low (10^0 to 10^2 CFU/ml) target concentrations. Inoculated surfaces were swabbed with World Bioproducts EZ Reach environmental swabs (WBEZ) with HiCap (WBHC) and Dey-Engley (WBDE) neutralizing broths, and 3M environmental swabs (3M) with Dey-Engley neutralizing broth. 3M *Listeria* Environmental Plate and Aerobic Plate Count petrifilm enumeration methods and U.S. Food and Drug Administration, AOAC BAX, and modified United States Department of Agriculture enrichment methods were used to compare sensitivity of recovery between environmental swabs.

Results: At high concentrations, enumeration results showed that all swab formats performed equally on all environmental surfaces. At low concentrations, 3M and WBEZ swabs recovered *Listeria* spp. from 89.7% of plastic, stainless steel, and dairy brick surfaces, but only 60.5% of wooden surfaces; 11.92, 46.3, and 65.3% were recovered at 10^0, 10^1, and 10^2 CFU/ml, respectively ($P<0.05$). Differences in recovery (WBDE [86.1%], WBHC [78.5%], 3M [82.4%]) from all surfaces were observed, where WBDE performed optimally. Strain influenced recovery, with *L. monocytogenes* 19115 recovered more effectively from wooden surfaces by 3M and WBEZ swabs, followed by *L. monocytogenes* 1042 and *L. innocua* ($P<0.05$).

Significance: Results may be discrepant due to media, strain, and variation in porosity of environmental surfaces; this should be taken into consideration when implementing environmental sampling plans.

P3-149 Exposure of *Bacillus cereus* Spores to Sublethal Stresses Prior to Spray Drying Increase Their Survival and Recovery in Milk Powder throughout a Storage Period of 180 Days

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❖ ***Developing Scientist Competitor***

Introduction: Bacillus cereus is a sporeforming bacteria with high incidence in the milk supply chain. Despite the thermal processes employed, spores may resist and be found in milk and dairy products such as milk powder. If appropriate conditions are provided, *B. cereus* can germinate and cause product spoilage or even produce toxins.

Purpose: This study aims to compare the survival of *B. cereus* spores on powdered milk under different spray drying conditions after exposure of sublethal thermal stress.

Methods: Three strains of *B. cereus* (436, B63, and 540) were exposed to four sublethal stress conditions. In treatments 1, 2, and 3, heat shocks were applied at different temperatures (80 and 90°C) from 10 to 30 min; in treatment 4, a combination of heat shock (72°C/15 s), cold shock (4°C/12 h), and new heat shock (72°C/15 s) was employed. After the drying process (190°C) in a bench-scale spray dryer, *B. cereus* counts were performed: fraction 1 (subjected to heat shock at 75°C/20 min) and fraction 2 (not subjected to heat shock). The milk powders produced were stored at room temperature for 180 d, and the two fractions of spores were enumerated.

Poster

Results: The application of the sublethal treatments increased the survival of B63 and 540 *B. cereus* strains in the drying process and their persistence in the samples produced after the storage period. Spore populations after 180 d of storage ranged from <1.00 to 4.41 log spores/g dry weight.

Significance: These results show that previous exposure of sporeforming bacteria such as *B. cereus* to sublethal stresses can increase their resistance to spray drying conditions, as well as lead to their augmented persistence during product storage. These findings can help to explain the dominance of some *B. cereus* strains in milk powder processing plants.

P3-150 Comparison of 3M Petrifilm Rapid Aerobic Count to Petrifilm Aerobic Count with a Bovine Raw Milk Matrix

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Introduction: Aerobic bacteria present in dairy products serve as an indicator of food spoilage and are crucial for determining compliance with the Grade "A" Pasteurized Milk Ordinance. Recently, the U.S. Food and Drug Administration (FDA) and the National Council on Interstate Milk Shipments (NCIMS) approved the use of the 3M Petrifilm Rapid Aerobic Count (RAC) medium. The Florida Department of Agriculture and Consumer Services (FDACS) Bureau of Food Laboratories (BFL) set forth to verify RAC application against the 3M Petrifilm Aerobic Count (PAC) medium.

Purpose: The purpose of this verification is to demonstrate that the RAC medium is comparable to the currently accepted PAC platform.

Methods: Forty-nine bovine raw milk samples were analyzed using the FDA/NCIMS 2400a-4 Petrifilm Aerobic & Coliform Count method. Calculated results from the RAC counts at 24±2 h were compared to calculated results obtained on PAC at 48±3 h. This comparison was made with the application of the mean log difference of CFU/ml in the countable range.

Results: Out of 49 raw milk samples tested, 23 were within the countable range and used in the analysis. Comparison of the average of the difference in log CFU/ml between RAC and PAC resulted in a mean log difference of -0.09. In addition, the mean percent recovery when comparing the log of RAC and log of PAC was 98%.

Significance: The RAC medium can determine the aerobic bacterial count in raw milk 24 hours faster than the PAC matrix. This improved turnaround time enables more rapid regulatory action that will enhance public health and food safety.

P3-151 Microbial Quality of Unpasteurized Ruminant Milk for Retail Sale in Maine, 1997 to 2008

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Introduction: Maine is one of few states in the United States that permits the sale of unpasteurized fluid milk in retail stores. Producers are required to obtain a state license and to submit product for testing a minimum of four times every six months. The Maine Milk Quality Laboratory conducts required tests and maintains a database of results. Analysis of this uniquely large data set can provide a number of insights relevant to the safety of unpasteurized dairy products.

Purpose: The objective of this analysis is to assess the factors that consistently affect the microbial quality of unpasteurized milk.

Methods: Data were obtained from the Maine State Milk Quality Laboratory and blinded for producer identity prior to receipt. Overall differences in microbial quality between cow and goat milk were determined using one-way analysis of variance. Effects of producer, month, and year on coliform count and standard plate count were assessed using GLM in JMP v. 13.

Results: Samples tested were more likely to violate legal limits based on coliform count regardless of species (41 and 31% fail rate for goat and cow milk, respectively). Mean values for standard plate count and coliform count were significantly higher in goat milk samples compared to cow milk (P<0.01), but there was no statistical difference in the percentage of samples violating legal standards by species. There was no significant effect of month or year on standard plate count (P>0.05), but the effect of month was significant for coliform counts (P<0.05), with August demonstrating the highest mean values.

Significance: A better understanding of the microbial quality of unpasteurized dairy can inform research directions for increasing safety of these products and be used in extension and outreach to educate licensed producers and consumers of such products.

P3-152 Assessment and Mitigation of Aflatoxin and Fumonisin Contamination in Animal Feeds and Aflatoxin M1 in Milk in Rwanda

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Introduction: Aflatoxins and fumonisins are fungal metabolites that contaminate crops and animal feeds under favorable growth conditions and have negative impact on public and animal health. Humans can be exposed directly through contaminated crops or via animal products when they are fed contaminated feeds. However, there is a scarcity of data in Rwanda regarding aflatoxin and fumonisin contamination in animal feeds.

Purpose: The main objective of this study is to assess the prevalence of aflatoxins and fumonisins in animal feeds and aflatoxin m1 in milk.

Methods: The total number of samples collected during each of the six rounds was 615 (219 from dairy cattle farmers, 317 from poultry farmers, 70 from animal feed vendors, and 9 from animal feed processors/manufacturers) for total aflatoxins and fumonisins analysis. A total of 170 milk samples were collected at different dairy farms for aflatoxin m1 analysis. Enzyme-linked immunosorbent assay (ELISA) and fluorometry were used to analyze feed and milk samples, respectively.

Results: In preliminary results, the aflatoxin means were 160.64±36.36, 170.06±50.52, 98.07±47.11, and 136.96±34.94 mg/kg for dairy farms, poultry farms, feed processors, and feed vendors, respectively. The fumonisin levels were 1.83±0.43, 1.52±0.5, 1.26±0.55, and 1.83±0.88 mg/kg for dairy farms, poultry farms, feed processors, and feed vendors, respectively. The aflatoxin m1 level was 0.89±1.64 mg/L with the maximum of 14.5 mg/L in milk samples.

Significance: There is an urgent need for mitigation strategies to reduce the high contamination level of mycotoxins in feeds and milk, hence protecting public health.

P3-153 Neomycin Selects for Antibiotic Resistance Genes in the Cecal Microbiome of Commercial Turkey Poults

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❖ ***Developing Scientist Competitor***

Introduction: Prolonged antimicrobial usage is considered the most important factor promoting the emergence, selection, and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine. As the human population grows steadily, there is even more pressure on the food systems and elimination of antimicrobial usage in agriculture does not seem like a viable option at this time. It would be of public health importance to determine how soon these microbes change to accommodate our use of antimicrobials.

Purpose: This study determines the effects of neomycin usage on the cecal microbiome of turkey poults within two weeks of administration

Methods: Cecal cores were obtained from a commercial turkey farm (10 pooled neomycin samples and 10 pooled control samples). The sample DNA was extracted using the MoBio PowerSoil Kit and sequenced using Illumina MiSeq. The sequence analysis was performed on the CosmosID web interface.

Results: The results indicate a shift in microbial profile from predominantly *Escherichia coli* to *Enterococcus faecium* and *Enterococcus faecalis*, with more richness in the poults treated with neomycin. The turkey poults treated with neomycin had a higher number and wider variety of antibiotic resistance genes and virulence factors compared to the control poults. The viral profile also shifted from viruses associated with *E. coli* to those associated with *E. faecium* and *E. faecalis*.

Significance: The data from this work suggests that resistance genes are selected within a few weeks of antibiotic usage. *E. faecium* and *E. faecalis* have emerged in the last decade as important bacterial pathogens in human nosocomial infections. These organisms are particularly challenging to eliminate because of their ability to adapt to environmental stresses.

P3-154 Report of Macrolide Resistance Gene *Erm(B)* in *Campylobacter* in the United States

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Introduction: Campylobacter is a leading cause of bacterial foodborne illness in the United States. *Campylobacter* causes a self-limited diarrheal illness; antimicrobial therapy is rarely necessary. With severe illness or high-risk patients, macrolides are recommended. Macrolide-resistant *Campylobacter* isolates have been described globally, and resistance is largely due to 23S rRNA mutations. Macrolide-resistant *erm(B)+ Campylobacter* have primarily been reported in China, and to the best of our knowledge have not previously been observed in the United States.

Purpose: The purpose of this study was to characterize an *erm(B)+ Campylobacter* isolated in the United States by genetic and phenotypic methods and understand the epidemiology of this case.

Methods: The Centers for Disease Control and Prevention's National Antimicrobial Resistance Monitoring System (NARMS) conducts antimicrobial resistance surveillance for human *Campylobacter* isolates. Illumina MiSeq sequencing of multi-drug resistant (MDR) isolates was conducted. Read-mapping procedures were used to detect relevant mutations contributing to resistance. Assembly-based and assembly-free methods were used to identify resistance genes. Broth micro-dilution antimicrobial susceptibility testing (AST) was conducted according to standard NARMS methodology. Epidemiological information was obtained from a case report submitted to the Foodborne Diseases Active Surveillance Network (FoodNet).

Results: Sequencing revealed a single *erm(B)+ C. jejuni* isolate. The isolate possessed several other resistance determinants and *erm(B)* was present in a novel genetic context. AST revealed the isolate was MDR, including resistance to macrolides. The isolate was cultured from the stool of an ill patient in 2016. Illness was reported after three weeks of travel in Malaysia and before returning to the United States.

Significance: We report an *erm(B)+ Campylobacter* in the United States. The *erm(B)* context is novel compared to previously reported *erm(B)+ Campylobacter*. Illness was likely acquired while travelling, consistent with previous reports of *erm(B)+ Campylobacter* found outside the United States.

P3-155 Identification and Characterization of a Multidrug-resistant *Salmonella enterica* serotype Heidelberg Outbreak Associated with Dairy Cattle in the United States

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Introduction: *Salmonella enterica* serotype Heidelberg is a common serotype which causes foodborne illness outbreaks typically associated with poultry in the United States. Antimicrobial resistance has remained level in *Salmonella* Heidelberg and multi-drug resistance (MDR) to five or more drug classes is uncommon. However, in November 2016, the Centers for Disease Control and Prevention (CDC) reported a multi-state outbreak of MDR *Salmonella* Heidelberg linked to exposure from dairy bull calves.

Purpose: To better understand the epidemiology of this outbreak, the resistance mechanisms and associated plasmids of MDR *Salmonella* Heidelberg isolates associated with this outbreak were characterized.

Methods: Whole genome sequencing (WGS) was performed on a subset of outbreak isolates (n=99) from human, animal, and environmental samples. Resistance determinants and plasmids were detected using ResFinder 3.0 and Plasmid Finder 1.3 databases. Plasmids were assembled in Geneious and annotated using Prokka, IS Finder, and MARA. Phenotypic testing was performed using broth microdilution (Sensititre, Oakwood Village, OH) and Clinical and Laboratory Standards Institute interpretive criteria.

Results: Of the 99 isolates tested, 91 were non-susceptible to 11 of 14 antimicrobials (from eight of 10 Clinical & Laboratory Standards Institute drug classes) tested, including clinically relevant 3rd generation cephalosporins and fluoroquinolones. This resistance profile for *Salmonella* has not previously been reported by National Antimicrobial Resistance Monitoring System at CDC. This resistance phenotype was conferred by 13 plasmid-borne genes, including a putative novel trimethoprim resistance gene. Twelve of 13 genes were carried on an IncA/C plasmid, a common cattle-associated MDR plasmid family. A *qnrB* gene was located on a ColE plasmid. IncA/C plasmids from this outbreak clustered into three distinct subgroups, based on mutations within the resistance regions, allowing us to track the spread of this MDR-plasmid and offering a unique insight into strain transmission dynamics.

Significance: Phenotypic and genotypic analyses of this outbreak revealed a novel MDR profile conferred by a cattle-associated plasmid in a typically poultry-associated *Salmonella* serotype.

P3-156 Transfer of Class 1 Integron-mediated Antibiotic-resistant Genes from *Salmonella* of Fly Origin to Susceptible *Escherichia coli* and *Salmonella* Strains

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❖ ***Developing Scientist Competitor***

Introduction: Foodborne bacterial pathogens, especially those that have developed resistance to antibiotics, are a serious threat to human health. Mobile DNA elements such as integrons are a critical source of antibiotic-resistant genes.

Purpose: The purpose of this study was to determine the prevalence of integrons in *Salmonella* strains isolated from flies captured on cattle farms, characterize the integron structures, and determine the transferability of identified integrons.

Methods: Presence of integrons and integron gene cassettes were screened using PCR in 606 *Salmonella* isolated from flies captured on cattle farms. DNA sequences of the identified gene cassettes were determined and compared against those deposited in the National Center for Biotechnology Information database. Integron-positive *Salmonella* strains were subsequently conjugated with an integron-negative *Salmonella* strain of fly origin and *Escherichia coli* C600 on tryptic soy agar (TSA) and selected farm samples including calf milk powder, three bovine feeds, drinking water, tail hair, bedding sand, and bovine feces.

Results: Two (0.3%) out of 606 isolated *Salmonella*, 438 and 442, harbored class 1 integrons. *Salmonella* 438 carried a gene cassette with *aadA7* (ca. 1.1 kb) and *Salmonella* 442 had a cassette with *drfA12-orfF-aadA2* (ca. 2.0 kb). The two integrons were transferrable through conjugation on TSA to the *Salmonella* strain of fly origin and *E. coli* C600 at efficiencies ranging from 1.47×10^{-6} to 4.25×10^{-5}. However, only *Salmonella* 442 was able to transfer its integron to the recipient cells on three out of the eight farm samples, namely cattle hair, bedding sand, and drinking water, with conjugation efficiencies ranging from 4.26×10^{-10} to 1.36×10^{-8}. Antibiotic-resistant genes not carried by integrons were co-transferred with integron-mediated antibiotic resistance genes.

Significance: These data revealed that *Salmonella* isolated from flies on cattle farms carried integrons which could disseminate antibiotic resistance genes through horizontal gene transfer in farm environments.

P3-157 Detection and Molecular Characterisation of *Escherichia coli* O26 from Cattle Faecal Samples in the North-West Province of South Africa

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Introduction: Shiga toxin-producing *Escherichia coli*, and particularly strains belonging to serotype O26, are considered an emerging pathotype and have been associated with a variety of infections in humans through the consumption of contaminated food products. Contamination of fresh produce, rivers, and borehole water is mostly through contact with faeces from cattle.

Purpose: This study was aimed at detecting and characterizing *E. coli* O26 strains from cattle using their virulence and resistance genes determinants.

Methods: A total of 600 faecal samples were collected during February 2015 to March 2017 from different cattle farms in the North-West Province of South Africa. Samples were analysed using sorbitol MacConkey agar. Thereafter, susceptibility, virulence and resistance genes profiles of the isolates were determined according to standard methods.

Results: A total of 450 isolates were confirmed as *E. coli* using *E. coli uidA* primer. Of the total, seven isolates were positive for characters of O26 serotype based on amplification of a specific target gene in the *wzx-wzy* region. BLAST search results confirmed these isolates as *E. coli* O26:H11 with assigned accession numbers. Of the seven isolates, five were obtained from beef cattle and two from dairy cattle in one sampling region (Rooigrond). All five isolates from beef were positive for *eae*, *hly*, *stx*1, and *stx*2, meanwhile two isolates from dairy were positive only for *hly* and *stx*2. Out of six antimicrobial genes tested, five isolates from beef cattle and none from dairy cattle were resistant to tetracycline, ampicillin, and sulfhonamide. Antimicrobial resistance gene analysis corroborated with susceptibility test in this study.

Significance: These findings are of great importance considering the fact that the *E. coli* O26 serotype has been overlooked in the area of study coupled with the fact that resistance and toxic genes can be transferred to other microorganisms in the animal gut, humans, and the environments, posing a threat to the food and medical industry.

P3-158 Efficacy of Ferrous and Alkaline Activated Persulfate in Inactivating *Escherichia coli* O157:H7

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❖ ***Developing Scientist Competitor***

Introduction: Chlorine-based sanitizers can react with organic matter and produce toxic disinfection byproducts (DBPs) during produce sanitation. Activated persulfate can generate reactive free radicals and has potential for produce sanitation without production of DBPs.

Purpose: We aimed to investigate the efficacy of activated persulfate to inactivate *Escherichia coli* O157:H7.

Methods: A five-strain cocktail of *E. coli* O157:H7 was treated with sodium persulfate activated by ferrous sulfate or sodium hydroxide for 2 min. The initial concentrations were 10 to 80 mM for persulfate, 2 to 160 mM for ferrous, and 5 to 30 mM for sodium hydroxide. The treated samples were plated on tryptic soy agar. The steady-state concentrations of free radicals were quantified with anisole and nitrobenzene using high-performance liquid chromatography coupled with diode array detection. Isopropanol, butanol, and benzoquinone were used to identify the major radicals in pathogen inactivation. The entire experiment was repeated three times.

Results: Both ferrous and alkaline activated persulfate showed high efficacy in inactivating *E. coli* O157:H7. The ratios between persulfate and activators had a significant effect. For ferrous activation, persulfate to ferrous ratio at 3:1 achieved the highest reduction (7.77 log CFU/ml). Further increase or decrease of ferrous content resulted in significantly ($P<0.05$) lower reductions. For alkaline activation, higher amount of sodium hydroxide resulted in higher reductions. Higher initial persulfate concentration also achieved higher reductions. In addition, activated persulfate treatments that achieved high microbial reductions were found to contain high concentrations of sulfate and hydroxyl radicals. Hydroxyl radicals were found to be the major contributing radical in inactivating *E. coli* O157:H7 for ferrous activation, while superoxide radicals were the major contributor for alkaline activation.

Significance: This study demonstrated the high efficacy of activated persulfate to inactivate *E. coli* O157:H7 and reveals the potential of activated persulfate for food safety applications.

P3-159 Antibiotic Resistance Profile of *Salmonella* Isolated from Leafy Green Vegetables in Ghana

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❖ ***Developing Scientist Competitor***

Introduction: Antibiotic-resistant pathogens, including *Salmonella*, have been isolated from leafy green vegetables in the United States and elsewhere. However, similar work has not been done in Ghana and surrounding countries.

Purpose: The purpose of this study was to determine: 1) the antibiotic resistance profile of the *Salmonella* strains isolated from leafy green vegetables (n= 328) collected from 50 farms and 37 market vegetable sellers in Accra, Ghana, and 2) the distribution of integrons among identified antibiotic-resistant *Salmonella* strains.

Methods: Antibiotic resistance profiles of isolated *Salmonella* to 12 antibiotics were determined using disc diffusion assay. The MICs of five antibiotics most commonly resisted by the *Salmonella* isolates were determined using the two-fold macrodilution method. PCR was used to detect the presence of integrons and integron gene cassettes in *Salmonella* cells. Amplified integron gene cassettes were purified and sequenced using Sanger sequencing techniques.

Results: Thirty-three *Salmonella* were isolated from sampled leafy green vegetables. The *Salmonella* isolates resisted at least one antibiotic and approximately 30% (10 of 33) of the isolates were multi-drug resistant. Most of the isolates were resistant to sulfisoxazole (27 of 33) and cefoxitin (12 of 33). The MICs of ampicillin, cefoxitin, streptomycin, tetracycline, and sulfisoxazole were ≥64, 32, 64, 16, and >1,024 µg/ml, respectively. Eight different patterns of antimicrobial resistance were observed among the *Salmonella* isolates, and the most common MDR pattern was AAuFox. One (3%) out of the 33 *Salmonella* isolates tested positive for integrase gene and class 1 integron gene cassette (800 kb in size). Nucleotide sequencing revealed the class 1 integron carried a single gene of *dfrA7*.

Significance: This study shows that leafy green vegetables grown or sold in Ghana are contaminated with *Salmonella* with mobile antibiotic resistance genes. Consumption of contaminated vegetables can be a possible route for acquiring antibiotic-resistant *Salmonella*.

P3-160 Withdrawn

P3-161 A Novel Peracetic Acid-based Meat Grinder Sanitation Process Optimization

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Introduction: There is a need for new rapid interventions which could be employed during ground beef processing to improve the overall safety of ground beef.

Purpose: The objective of this study was to optimize a rapid clean-in-place type meat grinder sanitation process using antimicrobial ice and solutions.

Methods: Four different types of antimicrobial ice and solutions were prepared from peracetic acid (PAA, 350 mg/L) and a combination of PAA with 2% FreshFX (PAAF), 2% Paradigm (PAAP), and 2% lactic acid (PAAL). The meat grinders were contaminated by processing 400-g beef trims spiked with either 3 or 6 log CFU/g *Escherichia coli* O157:H7 or *Salmonella* Typhimurium DT 104. The effectiveness of antimicrobial ice and solutions were tested by processing 1,000 g antimicrobial ice and 500 ml of corresponding solution through the meat grinders. After each ice treatment, 400 g un-inoculated beef was processed through the meat grinder to determine the pathogen transfer from the meat grinder to un-inoculated ground beef. Findings of the study were compared with deionized water ice (DI) and no treatment (NT) controls.

Results: For low levels of inoculation, all ice treatments except DI were able to to reduce both pathogens to non-detectable levels. Recoveries from the meat grinder inoculated with high levels *E. coli* O157:H7 were 5.95, 4.26, 3.79, 3.58, 3.54, and 3.50 log CFU/g and *S.* Typhimurium DT104 recovery was 5.86, 4.18, 3.63, 3.23, 3.35, and 3.46 log CFU/g for NT, DI, PAAP, PAAL, PAAF, and PAA treatments, respectively. All antimicrobial ice treatments were significantly ($P\leq0.05$) more effective in reducing cross-contamination in comparison to NT and DI controls. However, no significant difference between antimicrobial efficacies of different ice treatments were observed ($P\leq0.05$).

Significance: These antimicrobial ice treatments could serve as an easily applied antimicrobial intervention to improve the overall safety of ground beef.

P3-162 MICs of Eco-Friendly and Traditional Sanitizers against *Listeria monocytogenes*

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Introduction: Effective cleaning and sanitation programs are essential for controlling *Listeria monocytogenes* in food processing environments. As part of the "Design for the Environment" initiative by the U.S. Environmental Protection Agency, more antimicrobials with eco-friendly designations are becoming available. However, little is known about the ability of these antimicrobials to effectively control *L. monocytogenes*.

Purpose: To investigate the MIC of eco-friendly and traditional sanitizers against *L. monocytogenes* and the effect of temperature on the efficacy of a quaternary ammonium compound (QAC)-based sanitizer.

Methods: Six *L. monocytogenes* strains (Scott A, OS4-328, Ohio, California, ATCC 19115, and ATCC 19116) were exposed to QAC benzalkonium chloride (BAC) and three commercially available sanitizers: two eco-friendly (alcohol-[ALB] and citric acid-based [CAB]) and one traditional (chlorine-based [CLB]). BAC MICs were measured by agar dilution method (blood agar plates with 1 to 30 µl/ml BAC; incubated at 4°C for 7 days, 15°C for 72 h, or 30°C for 48 h). Microbroth two-fold dilution method in tryptic soy broth was used to measure MICs for ALB (1.15 to 147 µl/ml), CAB (0.025 to 3.3 µl/ml), and CLB (3.91×10^{-4} to 0.025 µl/ml) at 30°C for 48 h. Inoculum levels were 6 to 7 log CFU/ml.

Results: All strains exhibited similar MICs for ALB (30.6 to 49 µl/ml), BAC (<1 to 3 µl/ml), and CLB (0.003 µl/ml). *L. monocytogenes* Ohio was more susceptible to CAB (MIC 0.31 µl/ml) compared to other strains (MICs 1.4 to 2.2 µl/ml). MICs were lower than the manufacturer recommended concentrations (MRC) for ALB (6 to 10×), CAB (3 to 10×), and CLB (33×). CLB was the most effective at controlling *L. monocytogenes* growth. Lower BAC MICs were observed at 4 to 15°C for all strains compared to 30°C.

Poster

Poster

Significance: All tested *L. monocytogenes* showed susceptibility to eco-friendly and traditional sanitizers at concentrations below MRCs. Notably, BAC was more effective at lower temperatures. This study highlights the importance of sanitizer selection and temperature application as considerations in sanitation programs in the food industry.

P3-163 Antibiotic-resistance Gene Profiles of *Escherichia coli* Isolated from Fresh Produce Sold at Informal Market in Tembisa, Gauteng Province, South Africa

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Introduction: The emergence of foodborne bacteria carrying antibacterial resistance (ABR) genes has been recognized as a serious threat to human health, especially due to the increase in number and variety of resistance genes that confer a wide range of resistance traits to those microorganisms.

Purpose: The purpose of the study was the identification of the ABR genes carried by the *Escherichia coli* isolates obtained from fresh produce.

Methods: A total of 22 *E. coli* isolates were analyzed using uniplex PCR targeting 43 selected genes related with the resistance to tetracycline (13), cefoxitin (1), aminoglycosides (1), fluoroquinolones (2), phenicols (3), sulfonamides (2), penicillin (1), quinolones (2), β-lactams (18), and nitrofurantoin (2). PCR reactions were conducted following conditions previously described, as well as in the instructions included with the commercial PCR Master Mix. Positive controls were included for some genes according to the availability of controls at the time of the study. For those genes with no available controls, touchdown PCR was performed to identify potential positive samples. All presumptive positive amplicons were further sequenced to confirm specificity and to identify the corresponding allele in those genes with multiple alleles.

Results: Results shown that 100% (22) of isolates carry the *blaTEM* gene (β-lactams); 77.3% (17) *tetA*, 4.6% (1) *tetB*, 9.1% (2) *tetE*, and 32% (7) *tetM* (tetracyline); 95.5% (21) *sulI,* and 100% (22) *sulII* (sulfonamides); 9.1% (2) and 4.6% (1) showed mutations on *gyrA* and *parC* genes, respectively; finally, mutations in *nfsA* and *nfsB* genes were identified in 13.7% (3) and 18.8% (4) of isolates, respectively.

Significance: This study has provided relevant data about the presence of ABR genes in *E. coli* isolated from fresh produce sold informally in South Africa. This study has laid the foundation for the development of a routine method for the screening of ABR genes.

P3-164 Comparison of the Effectiveness of Antimicrobial Interventions on Reducing Antibiotic-resistant and Susceptible Beef-associated *Salmonella*

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Introduction: Concerns about pharmacologic antibiotic use in beef cattle production have arisen due to the perceived possibility of the emergence and transmission of antimicrobial resistant (AMR) bacteria into the beef supply. However, it is not fully known if AMR bacteria strains have enhanced ability to resist the bactericidal efforts imposed by commercial antimicrobial interventions (AMIs) used in beef processing.

Purpose: The objective of this study was to compare the efficacy of different AMIs on reducing AMR and non-AMR *Salmonella* contamination on fresh beef.

Methods: Two approaches were employed. In approach 1, a laboratory experiment was designed to determine whether AMI alone or sequential treatments equally affect inoculated AMR and non-AMR *Salmonella*. In approach 2, systematic review and meta-analysis were conducted to summarize what is known about the impacts of commercially available AMIs on beef-associated AMR and non-AMR *Salmonella* strains.

Results: Experiment results showed there was no significant difference in the concentration reduction between AMR and non-AMR *Salmonella* on the fresh beef surface treated with the same antimicrobial. The order of decontamination efficacy is: lactic acid (LA) = LA + peracetic acid (PAA) > PAA, where LA + PAA is for the combination of LA and PAA in sequence. From the systematic review, nine citations with eligible data were used for meta-analysis, from which acid, oxidizer, physical, thermal, and other interventions were evaluated. Efficacy ratio for a particular intervention was computed as the ratio of the efficacy of eliminating AMR strains to the one for non-AMR strains. Findings showed most of the efficacy ratios were around 1.0.

Significance: Results indicate that most antimicrobial interventions employed at the beef processing can exert similar bactericidal effects regardless of the antibiotic resistance profile.

P3-165 Use of a Drip Flow Reactor to Evaluate Foodborne Pathogen Biofilm Formation and Interventions in Meat and Poultry Processing Environments

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Introduction: Listeria monocytogenes causes 19% of foodborne illness cases that result in death in the United States. Unfortunately, this pathogen is capable of forming biofilms on surfaces and has become part of the resident flora of food processing environments. New technologies to evaluate attachment, growth, and biofilm formation are continuously needed.

Purpose: Evaluation of antimicrobial treatments, current, and novel non-thermal intervention technologies to inhibit biofilm growth of foodborne pathogens on food processing surfaces.

Methods: A drip flow biofilm reactor (compliant with ASTM E2647-13) was used to evaluate the growth of *L. monocytogenes* on stainless steel in comparison to biofilm formation on an antimicrobial copper surface (Alloy C706). Surface coupons of 16 cm^2 were inoculated into the reactor with 16 ml of a three-strain *L. monocytogenes* (ATCC 19118/49594/51414) cocktail (8 log CFU/ml concentration), allowed to attach for 6 h, followed by a continuous phase with a supply of 50 ml/h of 3 g/L of tryptic soy broth to promote biofilm growth. Samples were taken at fixed intervals, rinsed to remove planktonic cells, scraped to remove biofilm, and homogenized at 22,000 rpm. Samples were plated onto modified Oxford agar and incubated at 37°C to enumerate viable cells as log CFU/cm^2.

Results: An initially attached population of 6.72 log CFU/cm^2 can grow as a biofilm on the surface up to 8.52 log CFU/cm^2 on stainless steel, increasing 1.81 log CFU/cm^2 (95% confidence interval ±1.03 log CFU/cm^2, two-sample *t* test, *P*=0.004) after 48 h at 37°C. On the other hand, there were no recovered attached/biofilm cells on the surface of C706, either at time 0 H or 48 H (detection limit 2.1 log CFU/cm^2), which is statistically different from stainless steel (one-sample *t* test, *P*<0.001 in both cases).

Significance: Results can be used to evaluate the use of antimicrobial surfaces and other interventions for their capacity to inhibit biofilm formation of the foodborne pathogen *Listeria monocytogenes.*

P3-166 Microbial Profiling and Pathogen Inactivation by Copper-containing Coating Materials and Drains at Poultry and Pork Processing Facilities

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Introduction: Copper is an antimicrobial material with multiple applications in medical and institutional facilities. Pathogen reductions of more than 5 log CFU/cm^2 have been demonstrated in our laboratory against *Listeria monocytogenes* and *Salmonella* spp. with copper-containing alloys C706 and C710; therefore, an intervention based on the use of coating materials that contain copper as an antimicrobial has been developed and tested during commercial processing conditions.

Purpose: Evaluate the effectiveness of copper-containing coatings to reduce counts of microbial indicators and contribute to disinfection of drains in poultry and pork processing facilities.

Methods: Different drainage configurations were selected as high-risk areas of harboring foodborne pathogens. Samples were taken from three processing plants (one poultry, two swine) at different days of the week, at different steps of processing (five locations), and also at different times after the beginning of operation (0 to 16 h). Surfaces of 200 cm^2 were swabbed before and after intervention (2016 and 2017) and sent for laboratory analysis to quantify: aerobic plate count (APC), *Enterobacteriaceae*, and detection of *L. monocytogenes* (*n*=60; *n*=30 for each plant). The results were compared with a general linear model including all the variables and least squares regression.

Results: In the poultry plant, the APC was reduced by 1.01 log CFU/cm^2 and *Enterobacteriaceae* were reduced by 1.76 log CFU/cm^2 after 16 h of operation; however, low detection of *L. monocytogenes* was not enough to observe statistically important differences (6.7% control versus 0% treatment). The swine plant presented similar results: reduction of 1.04 log CFU/cm^2 for APC, 0.54 log CFU/cm^2 for *Enterobacteriaceae*, and significant reduction of *L. monocytogenes* (30% control versus 0% treatment; intervention variable *P*<0.05).

Significance: Results are promising, showing the effectiveness of an intervention with copper paint on drainage configurations of food processing plants.

P3-167 Characterization of Antimicrobial-resistant Genes and Plasmids of *Salmonella* Enteritidis Isolated from Clinically Ill Children in Shanghai, China

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Introduction: Salmonella species are recognized as a common cause of childhood infections, particularly gastroenteritis, bacteremia, and typhoid (enteric) fever all over the world.

Purpose: Our study investigated the distribution of antimicrobial resistant genes and the distribution of plasmid incompatible groups in 92 *Salmonella enterica* serovar Enteritidis isolated from children younger than 10 years old with clinical illness in Shanghai from 2010 to 2012 in order to better understand the generation and transmission of *Salmonella* resistance and also provide data for epidemiology.

Methods: The antimicrobial resistant genes of 92 isolates of *Salmonella* Enteritidis were screened by PCR. The 18 plasmid incompatible groups of 75 multidrug resistant isolates were analyzed by PCR replicon typing.

Results: The *sul*1, *sul*2, and *gyr*A genes were the highest in 92 isolates of *S.* Enteritidis and all of them were 100%, followed by *tet*A (98.9% [91]), *par*E (98.9% [91]), *par*C (97.8% [90]), *gyr*B (96.7% [89]), *tet*B (95.7% [88]), *Aph(3')*-Ⅱa (94.6% [87]), *aac(6')*-Ib-cr (94.6% [87]), *sul*3 (94.6% [87]), bla_{CTX-M} (92.4% [85]), bla_{TEM} (91.3% [84]), *tet*G (82.6% [76]), bla_{OXA} (70.7% [65]), bla_{CMY} (66.3% [62]), and *Cat* (1.1% [1]), while *bla*PSE, *aad*A, and *qnr*B carrying rates were 0% (0 of 92). Among 75 isolates of MDR *Salmonella* Enteritidis, 63 isolates contained Inc Y plasmid groups with carrying rates of 84.0%, followed by Inc FIIs groups and Inc FIB groups, 75.0 and 69.3% respectively. And for Inc I1 groups, Inc FrepB groups, Inc FIC groups, Inc FIA groups, Inc N groups, Inc HI1 groups, Inc HI2 groups, and Inc A/C groups , carrying rates were 53.3, 41.3, 29.3, 16.0, 14.7, 12.0, 1.3, and 1.3%, respectively. In addition, all MDR isolates did not carry Inc K groups, Inc B/O groups, Inc X groups, Inc L/M groups, Inc W groups, Inc T groups, and Inc P plasmid groups.

Significance: Salmonella Enteritidis carrying multiple antimicrobial resistant genes has become a common phenomenon. There is a certain correlation between resistant plasmids and their host cells with resistant genes. However, there is no direct relationship between resistant plasmids and the resistant phenotype.

P3-168 Withdrawn

P3-169 High Prevalence of Antibiotic Resistance Associated with Urban Agricultural Environment with the Potential of Horizontal Gene Transfer

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❖ ***Developing Scientist Competitor***

Introduction: Urban agricultural environments are a potential reservoir of antibiotic resistance, as many soil bacteria can produce antibiotic substances and are antibiotic-resistant. The nature and extent of this antibiotic resistance reservoir is yet to be determined.

Purpose: This study was aimed at examining the antibiotic resistance phenotypes, soil bacterial composition, and the potential of horizontal gene transfer associated with urban agriculture.

Methods: Bacteria were isolated from 15 soil samples and 45 vegetable samples collected from three urban gardens located in the metro area of Detroit, Michigan. Antibiotic resistance profiles of soil bacteria were determined using the Sensititre Antimicrobial Susceptibility System. MiSeq Illumina sequencing was performed on soil DNA to reveal the bacterial composition in soil. A conjugation experiment was designed to demonstrate the horizontal transfer of tetracycline resistance and confirmed by PCR amplification of *tet*M in the transconjugants.

Results: A total of 226 bacteria were isolated from soil and vegetable samples, with the majority of isolates being gram negative. Gram negative bacteria showed highest resistance to ampicillin (85.0%), followed by cefoxitin (80.56%), amoxicillin/clavulanic acid (67.78%), chloramphenicol (62.78%), ceftriaxone (60.56%), and gentamicin (45.56%). All gram positive bacteria (100%) were resistant to penicillin, gentamicin, and kanamycin. *Proteobacteria* was the most prevalent phylum and identified in 37.32% of the MiSeq reads, followed by *Actinobacteria* (19.45%), *Firmicutes* (12.28%), and *Bacteroidetes* (9.76%). *Granulicella* (2.78%), *Rhodoplanes* (2.78%), *Flavobacterium* (1.98%), *Kaistobacter* (1.85%), and *Niastella* (1.43%) were the top five genera detected. Four out of seven gram negative bacteria with appropriate antibiotic resistance phenotypes successfully transferred tetracycline resistance to *E. coli* DH5α (amp^r, kan^r). The transfer rate was 1.50×10^{-4} to 2.15×10^{-3}/recipient cell.

Poster

Significance: Antibiotic resistance is prevalent in urban agricultural environments and has the potential for horizontal gene transfer.

P3-170 Comparative Assessment of Antimicrobial Resistance in *Escherichia coli* isolated from Beef Production Systems and Human Sewage

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❖ ***Developing Scientist Competitor***

Introduction: Antimicrobial-resistant (AMR) bacteria can be disseminated through contaminated food, water, and contact with humans and animals.
Purpose: This study examined AMR in putative extended spectrum β-lactamase *Escherichia coli* isolates (ESBLs) from a beef production system and human sewage in Alberta, Canada.
Methods: A total of 713 putative ESBLs were obtained from multiple points of the beef production system. Samples were either enriched in cefotaxime prior to selective plating onto MacConkey agar containing ceftriaxone or selectively plated without enrichment. ESBL isolates were obtained from cattle faeces (CF) (*n*=385), catch basin water (CB) (*n*=112), municipal sewage (ST) (*n*=98), surrounding streams (SW) (*n*=89), and beef processing plants (BP) (*n*=29). Non-selected isolates (689) were obtained directly on MacConkey agar without enrichment or antibiotic selection, CF (*n*=142), CB (*n*=142), ST (*n*=97), SW (*n*=130), soil (*n*=10), and BP (*n*=159). All isolates were screened for resistance to 12 antimicrobials using the disc diffusion method according to CLSI guidelines. ESBLs were screened for ESBL genes *SHV*, *TEM*, *OXA*, *CMY*, and *CTX-M*.
Results: Putative ESBLs from ST showed the highest multidrug resistance (MDR) (98.0%), followed by CF (97.1%), CB (71.8%), SW (60.2%), and (BP) 50.0%, (*P*=0.0001). Overall MDR was 86.5% in putative ESBLs, as opposed to only 27.1% of non-selected isolates. MDR in non-selected *E. coli* was lower in CF (45.1%), soil (40.0%), CB (36.6%), SW (26.2%), ST (13.6%), and BP (10.7%) (*P*=0.0001). Putative ESBLs (95.9%) possessed at least one ESBL gene.
Significance: MDR levels were highest in isolates from human sewage, enriched beef cattle feces, and much lower in isolates from the broader environment and processing plants. Equally high MDR levels in ST isolates, obtained without enrichment as compared to enriched CF isolates, imply that MDR in ST were far higher. Our work suggests that ESBL prevalence is highest in sources closest to the point of antimicrobial use, i.e., in isolates obtained from sewage or beef cattle feces.

P3-171 Antimicrobial-resistance Profiling of Bacteriophage-insensitive *Salmonella enterica* Mutants

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❖ ***Developing Scientist Competitor***

Introduction: Exploitation of bacteriophages (phages) to control foodborne pathogens has shown considerable promise. However, little is currently known regarding bacteriophage-insensitive mutants (BIMs) and their response to other antimicrobials.
Purpose: To evaluate the sensitivity of *Salmonella* Agona S5-517 BIMs to tetracycline, which wildtype *Salmonella* Agona is resistant to, and chlorine, a sanitizer commonly used in the food industry.
Methods: BIMs (*n*=5) were isolated by spotting SI1, a phage previously isolated by our lab, on a lawn of *Salmonella* Agona and serially culturing colonies that grew in the centers five times. Resistance was confirmed by spot-testing SI1 on lawns of putative BIMs. To test for the tetracycline sensitivity, wildtype and BIMs were spectrophotometrically assayed in Mueller-Hinton broth supplemented with varying concentrations of tetracycline for 16 h at 25°C. Determination of the lag phase duration (OD_{600}<0.2) was performed for all isolates. To assay for chlorine resistance, wildtype and BIMs of *Salmonella* Agona were treated with 125 ppm chlorine for 5 min and spread onto tryptic soy agar for enumeration. All experiments were conducted in triplicates. Lag phase duration and log reduction values were analyzed with a one-way analysis of variance and a post-hoc Tukey's Honest Significant Difference test to compare resistances between isolates (α=0.05).
Results: The lag phase of BIMs subjected to 1.28 mg/ml tetracycline significantly increased by 3±0.5 h to 5.5±0.5 h compared to wildtype *Salmonela* Agona (*P*<0.05), indicating attenuated resistance to tetracycline. Conversely, log reduction values of the BIMs upon chlorine treatment ranged from 6.56±0.77 to 7.51±0.25 log CFU/ml, compared to 9.96±0.00 log CFU/ml of the wildtype, indicating significantly enhanced resistance of the BIMs to chlorine (*P*<0.05).
Significance: The results describe the diverse phenotypes of *Salmonella* BIMs. Attenuation or enhancement of antimicrobial resistance should be taken into consideration when designing phage therapies for the food industry.

P3-172 The Effect of Third Generation Cephalosporin Use on Antimicrobial Resistance in Dairy Farms in Korea

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❖ ***Developing Scientist Competitor***

Introduction: Antimicrobial resistance to third-generation cephalosporins (3G-Ceph) in gram negative bacteria is an emerging concern worldwide. The prevalence of 3G-Ceph resistant bacteria in livestock has also increased in the past few years in Korea. It is important to elucidate the relationship between the use of 3G-Ceph and the increased antimicrobial resistance to these antimicrobials on local farms.
Purpose: The current study was performed to evaluate the effect of 3G-Ceph (ceftiofur) use on the increase of antimicrobial resistance by comparing the antimicrobial resistant rates between farms that use 3G-Ceph and those that do not.
Methods: A total of 490 raw milk samples were collected from farms using ceftiofur (*n* =240) and non-using farms (*n*=250). *E. coli* were isolated using EC broth enrichment followed by streaking on ECC agars and confirmed by a specific PCR. Antimicrobial susceptibility tests were conducted according to the guidelines of the Clinical Laboratory Standards Institute. Antimicrobial resistance genes related to 3G-Ceph resistance were detected by specific PCRs.
Results: A total of 160 *E. coli* (123 from using farms and 37 from non-using farms) were isolated. The rate of antimicrobial resistance to 3G-Ceph was significantly higher in 3G-Ceph using farms (9.7%) than in non-using farms (2.7%). The MICs of ceftiofur were measured as less than 8 ug/ml in isolates from non-using farms and 64 to 1,024 ug/ml from using farms. All the resistant isolates from using farms harbored a CTX-M gene, whereas none from non-using farms did.
Significance: Contaminated raw milk can be a vehicle for transmission of antimicrobial resistant bacteria from dairy to humans. The current study indicates the overuse of 3G-Ceph in dairy farms could increase the risk of transmission of 3G-Ceph resistant bacteria to a human community. Therefore,

prudent use of this category of antimicrobials in dairy farms is warranted to prevent the dissemination of 3G-Ceph resistant bacteria from animals to humans.

P3-173 Prevalence and Antimicrobial Susceptibility of *Acinetobacter* spp. on Swine Farms in Korea

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❖ ***Developing Scientist Competitor***

Introduction: The genus *Acinetobacter*, including *A. baumannii*, is one of the most life-threatening pathogens due to increased antimicrobial resistance. However, limited information is available on antimicrobial-resistant (AR) *Acinetobacter* spp. from livestock. It is important to investigate the prevalence of AR *Acinetobacter* in livestock to evaluate the potential risk of transmission to humans.
Purpose: The purpose of this study was to investigate the prevalence of AR *Acinetobacter* in swine farms by sampling from pigs and pig-associated workers to gain the basic information to evaluate the potential risk of transmission of AR *Acinetobacter* from animals to humans.
Methods: A total of 911 swab samples (760 from pigs and 151 from humans) were collected from swine farms in Korea. *Acinetobacter* spp. were isolated by using the CHROMagar-*Acinetobacter* agars, and the species were confirmed by 16S rRNA sequencing followed by blasting in GenBank. An antimicrobial susceptibility test was performed against 13 antimicrobials (nine different classes).
Results: A total of 87 *Acinetobacter* spp. (80 from pigs and 7 from humans) were isolated. Among them, 36 isolates were confirmed as *A. haemolyticus*, 12 as *A. variabilis*, nine as *A. baumannii*, and 30 as other *Acinetobacter* spp. The overall AR rates were relatively low, with the highest AR rate to ciprofloxacin (4.6%). AR rates to minocycline, tigecycline, piperacillin, ceftazidime, cefepime, imipenem, and tobramycin were less than 3%, and all the isolates were susceptible to other tested antimicrobials. Interestingly, all *A. baumannii* were susceptible to all the tested antimicrobials.
Significance: Although massive use of antimicrobials in swine industry was reported, the results of this study showed overall low antimicrobial resistance rates in *Acinetobacter* spp. from swine farms. However, the use of antimicrobials and resistance are closely related. Therefore, continuous surveillance is needed to prevent the emergence and dissemination of AR *Acinetobacter* spp. in the porcine industry.

P3-174 Biofilm Formation of Wild-type and Pressure-stressed *Cronobacter sakazakii* and *Salmonella* Serovars and Their Sensitivity to Sodium Hypochlorite

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❖ ***Developing Scientist Competitor***

Introduction: Aggregate cells are the predominant physiological mode of bacterial proliferation in food processing and clinical settings, and microbial biofilms are responsible for as high as 80% of all bacterial infections.
Purpose: Current study discusses biofilm formation of two pathogenic species on an abiotic surface and validates a decontamination intervention against wild type and pressure-stressed phenotypes of the bacteria.
Methods: Four-strain mixtures of *Cronobacter sakazakii* and *Salmonella* serovars were used for biofilm formation for up to 14 days. Biofilm formation/enumeration/decontamination was conducted on the surface of stainless steel coupons (finish 2b) at 7 and 25°C. After removal of loosely attached cells, samples were neutralized using D/E neutralizing broth and separated from coupons using glass beads method prior to culture dependent analyses. Pressure-stressed phenotypes were prepared by exposing the isolates to sub-lethal elevated hydrostatic pressure at 15,000 PSI (approximately 100 MPa) for 15 min. The experiments were conducted in two biologically independent repetitions as blocking factors of a randomized complete block design and analyzed statistically using OpenEpi software.
Results: In excess of 3.26 and 2.17 log CFU/cm^2 increase (*P*<0.05) in biofilm mass on stainless steel coupons was observed during 14 days for wild type and pressure-stressed phenotypes of *Salmonella*, respectively, at 25°C. Treatment on days 0, 7, and 14 of biofilm formation were responsible for reductions of 2.54, 1.78, and 1.88 log CFU/cm^2 (*P*<0.05) of biofilms of wild type *Cronobacter sakazakii*, respectively, at 25°C and led to reductions of 2.76, 1.62, and 0.99 log CFU/cm^2 (*P*<0.05) of biofilms of pressure-stressed *Cronobacter sakazakii.*
Significance: Overall, wild type and pressure-stressed phenotypes of *Salmonella* serovars and *Cronobacter sakazakii* exhibited similar biofilm formation capability and sensitivity to the sanitizer. Sodium hypochlorite, at highest concentration recommended by manufacturer, was efficacious only against planktonic cells and unable for complete elimination of one- and two-week mature bacterial biofilms.

P3-175 Antimicrobial Resistance Profiles of *Escherichia coli* from European Starlings (*Sturnus vulgaris*) Associated with Concentrated Animal Feeding Operations

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Introduction: Bird species that are in frequent contact with livestock production may play an important role in the dissemination and propagation of antimicrobial resistance (AMR). In this study, we investigated AMR *Escherichia coli* isolated from whole GI tracts of European starlings (*Sturnus vulgaris*) associated with concentrated animal feeding operations via genetic methods following culture-based isolation.
Purpose: To identify common genetic determinants conferring extended-spectrum and AmpC-type (plasmid-mediated *ampC* gene of *Citrobacter freundii* origin) β-lactamase activity within these *E. coli* isolates and classify these isolates into relevant phylogenetic groups.
Methods: Isolation involved cefotaxime- (CTX) and ciprofloxacin- (CIP) supplemented media for selective recovery of specific AMR phenotypes. Confirmation was conducted via matrix-assisted laser desorption ionization time of flight mass spectrometry, followed by determination of susceptibilities to 18 antimicrobials. A total of 1,477 European starlings were examined from Colorado (four farms), Iowa (one farm), Kansas (six farms), Missouri (two farms), and Texas (five farms), yielding 61 CTX-selected isolates and 177 CIP-selected isolates (4 and 10% of European starlings, respectively). We screened these isolates for genetic determinants of β-lactam resistance, focusing on predominant class A ß-lactamase genes bla_{CTX-M}, bla_{SHV}, bla_{TEM} and CIT-type AmpCs in combination with phylogenetic typing.
Results: β-lactamase genetic determinants identified included $bla_{AmpC-CIT}$ (*n*=57), bla_{CTX-M} (*n*=35), and bla_{TEM} (*n*=90). CIT-type AmpCs mediated resistance to all β-lactams tested except imipenem (penicillins, β-lactam/β-lactam inhibitors, monobactams, cephalosporins), while bla_{CTX-M} and bla_{TEM} were also identified in multiple isolates, primarily mediating resistance to third generation cephalosporins and penicillins, respectively. Phylotyping permitted determination of 29 isolates as belonging to group A, 121 to group B1, 37 to group D, and only one isolate each to the groups C and B2.

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Significance: European starlings carry *E. coli* that are resistant to antimicrobials categorized by the World Health Organization as critically important, highly important, and important, as well as class A ß-lactamase genes, posing a risk for the maintenance and dissemination of AMR in agricultural operations by this invasive bird species.

P3-176 Low Levels of Antimicrobial Resistance among Indicator Bacteria Isolated from Wildlife Associated with Produce Fields

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Introduction: Environmental inputs contribute to the maintenance and spread of antimicrobial resistance (AMR) in agricultural operations. Wildlife may serve as reservoirs of AMR bacteria and their associated genes. When wildlife visit produce fields, fecal shedding of AMR bacteria and pathogens can occur, contaminating produce fields, harvesting equipment, or irrigation systems, leading to contamination of produce with AMR bacteria.

Purpose: To investigate the role of wildlife as a vehicle for AMR transmission to produce fields located in an area with limited anthropogenic activity through isolation and characterization of the indicator bacteria *Escherichia coli* and *Enterococcus* spp. with priority AMR phenotypes from produce and produce-associated wildlife.

Methods: A total of 253 wildlife fecal samples and 176 leafy green samples were collected between June and September 2015 from produce farms in the San Luis Valley, Colorado. Isolation was performed on MacConkey agar, Levine's Eosine-Methylene Blue agar (*E. coli*), Enterococcosel agar (*Enterococcus* spp.), or the same media supplemented with ciprofloxacin, cefotaxime, erythromycin, or tetracycline. Confirmation of isolates was accomplished via matrix-assisted laser desorption ionization biotyping. Antimicrobial susceptibility testing was performed by disk diffusion according to Clinical & Laboratory Standards Institute guidelines.

Results: Among *Enterococcus* isolates from fecal samples, *E. faecalis* (*n*=241) showed resistance mainly to rifampin (39.8%) and quinupristin-dalfopristin (37.8%) and *E. mundtii* (*n*=95) primarily to quinupristin-dalfopristin (30.5%). The highest percentages of resistance in the other *Enterococcus* spp. (*n*=38) were to quinupristin-dalfopristin (39.5%), rifampin (28.9%), and fosfomycin (15.8%). *Enterococcus* isolates obtained from produce samples (*n*=43) showed high levels of resistance mainly to quinupristin-dalfopristin (42%) and rifampin (30.2%). Among *E. coli* isolates (*n*=142), little to no resistance was observed to antibiotics of human clinical importance.

Significance: In the absence of marked anthropogenic sources, isolates from wildlife and produce carried primarily intrinsic or common AMR resistances, indicating a minor role in these settings for wildlife-mediated dissemination of AMR to produce.

P3-177 Antibiotic Susceptibility-resistance Profiles of Super-shed *Escherichia coli* O157:H7

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Introduction: Escherichia coli O157:H7 (O157) can cause diarrhea and serious secondary sequelae, including kidney failure and death in humans. With antibiotics like fosfomycin, colistin, and azithromycin, which do not stimulate toxin expression by O157, being considered for treatment of early gastrointestinal disease, it is important to evaluate antibiotic resistance in these *E. coli*, as it could hinder such therapies.

Purpose: Cattle are reservoirs of O157 and super-shedding cattle can shed O157 at ≥10^4 CFU/g of feces. Taking into account the potential for increased dissemination, we evaluated a genetically diverse set of bovine super shed O157 (SS-O157) isolates for their antibiotic resistance (AR) profiles and presence of AR genes.

Methods: SS-O157 isolates (*n*=53) were tested for sensitivity to 17 antibiotics using the Kirby-Bauer disc diffusion method and screened for the presence of 30 AR genes by PCR and sequencing. MIC tests were used to verify resistance (R) and intermediate-resistance (IR) phenotypes. The AR profile of bovine, non-O157 *E. coli* (*n*=20) and sequenced O157 strains (*n*=7) was similarly analyzed for comparison.

Results: A combination of 12 or more resistance genes was identified among the SS-O157 isolates, similar to non-O157 *E. coli*, by PCR and sequencing. While all isolates were sensitive to fosfomycin and colistin, 94% SS-O157 and 5% non-O157 *E. coli* demonstrated IR or R phenotypes with azithromycin, respectively. Sulfisoxazole resistance was observed in 83% SS-O157 and 40% non-O157; isolates also demonstrated resistance to tetracycline, streptomycin, chloramphenicol, nalidixic acid, cefotaxime, cefoxitin, gentamicin, amoxicillin, and ampicillin. MIC of these antibiotics is presently being evaluated to verify the results and compare overall data to AR genes characterized by PCR.

Significance: SS-O157, like other *E. coli*, can demonstrate varying antibiotic susceptibility profiles. Resistance to antibiotics could have important clinical implications and hence should be comprehensively verified.

P3-178 Clonal Spread of *Bla*$_{CMY-2}$-producing *Salmonella* Heidelberg ST15 Isolated from Commercial Chicken Meat in Brazil

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❖ ***Developing Scientist Competitor***

Introduction: Salmonella enterica is frequently recovered along the poultry production chain; *Salmonella enterica* Heidelberg is one of the most common serotypes recovered from poultry. In addition to the recovery of *Salmonella*, the detection of multidrug resistant (MDR) strains from chicken meat, particularly those harbouring extended-spectrum β-lactamase (ESBL) genes, is of global concern.

Purpose: To investigate the presence of ESBL genes in *S. enterica* isolated from retail chicken meat in São Paulo, Brazil.

Methods: Forty-one chicken meat samples (breasts and thighs [*n*=20 each], and liver [*n*=1]) were collected from 12 supermarkets in four regions (*n*=3/region) and cultured using standard methods. Antimicrobial susceptibility profiles and MIC values of cephalosporin were determined by disc diffusion and agar dilution, respectively. ESBL and pAmpC genes were screened by PCR and sequenced. Replicon type was determined for ESBL isolates; pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) were also conducted.

Results: Seven *Salmonella* isolates were recovered from chicken meat (17%; 7 of 41) in the south (*n*=1; *Salmonella* Heidelberg), east (*n*=3; 4,12:i:-), and west (*n*=3; *Salmonella* Heidelberg) regions of São Paulo city. Breast meat was most often positive (30%; 6 of 20), followed by the liver sample (100%; 1 of 1). Overall, two *Salmonella* Heidelberg isolates were positive for bla$_{CMY-2}$ genes and all isolates were found to be related by PFGE. The two clonal ESBL strains were from unrelated locations (west and south). MLST analysis identified sequence type ST15. IncA/C2 was identified in both ESBL strains through sequence analysis.

Significance: ST15 is widely identified in *Salmonella* Heidelberg from both poultry and humans and is also associated with production of ESBLs. The presence of IncA/C2 has also been associated with the presence of *bla*$_{CMY-2.}$ Collectively, the recovery of ESBL *Salmonella* Heidelberg ST15 is a first observation from poultry meat in Brazil and mitigation strategies to curb further dissemination of ESBL-producing strains are warranted.

P3-179 Prevalence of Antimicrobial-resistant *Enterobacteriaceae* and Survival of *Salmonella* and *Escherichia coli* in Plant-based Milk

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Introduction: With the current changes in food preferences, healthier lifestyles, milk intolerance, and more inclination towards vegan diets, plant-based milk has become popular. Although plant-based milk has become a choice for many consumers, limited data is available on the prevalence of antibiotic-resistant bacteria in this type of milk.

Purpose: The purpose of this study was to evaluate the prevalence of antimicrobial resistant *Enterobacteriaceae* in almond milk. In addition, the effect of storage time and temperature on the survival of *Salmonella* and *Escherichia coli* in almond, soy, and cashew milk was determined.

Methods: Almond milk was extracted from organic and convectional raw almonds purchased from a local store. *Enterobacteriaceae* was isolated from both almond milk and almond skins, and thereafter identified using API 20E method. Antimicrobial susceptibility of the isolates was determined by using Bauer and Kirby disk diffusion technique. Pasteurized almond, cashew, and soy milk samples were inoculated with *E. coli* and *Salmonella,* and thereafter evaluated for 10 h at 2-h intervals at 23°C and 9 days at 3-day intervals at 4°C.

Results: Our results indicate that raw almond milk and skins were contaminated with antibiotic resistant *Esherichia vulneris, Pantoea*, *Enterobacter cloacae*, *Klebsiella pneumoniae* spp., and *Citrobacter* spp. Of the 54 isolates, vancomycin (100%) and novobiocin (82.5%) significantly (P<0.05) indicated the highest resistance compared to tetracycline (42.6%), cefpodoxime (26%), and kanamycin (3.7%). None of the isolates were resistant to nalidixic acid or imipenem. At 10-h storage, *Salmonella* and *E. coli* levels increased significantly (P<0.05) from 4.3 to 6.1 CFU/ml and from 4.8 to 6.2 CFU/ml, respectively. For the 9-day storage, there was an increase (1.0 CFU/ml) in growth for both bacteria

Significance: Our results indicate that plant-based milks harbor antibiotic-resistant bacteria of clinical importance. *Salmonella* and *E. coli* may also survive and grow in plant-based milk stored at room and refrigerated temperatures.

P3-180 Control of *Salmonella* spp. by Food Grade Antimicrobials Following Various Stressors

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✦ ***Undergraduate Student Award Competitor***

Introduction: Using multiple stressors (usually physical or chemical) during food animal processing is a common technique used to control pathogens. Ascertaining whether stressors commonly encountered by *Salmonella* during processing impact antimicrobial efficacy can assist producers and regulators in determining chemical concentrations for judicious use.

Purpose: The purpose of this study was to ascertain if subjecting *Salmonella* to common slaughter/fabrication stressors prior to antimicrobial exposure impacts the MIC of said sanitizers.

Methods: Salmonella Montevideo, Newport, and Typhimirum were independently grown in tryptic soy broth (TSB) for 18 h at 37°C and exposed to one of seven stresses: no stress (control), salt, acid, heat, cold, freeze-thaw, and alkaline. 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 the antimicrobials lauric arginate (LauArg), citric acid plus hydrochloric acid (CA+HCl), peroxyacetic acid plus acetic acid and hydrogen peroxide (PAA+AA), lactic acid plus citric acid (LA+CA), and lactic acid (LA). MICs were determined from absorbance value (450 nm) interpretations following 18-h growth at 37°C.

Results: For all serotype and sanitizer combinations investigated, concentration of sanitizer was significant (P<0.0001). Stress was significant for *Salmonella* Montevideo subjected to LauArg, CA+HCl, and PAA+AA (P<0.05). In regards to *Salmonella* Newport, stress was significant when subjected to LA+CA, while concentration*stress was significant when exposed to CA+HCl, PAA+AA, and LA (P<0.05). Pertaining to *Salmonella* Typhimurium, stress was significant for subjected to LA+CA, while concentration*stress was significant for CA+HCl, PAA+AA, and LA (P<0.05).

Significance: Use of common processing stressors in concert with sanitizers may increase or decrease sanitizer concentration required to destroy *Salmonella*. Continued monitoring of changes in efficacy related to processing stressors is warranted as ecological changes in microbial populations continue to occur and bacteria continue to evolve.

P3-181 Validation of Electrostatic Antimicrobial Application on Surrogate-inoculated Poultry and Beef in a Continuous Flow System

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Introduction: Current antimicrobial application systems in meat and poultry plants require large amounts of water to reduce pathogen contamination on products, which is both environmentally and economically impractical. Developing an application method that reduces water and chemical usage while providing bacterial reductions is critical.

Purpose: This study evaluated the efficacy of a continuous application electrostatic cabinet in reducing inoculated populations of non-pathogenic *Escherichia coli* biotype I, serving as surrogates for Shiga toxin-producing *E. coli* (STEC) and *Salmonella enterica*, on two types of poultry and beef products.

Methods: Skin-on chicken wings, boneless skinless chicken breasts (BSCB), beef cheek meat, and beef navels were inoculated (chicken: 5 to 6 log CFU/ml of rinse solution; beef: 6 to 7 log CFU/g) with a five-strain mixture of rifampin-resistant (100 mg/ml) *E. coli* biotype I. Each of the tissue types were inoculated via two inoculation methods: (i) spot inoculation followed by spreading the inoculum with a disposable spreader over each flat surface, and (ii) distributing the inoculum over the entire sample surface using gloved hands. Inoculated tissues were treated for 20 s (approximately 200 ml of solution per treatment [30 pieces]) in the continuous flow electrostatic system with water, 10% sodium hypochlorite (200 ppm; 10-Chlor), or 2,000 ppm peroxyacetic acid (PAA). Untreated and treated samples (*n*=3 per treatment) were analyzed for surviving bacterial counts on tryptic soy agar supplemented with rifampin (100 µg/ml).

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Results: Overall, the two inoculation methods resulted in similar initial counts on untreated samples. Regardless of inoculation method, PAA effectively reduced (P<0.05) bacterial counts by 0.9 to 1.2 and 0.6 to 1.1 log CFU/ml on wings and BSCB, respectively, and 0.2 to 0.8 and 0.9 to 1.3 log CFU/g on cheek meat and navels, respectively.

Significance: These data suggest that the continuous flow electrostatic application of antimicrobials is a viable option for reducing bacterial contamination on chilled products, as well as water and chemicals used.

P3-182 The Efficacy of Wash Water Antimicrobials in Inactivating MS2 Bacteriophage on Strawberries Prior to and after Refrigeration and Frozen Storage

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Introduction: Foodborne illness caused by viruses such as Noroviruses and Hepatitis A (HAV) on fresh produce remains a concern worldwide. Raw and RTE vegetables and fruits contaminated at the point of production serve as a vehicle for virus transmission. In this study, commercial and home processing and storage practices of strawberries were evaluated for inactivation of bacteriophage MS2.

Purpose: Evaluate practices that facilitate virus inactivation to improve microbial safety of strawberries.

Methods: The MS2 bacteriophage was used as a surrogate of Norovirus. Strawberries were spot inoculated to achieve 6.6 log PFU/g. The inoculated strawberries were washed for 90 s using tap water, electrolyzed water (50 ppm free chlorine), or 50 ppm chlorine. After washing, the strawberries were separately stored at 4°C for 2 days, or -20 or -80°C for 30 days. Samples were processed and plaque assay was conducted to determine the population of MS2 remaining on strawberries at pre-determined days of storage.

Results: Frozen and refrigerated storage had little effect on inactivating MS2. No significant difference in MS2 population was observed between -20 and -80°C storage. At the end of storage period (30 days), 5 log PFU/g MS2 remained active on the strawberries. Washing of berries in water containing chemical antimicrobials had little effect (≤1 log PFU/g) on inactivating MS2, regardless of whether berries were washed prior to and after storage. However, the level of inactivation was lower when water alone was used.

Significance: Under conditions evaluated using water antimicrobials and refrigerated or frozen storage MS2 on strawberries remained active. Regardless, it is recommended to wash strawberries using water antimicrobial prior to consumption or further processing.

P3-183 Assessment of Combined Effect of Polysaccharide Gums and Antimicrobial Agents on Susceptibility and Protein Expression of Select Pathogens in Milk

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❖ ***Developing Scientist Competitor***

Introduction: There is increasing demand for the use of natural ingredients as preservatives and antimicrobial agents. Polysaccharide gums are usually used as emulsifying agents and preservatives.

Purpose: The objective of this study was to investigate effects of polysaccharides gums on the growth of *Escherichia coli* O157:H7, *Salmonella enterica*, *Listeria monocytogenes*, and *Staphylococcus aureus* and to evaluate the combined response of five different gums, including carrageenan-maltodextrin, locust bean pectin, xanthan and agar gums, and antimicrobial agents at the proteome level.

Methods: Antimicrobial susceptibility and protein concentration were determined by disc diffusion and Pierce BCA assay, respectively. The proteome pattern and the number of protein spots was determined by two-dimension electrophoresis.

Results: Although control remained resistant, treatment with gums caused *Salmonella enterica* to become susceptible against tetracycline and doripenem. The highest inhibition zones (20.50±0.70) were observed in *E. coli* O157:H7 exposed to carrageenan-maltodextrin-cefixime. The proteome pattern was impacted by the gums, with protein band of size 30 kDa being the most prominent band. The highest number of protein spots (n=35) were obtained in samples treated with locust bean pectin.

Significance: These findings indicated that tested gums affected microbial protein expression and were effective in inhibitory activity against tested pathogens, specifically *Escherichia coli* O157:H7, thus they hold great promise as antimicrobial agents. Further characterization of protein targets is necessary.

P3-184 In Situ Generation of Chlorine Dioxide for Decontamination of Sprout Seeds

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Introduction: Raw sprouts contaminated with bacterial pathogens have been implicated in outbreaks of foodborne illnesses. Contaminated sprout seeds are a source of pathogens into sprouts. Treatment with chlorine dioxide generated *in situ* on the surfaces of sprout seeds by sequential applications of sodium chlorite ($NaOCl_2$) and hydrochloric acid (HCl) may effectively decontaminate the seeds for sprout production.

Purpose: This study evaluated the sequential treatment for decontamination of bacterial pathogens on sprout seeds.

Methods: Mung bean and alfalfa seeds were inoculated with *Salmonella* spp., *Listeria monocytogenes*, and Shiga toxin-producing *Escherichia coli* (STEC) by soaking. The seeds were sequentially soaked in 1.6% $NaOCl_2$ and 6 mM HCl solutions for 5 to 30 min, with a 30-min drying between the soakings. Inoculated seeds were also treated in $NaOCl_2$ or HCl solution for comparison. Treated seeds were sprouted at room temperature to determine sprouting rates.

Results: With an inoculation level of 5.5 to 6.6 log CFU/g in mung beans, <1.0 log CFU/g of pathogens were reduced by HCl treatment alone, regardless of treatment time. $NaClO_2$ treatment for 5 to 30 min was more effective, causing reductions of 2.0 to 3.4, 1.6 to 2.4, and 3.5 to 4.2 log CFU/g of *Salmonella* spp., *L. monocytogenes*, and STEC, respectively. However, more effective reduction was observed with the sequential treatment, with reductions of 3.6 to 4.7, 3.3 to 5.5, and 4.3 to 6.7 log CFU/g of *Salmonella* spp., *L. monocytogenes*, and STEC, respectively. The effectiveness of treatment increased with the treatment time, and STEC was most susceptible to the sequential treatment. For alfalfa seeds, a 15-min treatment caused a pathogen reduction of >5.0 log CFU/g. The sequential treatment did not affect the sprouting rates of treated seeds.

Significance: The results of this study showed that the sequential treatment with $NaClO_2$ and HCl solutions was effective in decontaminating pathogens inoculated on sprout seeds and could be used by the industry to produce microbiologically safer sprouts.

Poster

P3-185 Antimicrobial Properties of *Artemisia afra* against Bacteria Isolated from Bulk Tank Milk

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❖ ***Developing Scientist Competitor***

Introduction: Plants produce secondary metabolites as natural protection against microbial pathogens. Essential oils (EO's), also known as volatile oils, are products of this secondary metabolism. Known for their antibacterial, anti-inflammatory, anti-carcinogenic, and anti-fungal activity, they may be potential antimicrobials for controlling and eliminating antibiotic-resistant bacteria in foodstuffs of animal origin.

Purpose: To investigate the antimicrobial properties of *Artemisia afra* against antibiotic-resistant bulk-tank isolated bacteria.

Methods: The efficacy of plant EO's against antibiotic resistant bulk-tank isolated bacteria was examined using the agar diffusion bio-assay. The study included the incorporation of EO's, or their major constituents, into agar to allow uniform dispersion of the substance throughout an agar surface. Scanning electron microscopy and transmission electron microscopy were used to assess the effect of the oils on bacterial morphology.

Results: Antimicrobial properties of *Artemisia afra* (Lengana) were observed against nine antibiotic-resistant psychotropic bacterial species (*Enterobacter cloacae*, *Hafnia alvei*, *Lelliottia amnigena*, *Pseudomonas* spp. [*P. oeovorans*, *P. fragi*, *P. ludensis*, *P. taetrolens*, *P. rhodesiae*], and *Serratia liquefaciens*). Scanning and transmission electron microscopy results observed showed that the oils have antibacterial activity against bacteria tested against the *A. afra* oil. Furthermore, scanning electron microscope (SEM) and transmission electron microscope (TEM) micrographs showed no influence on untreated (control) bacterial samples; well-separated, rod-shaped, firm, and intact bacteria were observed. This was different in treated organisms (organisms exposed to EO's), as the EO's affected both the external envelope of the cell wall and the cytoplasm leading to cell lysis/leakage and the presence of holes in the cell wall, an indication of activity of EO's against antibiotic-resistant bacteria.

Significance: The results observed for the SEM and TEM images prove that *Artemisia afra* oil has an antimicrobial effect on the gram negative bacteria isolated in the bulk tank milk in this study. The compounds of this oil have been shown to exercise their antibacterial action/activity through membrane perturbations.

P3-186 Antimicrobial Activities of Gaseous Essential Oils against Xerophilic Mold (*Penicillium corylophilum*)

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❖ ***Developing Scientist Competitor***

Introduction: Penicillium corylophilum is a xerophilic mold that has been known to cause a spoilage in low-moisture foods (LMF). Essential oils (EO) have potential as natural preservatives for inhibiting the growth of *P. corylophilum*. Gaseous EO in particular may have the advantage of not only inhibiting the growth of xerophilic mold, but also minimizing sensory changes of foods.

Purpose: This study was carried out to determine the MIC of EO gases and the combinations of EO gases with synergistic lethal effect against *P. corylophilum*.

Methods: From 97 types of EO gases, those with strong antimicrobial activities against *P. corylophilum* were screened using vapor-diffusion assay. The MICs of screened EO gases were determined on DG18 agar. Finally, the combinations of EO gases causing synergistic lethal effects against *P. corylophilum* were determined using checkerboard assay.

Results: Eight EO gases showed antimicrobial activities against *P. corylophilum* after vapor diffusion assays. The MIC values of garlic, cinnamon bark, may chang, citronella, thyme thymol, oregano, spearmint, and thyme linalool EO gases were 0.0390, 0.1563, 0.1563, 0.3125, 0.3125, 0.6250, 0.6250, and 0.6250 µL/ml, respectively. The combination of cinnamon bark and citronella EO gases (1:2 concentration ratio, fractional inhibitory concentration index [FICI]=0.5), and that of cinnamon bark, citronella, and may chang EO gases (1:2:1 concentration ratio, FICI=0.375) showed synergistic lethal effects (FICI≤0.5) against *P. corylophilum.*

Significance: This is the first study to determine the MIC values of EO gases and the combinations of EO gases with synergistic lethal effect against *P. corylophilum*. The results of this study will provide useful information in developing antimicrobial packaging technologies that can effectively inhibit the growth of *P. corylophilum* on LMF.

P3-187 The Use of (Bacterio) Phage for *Listeria* Lethality on Frozen Ready-to-Eat Vegetables

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Introduction: The U.S. Food and Drug Administration's Food Safety Modernization Act has a zero tolerance policy regulation for *Listeria* in the food supply, which has resulted in processors looking for ways to further reduce risk of foodborne illness and protect public health. As food safety practices evolve, processors seek new interventions for killing *Listeria* without impacting sensory or product quality. Phages can specifically target and lyse bacterial cells and are being exploited as a new technology to fight *Listeria* contamination of frozen vegetables.

Purpose: The efficacy of a commercially available anti-*Listeria* phage was evaluated when applied on post-blanch and frozen vegetables.

Methods: Samples of corn (30 g each, duplicate) were contaminated with 4 log *L. monocytogenes* and subsequently treated with 5×10^6 and 2×10^7 PFU/cm^2 of phage solution or tap water (negative control) at room temperature (68°F). Samples were held for 1, 10, and 30 min before freezing at -16°F. After 5 h, samples were removed from the freezer and bacteria retrieved and enumerated. Additional tests investigated phage efficacy when applied directly to frozen corn, edamame, chopped onions, and peppers contaminated as above, treated with phage (7×10^6 PFU/cm^2) and stored for 1, 10, and 30 days at -4°F. Duplicate samples were defrosted and *Listeria* enumerated.

Results: When applied on artificially contaminated corn after blanching, the phage solution reduced *Listeria* by >1.5 log 1 min after application. When applied directly on frozen vegetables >1-log kill was observed on all vegetable types at all time points.

Significance: Phage technology is an effective intervention during the processing of frozen vegetables and can be particularly useful for killing *Listeria* on imported and 3rd party products where processors and blenders do not have full control over the incoming supply chain.

Poster

P3-188 Genomic Characterization of a Novel *Aeromonas hydrophila*-Specific Phage and Confirmation of Its Lytic Activity for Use as a Biocontrol Agent

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❖ ***Developing Scientist Competitor***

Introduction: Aeromonas hydrophila has gained more attention due to its multidrug-resistance and the increasing number of outbreaks associated with a wide range of foods, including meats, fish, dairy products, and fresh produce. Since phages have been recognized as a "green approach" for controlling multidrug-resistant bacteria, our research group has isolated and purified an *A. hydrophila*-specific phage from a poultry processing plant for the development of a novel biocontrol agent against *A. hydrophila*.

Purpose: The purpose of this study was to identify the novelty of an *A. hydrophila*-specific phage and to investigate its lytic activity for use as a potential biocontrol agent.

Methods: Genomic DNA of an *A. hydrophila*-specific phage was sequenced using Illumina MiSeq platform, and open reading frames (ORFs) were predicted and annotated. Its comparative genome analysis was conducted with *Stenotrophomonas* phage IME15. For investigation of its lytic activity, *A. hydrophila* suspension was mixed with various concentrations of *A. hydrophila*-specific phage to obtain MOIs of 0.01, 1, and 100. The mixture was incubated at 37°C, and its optical density was measured at 3-h intervals for 24 h for investigation of lytic activity.

Results: The whole genome of the *A. hydrophila*-specific phage consisted of 40,071 bp with 53.06% GC content. Among the predicted 41 ORFs, 26 (63.4%) were predicted to have specific functions, and the others were identified as hypothetical proteins. Comparative analysis of the *A. hydrophila*-specific phage revealed low similarities with *Stenotrophomonas* phage IME15. Bacterial growth was maintained for 6 h, and there were no significant differences among various MOI values ($P<0.05$).

Significance: This study demonstrated the novelty of an *A. hydrophila*-specific phage and its potential as a biocontrol agent against *A. hydrophila.*

P3-189 Combination Effect of Four Essential Oils against *Escherichia coli* O157:H7*, Salmonella* Enteritidis*, Staphylococcus aureus,* and *Listeria monocytogenes* in Tryptic Soy Broth

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Introduction: Due to the hydrophobic interactions of essential oils (EOs) with food components, high doses of EOs are needed in food products to maintain their antimicrobial activity, which limits their applications. Synergistic combination of essential oils has been found to be an effective way to reduce each essential oil's dose; however, most studies focus on binary combination.

Purpose: It was aimed to compare the binary and ternary combinations of EOs. *Escherichia coli* O157:H7 ATCC 43895*, Salmonella enteritidis* ATCC 13076*, Staphylococcus aureus* ATCC 31885, and *Listeria monocytogenes* Scott A were used as bacterial strains.

Methods: Two- or three-dimensional checkboard assay was used to study the combination effect. In the double combinations, EOA was diluted two-fold along the *x*-axis of the 96-well microplate, whilst EOB was diluted two-fold along the *y*-axis. Six combinations between cinnamon, oregano, tea tree, and lemongrass oils were set up for the dual combinations. In the triple combinations, the third EO was distributed over the wells of dual combinations above. The fractional inhibitory concentration (FIC) was calculated as FICA+FICB in binary combination and FICA+FICB+FICC in ternary combination. The obtained results were interpreted as synergistic (FIC<1), additive (FIC=1), and antagonistic effects (FIC>1).

Results: All of the combinations showed either synergistic or additive effects (FIC≤1)) against the tested pathogens, except the ternary combinations, which showed antagonistic effect against *L. monocytogenes* (FIC>1). Ternary combinations can further reduce the MIC of each EO, compared to the binary combination.For example, the MIC of cinnamon oil was reduced from 0.4 mg/ml in binary combinations to 0.2 mg/ml in ternary combinations.

Significance: The mass transfer of EOs to the aqueous phase, which is the place that microorganisms are most likely to concentrate in food matrices, is very low, resulting in sub-lethal effect or no inhibition effect. However, the accumulation of low concentrations of multiple EOs of synergistic effect may act as lethal effect, indicating the potential of using appropriate triple EOs as antimicrobial formulations in food products.

P3-190 Antimicrobial Activities of Natural Antimicrobial Agents in Organic Foods

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❖ ***Developing Scientist Competitor***

Introduction: The importance of naturally occurring antimicrobial agents to extend the shelf life of organic foods has been emphasized. Natural antimicrobial agents such as organic acids and essential oils (EO) would be more favorable to consumers than synthetic chemical preservatives. However, the antimicrobial activities of natural antimicrobial agents in organic foods have rarely been reported.

Purpose: This study was done to determine the antimicrobial activities of 30 types of natural antimicrobial agents which are allowed in organic foods against *Bacillus subtilis*, *Enterococcus faecalis*, and *Escherichia coli.*

Methods: Organic acids (five types) and EOs (25 types) were selected and collected through literature review. Next, the natural agents having strong antimicrobial activities (inhibition zone ≥10.0 mm) against *B. subtilis, E. faecalis*, and *E. coli* were screened by a well-diffusion assay. Finally, the MIC and minimum lethal concentration (MLC) of the screened agents against *B. subtilis, E. faecalis*, and *E. coli* were measured by a resazurin broth microdilution assay.

Results: In total, four organic acids and four EOs of natural agents with strong antimicrobial activities were screened after the well-diffusion assay. The MICs of four types of organic acids (citric, malic, tartaric, and lactic acid) against *B. subtilis, E. faecalis*, and *E. coli* were ≤6.2500 mg/ml. The MICs of cinnamon bark, cinnamon leaf, oregano, and thyme thymol EO were ≤2.500 µl/ml. The oregano EO in particular showed the strongest antimicrobial activities (MIC=0.6250 µl/ml, MLC=0.6250 µl/ml) against tested *B. subtilis*, *E. faecalis*, and *E. coli*.

Significance: The antimicrobial activities of naturally occurring agents, which are allowed in organic foods, were determined against *B. subtilis*, *E. faecalis*, and *E. coli*. The results of this study will provide important information in developing methods to increase the microbiological safety of organic foods.

Poster

P3-191 Synergistic Lethal Effects between Gaseous Essential Oils in Inactivating *Listeria monocytogenes* in a Laboratory Medium and Radish Sprouts

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❖ ***Developing Scientist Competitor***

Introduction: Some essential oils (EO) have been known to have antimicrobial effects and can be used as liquids or gases. EO gases can be effective on large areas and may minimize the changes of sensory properties of foods, since they are not added directly into foods. However, the combinations of the EO gases which cause synergistic lethal effects against *Listeria monocytogenes* have not been reported.

Purpose: The objectives of this study were to determine the combinations of EO gases causing synergistic lethal effects against *L. monocytogenes* in laboratory media and to confirm their antilisterial activities on radish sprouts.

Methods: The MICs of EO gases (cinnamon bark, oregano, and thyme thymol) against *L. monocytogenes* were measured in nutrient agar with 1% D-glucose and 0.025% bromocresol purple. Next, synergistic lethal effects between two EO gases against *L. monocytogenes* were determined using a checkerboard assay. Finally, radish sprouts inoculated with *L. monocytogenes* were exposed to the combinations of the EO gases which had shown the synergistic antilisterial effects, and the populations of *L. monocytogenes* in radish sprouts were measured.

Results: The MICs of the three EO gases (cinnamon bark, oregano, and thyme thymol) were 0.0781 µL/ml. The combinations of EO gases that showed synergistic antilisterial effects were thyme thymol and oregano (fractional inhibitory concentration index [FICI]=0.375), cinnamon bark and oregano (FICI=0.375), and cinnamon bark and thyme thymol (FICI=0.5). When the combination of thyme thymol and oregano EO gases was applied to the radish sprouts containing *L. monocytogenes*, it significantly reduced ($P\leq0.05$) the number of *L. monocytogenes* compared to a single treatment of thyme thymol or oregano EO gas.

Significance: This is the first study which determined the combinations of EO gases with synergistic antilisterial effects in a laboratory medium and radish sprouts.

P3-192 Antimicrobial Properties of High Molecular Weight Water Soluble Chitosan against Gram Negative and Gram Positive Foodborne Pathogens

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❖ ***Developing Scientist Competitor***

Introduction: High molecular weight (HMW) chitosan is a new method for fast dissolving without any chemical modifications, eliminating the acid pungent odor of the HMW chitosan solution and making it more biocompatible.

Purpose: The aim of this study was to determine the antimicrobial properties of chitosan using a new fast-dissolving high molecular weight chitosan against gram negative and gram positive bacteria.

Methods: In this study, the bacteria were decimally diluted to give an initial inoculation of >6.5 log CFU/ml. Briefly, HMW with 789 kDa was dissolved in aspartic acid (AS) at 1%, 2%, and 4% w/v, while HMW with 1017 kDa was dissolved in AS at 1%, 2%, and 3% w/v. The 1% w/v 789 kDa and 1% w/v 1017 kDa chitosans in acetic acid (AC) as well as 1% AC and 3% AS solutions were also prepared. Treatments were serially diluted and plated onto the Mueller Hinton agar and the bacterial counts were determined on 0, 48, and 96 h.

Results: Escherichia coli bacterial counts were reduced by 2 log CFU/ml when treated with 789 AC 1% and 1,017 AC1% at 48 and 96 h, respectively. Only 789 AC1% significantly reduced *Salmonella* Typhimurium from the control sample with no treatment. *Vibrio parahaemolyticus* exhibited the greatest sensitivity to chitosan treatments, followed by *Vibrio vulnificus* and *Vibrio cholerae.* Chitosan 1,017 AS2% reduced *Staphylococcus aureus* and *Listeria monocytogenes* to <2 Log CFU/ml and *Bacillus cereus* to non-detectable levels (<10 CFU/ml) at 48 and 96 h.

Significance: This study demonstrated that HMW water-soluble chitosan is an effective antimicrobial against foodborne pathogens. The antibacterial activity of HMW chitosan differed depending on the chitosan MW and bacteria tested.

P3-193 Antibiofilm Effect of Chitosan and Oligochitosans against Biofilm-forming Foodborne Bacterial Pathogens

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Introduction: Biofilm refers to a group of microorganisms attached to a surface and encased in a hydrated polymeric matrix. Biofilm-forming bacteria have major implications in a variety of industries, including the food industry, as the biofilms cause a continuous source of contamination.

Purpose: The object of this study is to evaluate an antibiofilm effect of marine-derived compounds, chitosan and oligochitosans, against biofilm-forming foodborne bacterial pathogens (*Pseudomonas aeruginosa*, *Listeria monocytogenes,* and *Staphylococcus aureus*).

Methods: Chitosan (MW>250 kDa, 80% deacetylation) and oligochitosans (MW 1 to 3 kDa, 3 to 5 kDa, and 5 to 10 kDa) were used in this study. Antibacterial activity of oligochitosans against planktonic bacterial cells was investigated by MIC and minimum bacterial concentration (MBC) assay. The antibiofilm effects of oligochitosans were evaluated by determining biofilm inhibitory concentration (BIC) and biofilm eradication concentration (BEC). Biofilms were formed on bottom of microtiter plates by incubating for 24 h at 37°C.

Results: The marine-derived compounds, chitosan and oligochitosans, exhibited the highest antibacterial and antibiofilm activity against *L. monocytogenes*, followed by *P. aeruginosa* and *S. aureus*. Among of the compounds, the lowest molecular weight oligochitosan (MW 1 to 3 kDa) evidenced the strongest antibacterial and antibiofilm effects. The BIC and BEC of oligochitosans (MW 1 to 3 kDa) against L. *monocytogenes* KCTC 3569 strain were 256 and 4,096 µg/ml, respectively. Additionally, the sub-lethal concentrations of oligochitosan (MW 1 to 3 kDa) effectively prevented the biofilm formation by *L. monocytogenes*.

Significance: The oligochitosan with low molecular weight (MW 1 to 3 kDa) possess the activity to inhibit biofilm formation as well antibacterial activity against foodborne pathogenic bacteria.

Poster

P3-194 Evaluate the Effectiveness of Sodium Acid Sulfate to Reduce *Escherichia coli* O157:H7 from Chopped Bell Peppers

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❖ ***Developing Scientist Competitor***

Introduction: During the commercial produce packaging operation, produce is typically washed in recirculated wash waters before packaging. As a result, recirculating water is high in organic matter. Therefore, there is a need for alternative antimicrobials which are insensitive to organic load. Sodium Acid Sulfate (SAS) is one of such antimicrobial agents.

Purpose: The purpose of this research is to study the suitability of SAS as a produce wash sanitizer for chopped bell peppers.

Methods: Fresh bell peppers were chopped to produce approximately 1in² pieces. Individual pieces of bell peppers were inoculated by placing 50 µl of 7 Log_{10} CFU/mL *E. coli* O157: H7 or *Salmonella* Typhimurium DT 104. After inoculation, individual bell pepper pieces were allowed to air dry in a laminar flow hood for 60 min. Two pieces of inoculated bell peppers were subjected one of the five treatments; 3% SAS or 80 mg/L peracetic acid (PAA), PAA+SAS, SAS+ sodium dodecyl sulfate (0.5%), deionized water (W) and no treatment (NT) by submerging them into 25 ml treatment solution for 1 min. At the end of each treatment, bell peppers, and treatment solutions were analyzed for the presence of targeted pathogens. Each experiment was repeated at least three times.

Results: After the treatment, reduction of 1.23, 1.61, 1.68, 2.16 and 1.01 Log_{10} CFU/in² for PAA, SAS, PAA+SAS, SAS+SDS, and W, respectively. For *S.* Typhimurium, reductions of 1.41, 1.87, 2.22 Log_{10}CFU/in² were observed for W, PAA and PAA+SAS treatments. Treatments SAS and SAS+SDS were able to reduce *S.* Typhimurium to non-detectable levels (>2.95 Log_{10} CFU/in²). When treatment solutions were tested for the presence of the target pathogen, except for the DI water treatment, no pathogens were detected by direct plating. But, after enrichment, PAA treatment solution was tested positive for targeted pathogens.

Significance: SAS could become an effective alternate produce wash sanitizer which can provide protection against microbial cross-contamination during washing.

P3-195 Reduction of *Listeria monocytogenes* on the Surface of Commercial Brie Cheese by Electrostatic Spraying of Lactic Acid Bacteria (*Lactobacillus salivarius* L28 and *Enterococcus faecium* J19)

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✦ ***Undergraduate Student Award Competitor***

Introduction: The U.S. Food and Drug Administration recommends that pregnant women and other high-risk groups do not consume soft cheeses due to the risk of contamination with *Listeria monocytogenes*. Soft cheeses produced with pasteurized milk are generally safe, but can become contaminated on the surface by environmental contamination, thus supporting the growth of *L. monocytogenes*.

Purpose: The purpose of this study was to utilize novel strains of lactic acid bacteria (LAB), including *Enterococcus faecium* J19 and *Lactobacillus salivarius* L28, to reduce *L. monocytogenes* on the surface of brie cheese.

Methods: Brie wheels of a surface area of 50.27 in², a thickness of 1.25 in, and a weight of 2.25 lbs were used in this study. Cheese samples were inoculated with *L. monocytogenes* to a final surface concentration of 105 CFU/cm². The treatments were as follows: *L. salivarius*, *E. faecium*, and a combination of both at a 1:1 ratio. Treatments were performed with overnight bacterial cultures at a final concentration of 10^8 CFU/ml, and the control consisted of De Man, Rogosa, and Sharpe broth. The treatments and control were applied through an electrostatic sprayer for 30 sec on each side of the cheese, and treated cheeses were kept at refrigerated temperatures throughout the time points. Samples were diluted and enumerated on modified Oxford agar for the following time points: D0, D1, D3, D7, D14, D30, D45, and D60.

Results: Samples treated with *E. faecium* J19 strain showed improved results compared to the other treatments in this study. *L. monocytogenes* was reduced by 0.97, 0.94, 1.27, 0.69, 0.93, 0.71, 0.71, and 0.44 log CFU/cm² by J19 compared to control through the time points tested in this study; none of the treatments showed a statistically significant reduction (P>0.05).

Significance: Enterococcus faecium J19 is a probiotic LAB with potential in further inhibiting environmental *L. monocytogenes* contamination on brie and other types of cheeses.

P3-196 Reduction of Foodborne Pathogens on Low-moisture Foods Using Gaseous Chlorine Dioxide

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❖ ***Developing Scientist Competitor***

Introduction: The food safety of low-moisture foods (LMFs) had become a concern due to recent outbreaks. Gaseous chlorine dioxide (ClO_2) has been a great antimicrobial used for food sanitation.

Purpose: The main objective was to evaluate the efficiency of gaseous ClO_2 against Shiga toxin-producing *Escherichia coli* (STEC), *Salmonella*, and *Listeria monocytogenes* on LMFs, including almonds and black peppercorns.

Methods: A cocktail of pathogenic strains (six STEC, five *Salmonella* spp., and five *Listeria monocytogenes*), grown in tryptic soy broth and enriched on tryptic soy agar, were used to inoculate 1,200-g of food samples. Gaseous ClO_2 was generated on-site using dry precursors and monitored at various concentrations and time, depending on the food models. Almonds were treated with 0.27 and 0.40 mg of ClO_2/g of almonds for 4 and 6 h. Post-treatment heating was carried out at 50 and 65°C for 24 h. Peppercorns were treated with 0.29 and 0.40 mg of ClO_2/g of peppercorn for 2.5 and 4 h. The experiment was run in triplicates and samples were enumerated on selective media.

Results: Gaseous ClO_2 hurdled with heat at 65°C gave significantly higher reductions for all pathogens than that with heat at 50°C (P<0.05). Treatment at 0.27 mg of ClO_2/g of almonds for 4 h and hurdled with heat at 65°C obtained reductions of 4.58±0.32 and 4.10±0.27 log CFU/g for STEC and *L. monocytogenes,* respectively, and a reduction of 4.29±0.61 log CFU/g of *Salmonella* was achieved at 0.40 mg of ClO_2 for 6 h with 65°C of post-treatment heating. Bacterial reduction observed on peppercorns for *Salmonella* was 3.72±0.20 log CFU/g at 0.4 mg of ClO_2/g of peppercorn for 4-h treatment, and reductions of 3.68±0.15 and 2.97±0.2 log CFU/g were observed for STEC and *L. monocytogenes*, respectively.

Significance: Gaseous ClO_2 is a potential substitute for the food processing industry, as it displays an efficiently high bacterial reduction in food sanitation of LMFs.

Poster

P3-197 Efficacy of Lauric Arginate and Cetylpyridinium Chloride Applied Electrostatically to Pre-rigor Veal Carcasses Followed by an Acidified Peracetic Acid Spray Chill Application to Control Shiga Toxin-producing *Escherichia coli* (STEC)

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❖ ***Developing Scientist Competitor***

Introduction: Seven serotypes of Shiga toxin-producing *Escherichia coli* (STEC) are regulated as adulterants in non-intact raw beef and veal products. The United States Department of Agriculture has recognized veal as an important contributor to STEC-positive regulatory samples, establishing the need for validated intervention strategies in commercial operations.

Purpose: Evaluate the efficacy of: 1) electrostatically applying lauric arginate (LAE) and cetylpyridinium chloride (CPC) to pre-rigor veal carcasses, and 2) applying an acidified peracetic acid (aPAA) spray during a 20-h spray chill cycle in order to reduce STEC.

Methods: Three dressed veal carcasses were inoculated with a seven-serogroup STEC cocktail (~5.7 log CFU/cm²; 30-min attachment period) and treated using electrostatic application of 200 ml of 20% LAE solution, 30% CPC solution, or water (control). Carcasses were then split and subjected to either an acidified peracetic acid (200 ppm peracetic acid at ~1.3 pH) or water (control) spray chill cycle. Surviving STEC populations were enumerated from the top, middle, and bottom of each carcass side post-inoculation, post-electrostatic spray application, and post-spray chill cycle. Carcass color (L*, a*, and b*) was evaluated at each sampling point to determine quality impacts from the antimicrobial applications.

Results: The electrostatic applications of LAE and CPC were statistically (P≤0.05) different than water (control), reducing STEC populations by 1.1, 1.2, and 0.3 log CFU/cm², respectively. The aPAA spray chill application further reduced the population by 3.6 log CFU/cm² compared to water (control), which reduced populations by 1.3 log CFU/cm². Carcass color did not seem to be negatively impacted by the antimicrobial applications, even though chilled carcasses (post-spray chill application) were slightly lighter than the water (control) treated carcasses.

Significance: Low-volume concentrated LAE or CPC electrostatic spray applications to pre-rigor veal carcasses followed by an acidified peracetic acid spray chill application effectively reduces STEC populations and offers processors an additional level of STEC control without negatively affecting carcass color.

P3-198 Investigating the Inactivation of *Salmonella enterica* on Shell Eggs Using Commercially Available Natural Antimicrobial Rinses

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❖ ***Developing Scientist Competitor***

Introduction: Salmonella enterica is a foodborne pathogen that has been associated with outbreaks related to egg products. Although chlorinated washes are commonly used to sanitize whole shelled eggs within the poultry industry, consumers are now demanding alternative methods that are more natural and environmentally friendly. Because organic matter has the ability to bind to free hypochlorite ion in chlorine water, chlorine loses its effectiveness as an antimicrobial agent.

Purpose: The objective of this study was to investigate the ability of commercially available rinses to control the growth of *S. enterica* on shell eggs.

Methods: A five-serotype mixture of *S. enterica* was prepared at the concentration of ~7.8 CFU/ml. Shell eggs were purchased from a local retailer and sanitized using 70% alcohol prior to use in the study. Eggs were then inoculated by submersion in the *S. enterica* mixture for 2 min and allowed to attach for 60 min. After attachment, eggs were assigned to one of the following treatment groups: (A) distilled water (control), (B) 200 ppm chlorinated water, (C) citric acid-based rinse, and (D) lactic acid-based rinse. Two eggs per treatment were analyzed at 0, 15, 30, and 60 min. Survivors were determined by plating the samples on standard plate count media and xylose lysine deoxycholate agar. Colonies were counted after incubation (35°C) for 24 h.

Results: According to the analysis of variance, the greatest log reduction of 3.9 (P<0.05) occurred with Treatment D at 30 min. Although not significantly different (P>0.05) from that of the control of distilled water over time, Treatments A, B, and C had slight reductions over time.

Significance: The study demonstrated that some commercially available rinses may serve as alternatives to traditionally used sanitizers to control the growth of *S. enterica* on shell eggs.

P3-199 Evaluation of Antimicrobial Solutions, with and without a Surfactant, for Reducing Inoculated Bacterial Populations on Beef Trimmings, Chicken Wings, and Cantaloupes

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❖ ***Developing Scientist Competitor***

Introduction: Surfactants may decrease the surface tension of antimicrobials, allowing them to better penetrate the irregular surfaces of meat products and produce.

Purpose: Studies were conducted to determine whether the addition of a surfactant to various chemical solutions enhances their antimicrobial effects against inoculated bacterial populations on different food products.

Methods: Beef trimmings, skin-on chicken wings, and whole cantaloupes were inoculated with five-strain mixtures of non-pathogenic *Escherichia coli* (surrogates for Shiga toxin-producing *E.coli* and *Salmonella*; 6-7 log CFU/g), Salmonella (6-7 log CFU/ml of sample rinsate), and *Listeria monocytogenes* (8-9 log CFU/cantaloupe), respectively. Trimmings (n=10) were spray-treated (10 s, 20 psi) with peroxyacetic acid (PAA, 400ppm), a sulfuric acid+sodium sulfate blend (SSS, pH 1.1) or PAA (400ppm) acidified with SSS (pH 1.1; aPAA), without and with addition of a proprietary surfactant (0.4%) to the solution. Chicken wings (n=10) were immersed (15 s) in PAA (500ppm), SSS (pH 1.2) or aPAA (500ppm PAA and SSS, pH 1.2) solutions that were or were not supplemented with the surfactant (0.4%). Lastly, whole cantaloupes (n=10) were treated by immersion (30 s) into SSS (pH 1.0), SSS (pH 1.0)+surfactant (0.4%) or water. Untreated and treated trimmings, wings, and cantaloupes were analyzed for surviving *Enterobacteriaceae*, *Salmonella*, and *L. monocytogenes* counts, respectively. Data was analyzed using the mixed procedures in SAS.

Results: Regardless of food surface type, addition of surfactant did not (P ≥ 0.05) affect the efficacy of any of the antimicrobial treatments. All PAA-containing treatments effectively (P<0.05) reduced inoculated populations on both beef trimmings (6.5 log CFU/g) and chicken wings (6.0 log CFU/ml) by 0.5-0.6 log CFU/g and 1.7-1.8 log CFU/ml, respectively. The SSS-containing treatments applied to cantaloupes lowered (P<0.05) initial *L. monocytogenes* counts (8.2 log CFU/cantaloupe) by 1.7-2.1 log CFU/cantaloupe.

Significance: The results suggest that the antimicrobial effects of PAA and SSS were not enhanced with the addition of a surfactant.

Poster

P3-200 Plant Extracts for Control of Norovirus

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Introduction: Noroviruses are the most common cause of acute gastroenteritis. Norovirus causes inflammation in the intestines and the stomach, leading to stomach pain, nausea, and diarrhea. Approximately, 21 million Americans have infected with norovirus annually. Recent advances in molecular diagnostics have helped to establish norovirus as the most common cause of outbreaks of acute gastroenteritis across all ages. However, there is no effective or efficient treatment against norovirus infection. Conventional intervention techniques used to inactivate norovirus have shown lack of efficacy against human norovirus. Therefore, alternative antiviral agents such as crude extracts of *Zanthoxylum armatum* (ZA) and *Hibiscus sabdariffa* (HS) have received attention as potential norovirus inhibitors due to their relatively low toxicity and lack of side effects. This experiment has shown significant reduction of viruses with the extracts.

Purpose: To study the antiviral activities of crude and fractionated portions of *Zanthoxylum armatum* and *Hibiscus sabdariffa* against norovirus surrogate.

Methods: Murine norovirus 1 (MNV-1) a surrogate virus of human norovirus was infected with ATCC TIB 71 cell line, incubated with extracts of ZA and HS. Phytochemicals were extracted from the seeds and calyces of the plant using methanoic extraction. The study adopted techniques such as plaque assay, transmission electron microscope, fractionation, and ultra-performance liquid chromatography (UPLC). Fractionated portions of the crude extracts were subsequently used in both chromatographic and microbiological studies.

Results: Quercetin reduced the viral replication by approximately 14 virus log reductions per plaque forming unit (pfu) as against 15 virus log reduction for the control (non-treated) , myricetin has 6-7 log reductions, kaempferol has between 1.5-2 log reductions and luteolin has 3 log reductions.

Hexane fraction of the both extracts showed the highest viral log reduction {9-10 log reduction for *Hibiscus sabdariffa* (HS) and 5 log reduction for *Zanthoxylum armatum* (ZA)}.

Significance: Phenolic compounds are virucidal. Extracts of HS also exhibits anti-norovirus activity. Detailed studies involving animal study and elucidation of the effects of the studied extracts on human norovirus will be necessary. The results are anticipated to control/prevent the human norovirus infection.

P3-201 Thyme Oil and Thyme Oil Hydrosol Coating as Alternative to Synthetic Fungicides against *Phyllosticta citricarpa* Post-harvest

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Introduction: Citrus black spot (CBS) is a serious, widespread problem for citrus production around the world. It is caused by the fungus *Phyllosticta citricarpa*, which affects almost all commercial citrus species. Losses due to CBS can amount up to 25% of the total production, and in developing countries damage often exceeds 50%. Synthetic fungicides are the primary means to control most devastating fungal pathogens. However, fungal pathogens have shown a concerning trend of resistance against these fungicides. Additionally, they are known to remain on the plant or within its tissues following treatment resulting in potentially toxic and carcinogenic effects on human and food systems. To reduce the use of synthetic fungicides in the food chain, researchers have increased efforts to find alternative fungicides. Natural plant products such as essential oils (EO's) and hydrosol are gaining attention from researchers globally due to their safety, as well as their antimicrobial, biodegradable, eco-friendly, and economical properties.

Purpose: The purpose of the study was to investigate the use thyme oil and thyme oil hydrosol against citrus fungal pathogen *P. citricarpa* as an alternative to synthetic fungicides.

Methods: After cytotoxicity testing and MIC determination, semi-commercial trials were conducted. Thyme oils and thyme oil hydrosol were incorporated into a variety of semi-commercial citrus coatings. These amended coatings were applied postharvest to oranges in the absence of the synthetic fungicide. Thiabendazole synthetic fungicides were used as a control. Fruit quality was measured thereafter.

Results: Effective disease control was achieved with the amended coatings, while measured quality parameters indicated that overall fruit quality was maintained. Moreover, moisture loss was decreased significantly in fruit treated with essential oil enriched coatings.

Significance: The effectiveness of amended coatings as a possible alternative or supplement to existing fruit protection strategies was demonstrated in a semi-commercial trial.

P3-202 Potential Antimicrobial Combinations Controlling *Listeria monocytogenes* in Hot Dogs

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Introduction: Listeriosis is a fatal disease caused by the consumption of food contaminated with *Listeria monocytogenes*. The prevalence of *L. monocytogenes* under refrigerated conditions is always a concern for ready to eat meats such as hot dogs.

Purpose: The purpose of this study is to evaluate the antimicrobial effect of bisulfate of soda (SBS), nisin, and their combination on *L. monocytogenes* in hot dogs.

Methods: For Trial 1, hot dogs were inoculated with *L. monocytogenes* (initial population: 6.47 log CFU/g) and treated with water, SBS alone (3, 1.5, and 0.75%), nisin alone (2, 1, and 0.5%), and combinations (3% SBS+2% nisin, 1.5% SBS+1% nisin, 3% SBS+0.5% nisin, 1.5% SBS+0.5% nisin, and 0.75% SBS+0.5% nisin). For Trial 2, hot dogs were first treated under the same conditions, then inoculated with *L. monocytogenes* (initial population 6.32 log CFU/g). Hot dogs were treated for 2 min by shaking in each specific treatment, followed by 1 min of exposure to treatment. Afterward, hot dogs were removed and immersed in neutralizing buffer and plated. Data were analyzed using the GLM procedure of SAS software.

Results: For Trial 1, the treatment 3% SBS+2% nisin exhibited the greatest log reduction of *L. monocytogenes* ($P<0.001$). In Trial 2, there was less general reduction in *L. monocytogenes* but responses were still statistically significant ($P<0.001$), with the highest antimicrobial treatment being most effective.

Significance: The quantities of both antimicrobials when combined appeared to be capable of inhibiting *Listeria* without any change in the visual appearance of the hot dog. The results suggest that incorporating bisulfate of soda and nisin as an antimicrobial for hot dogs can be effective for decreasing *L. monocytogenes* populations.

Poster

P3-203 Replacement of Calcium Propionate in Bread with Natural Preservatives Based on Cultured Sugar and Natural Vinegar

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Introduction: Mold spoilage is a significant problem in bread and other baked goods. To date, chemical calcium propionate (Calpro) is the most commonly used agent for inhibiting mold growth in bread. However, natural preservatives are preferred by consumers, but since the available products are mostly based in cultured sugar or wheat, it has been difficult so far to match the shelf life that is reached with Calpro without compromising taste. It is therefore interesting to look for new combinations of natural preservatives.

Purpose: We analyzed the effect of new products based on mixtures of fermented sugar and natural vinegar (Corbion Verdad XM10 and MP100) on the shelf life of bread and compared the shelf life after the addition of Calpro.

Methods: White bread loaves containing a preservative (control, 0.5% Calpro, Verdad XM10, MP100) were prepared in a conventional manner. A testing panel scored each sample of bread on aroma and flavor on a nine-point scale. A cocktail of mold spores was inoculated at 10 locations per loaf at the beginning of shelf life. A total of 10 loaves of bread were inoculated for each preservative tested. The loaves were bagged and kept at room temperature.

Results: All breads with no preservative (control) had visible mold growth after seven days, whereas the breads with the tested preservatives were 100% molded between 13 to 18 days. The newly designed natural Corbion products, XM10 and MP100, provided inhibition of mold growth that was comparable to or better than Calpro. MD100 scored significantly better than Calpro in terms of aroma, whereas XM10 was on par. No difference in terms of flavor were observed.

Significance: The combination of cultured sugar and natural vinegar is a new concept that enables natural preservation of bread, reaching a shelf life at least comparable to chemical preservation without compromising aroma and flavor.

P3-204 Filamentation in *Salmonella*: A Transitional Morphotype in Response to Stress

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Introduction: Filamentous cells of *Salmonella* are formed during exposure to antimicrobials. They are atypical, long cells (>5 µm) containing multiple chromosomal copies. Previously, we described how exposing *Salmonella* to sub-lethal concentrations of pelargonic acid (PA), a nine-carbon saturated fatty acid present in tomato fruit exudates and also used as a herbicide, resulted in filament formation.

Purpose: Evaluate the fate of *Salmonella* filaments in presence or absence of the antimicrobial, PA, to investigate persistence of this morphological state.

Methods: Salmonella Newport was grown in presence of 20 mM PA for 16 h to obtain peak filamentation (3.46 filaments:1 regular cell). Cells were spun down, medium decanted, and suspended in fresh M9 minimal salts medium (M9) with and without pelargonic acid. Cell size, population, ratio of live to dead (L:D) cells, OD_{600}, and ratio of filamentous to regular (F:R) cells were measured at 0, 4, and 8 h.

Results: The initial population of *Salmonella* Newport cells in M9 media amended with PA was 6.4±0.1 log CFU/ml with an F:R ratio of 3.28 at 0 h. When this population was transferred to fresh M9 broth, *Salmonella* Newport counts increased significantly, reaching 9.9±0.1 log CFU/ml after 8 h ($P<0.01$) with an F:R ratio of 0.15, denoting a decrease in filamentation ($P<0.05$). Counts of *Salmonella* Newport cells transferred to M9 with PA remained unchanged after 8 h at 6.5±0.0 log CFU/ml, but the F:R ratio decreased to 0.18 ($P<0.05$), while the OD_{600} declined from 0.09 to 0.06 over 8 h. The OD_{600} of cells in M9 without PA increased from 0.07 to 0.16 over 8 h ($P<0.05$). The L:D ratio of cells in M9 with and without PA was 1.1 and 4.9, respectively, after 8 h ($P<0.05$).

Significance: Salmonella filaments are transitional morphotypes that fragment into regular-sized cells due to septa, formation irrespective of the presence or absence of a stressor; however, sustained stress curbs population growth.

P3-205 Modeling the Effect of Corpo Citrik Sanitizers on the Inhibition of *Pseudomonas aeruginosa*, *Salmonella* Typhi, and *Salmonella* Typhimurium on Stainless Steel Surfaces

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Introduction: In recent years, there has been great interest in the application of natural antimicrobials in food industry. Corpo Citrik is a company that has developed sanitizers derived from citrus products which are biodegradable, can replace sanitizers containing chlorine, iodine, or silver salts, and have a Kosher certification.

Purpose: This study was conducted to evaluate the effect of three Corpo Citrik sanitizers (Citrodex, Citrix-Agro, and CitrolK-Ultra) on the inhibition of *Pseudomonas aeruginosa*, *Salmonella enterica* serotype Typhi, and *Salmonella enterica* serotype Typhimurium on stainless steel surfaces through Weibul modeling.

Methods: Pseudomonas aeruginosa, *Salmonella* Typhi, and *Salmonella* Typhimurium were grown in tryptic soy broth at 35°C for 24 h. Cells (approximately 8 log CFU/ml) were added to stainless steel plates, and each microorganism was evaluated with its respective sanitizer. Every 3 min, a sample was taken (until 15 min of sanitizer action) and analyzed using aerobic plaque count with tryptic soy agar. With data obtained, curves were obtained and adjusted to the Weibul model.

Results: For the three microorganisms tested, log cycle reductions were obtained between 4 and 8. *Salmonella* spp. were the most resistant to the effect of Citrix-Agro and CitrolK-Ultra. Death curves had an adequate fit to Weibul's model (R^2=0.923), and biological parameters showed that inactivation rate was higher between 3 and 6 min of sanitizer application where the major microbial population died or was inactivated.

Significance: This study shows that Corpo Citrik sanitizers with natural antimicrobials are able to inhibit the surface growth of some foodborne pathogens.

P3-206 Indoor Fungi of Food Companies at Monterrey, Mexico

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Introduction: Fungi are ubiquitous organisms present in the air of any building that has contact with the exterior. Due to the presence of certain substrates and/or the environmental conditions required for their processes, food companies may be subject to losses due to the presence of fungi that could spoil their food and may even present an occupational hazard for their workers.

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Purpose: To evaluate the presence of fungi of indoor air at food companies in Monterrey, Mexico and its metropolitan area, since no information has previously been generated here regarding this topic.

Methods: A volume of 100 L of air was collected at four sites of 20 food companies; these sites were transition room, raw materials warehouse, production area, and packaging. An air sampler was employed. Air impacted to petri dishes containing appropriate media sites were sampled in triplicate. Plates were then incubated at 28°C and evaluated when fungal growth was visible. Colonies were counted and each colony was analyzed microscopically via adhesive tape preparations. Fungal genera were then identified by morphological characteristics and the number of colonies of each genus was determined. Yeasts were left unidentified unless showing a pink pigment.

Results: A total of 20 food companies were sampled. *Penicillium* spp. was the most common and abundant fungus, appearing in every company sampled, followed by *Cladosporium* spp. and *Aspergillus* spp. There was an expected correlation of fungal burden with room temperature, with the exception of meat production, where low concentrations of fungi were found. Company "Q" showed the highest concentration of fungi overall, with +1,000 more CFUs than the closest company.

Significance: Our results agree with other studies showing *Penicillium*, *Cladosporium*, and *Aspergillus* among the most common indoor fungi. Periodic evaluation of the indoor air microbiota could offer insights regarding sanitation strategies and potentially prevent losses and health hazards.

P3-207 Viability of *Listeria monocytogenes* on Commercial, Fully Cooked Pork Patties Formulated with and without Buffered Vinegar during Extended Refrigerated and/or Frozen Storage

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Introduction: Many food-grade chemicals are considered unacceptable as ingredients in "clean label", RTE meat products. Such products, however, may support outgrowth of *Listeria monocytogenes* over shelf life. Therefore, research is needed to find alternative antimicrobials for controlling the outgrowth of *L. monocytogenes* on clean label, RTE meats, especially those that aren't cured.

Purpose: Validate the efficacy of buffered vinegar or a blend of buffered vinegar and potassium lactate to inhibit *L. monocytogenes* on commercially prepared, fully cooked, uncured pork patties during storage at 4 and -20°C.

Methods: Fully cooked pork patties (ca. 38 g each) were formulated with or without buffered vinegar (BV; 1.0, 1.5, or 2.0%) or with a blend of buffered vinegar and potassium lactate (V-Lac; 2.0%) by a commercial processor. Patties were surface inoculated on both the top and bottom faces to a target level of ca. 2.3 log CFU/g with a five-strain cocktail of *L. monocytogenes,* placed into nylon-polyethylene bags, vacuum-sealed, and then stored at 4 or -20°C for up to 90 days.

Results: In the absence of antimicrobials, pathogen numbers increased by ca. 5.8 log CFU/g after 90 days at 4°C. Likewise, when pork patties were formulated with 1% BV or 2.0% V-Lac, *L. monocytogenes* numbers increased by ca. 4.1 or 5.1 log CFU/g, respectively, during storage at 4°C. When 1.5 or 2.0% BV was added to the formulation, pathogen numbers decreased by ca. 0.6 log CFU/g after 90 days at 4°C. However, when pork patties were stored at -20°C, regardless if antimicrobials were or were not included in the formulation, *L. monocytogenes* numbers remained relatively unchanged during storage.

Significance: In the event of post-process contamination, inclusion of ≥1.5% of BV would be effective as a clean label ingredient for inhibiting outgrowth of *L. monocytogenes* on fully cooked, uncured pork patties during extended refrigerated or frozen storage.

P3-208 *Lactobacillus salivarius* L28 in Dog Kibble Results in Shifts in Microbial Indicators in Pet Fecal Samples after Feeding

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Introduction: Certain probiotic lactic acid bacteria (LAB) have beneficial effects in the gastrointestinal tract (GI) of humans and pets. Fecal shedding of indicator microorganisms such as *Escherichia coli*, coliforms, *Enterobacteriaceae*, and *Salmonella* indictates the status of GI health.

Purpose: To evaluate the change in microbial indicators in pet fecal samples after feeding *Lactobacillus salivarius* L28 supplemented kibble.

Methods: A total of 47 domesticated dogs were assigned to four feeding groups, each group fed with different kibble: i) control (without probiotics), ii) L28, iii) L28 and commercial probiotic strains, and iv) commercial probiotic strains. Fecal samples from every dog were subject to microbial enumeration on days 3, 2, and 1 before feeding (to generate baseline profile of generic *E. coli*, coliforms, and *Enterobacteriaceae*) and on days 5, 10, 20, and 40 of feeding (to analyze their shifts). Presence of *Salmonella* in fecal samples was detected with Salmonella Real-Time BAX kit, followed by agglutination test.

Results: Reduction of *E. coli*, coliforms, and *Enterobacteriaceae* was higher for group 3, compared to the other feeding groups. In group 3, *E. coli* population was reduced on day 20 by 2.13 log CFU/g fecal matter. The average reduction of coliforms and *Enterobacteriaceae* for group 3 was highest on day 20 at 1.49 log CFU/g fecal matter and on day 40 at 1.93 log CFU/g fecal matter, respectively. The presence of *Salmonella* in fecal samples was rare. Fecal samples collected on day 40 from a dog fed with commercial probiotics were reported as *Salmonella* positive.

Significance: Results show a strong synergistic effect between L28 and commercial probiotic strains. L28 can be used in pet kibble together with commercial strains for improved GI health.

P3-209 Synergistic Antibacterial Effect of *Ishige okamurae* Extract in Combination with Antibiotics against Foodborne Bacteria and Cutaneous Pathogenic Bacteria

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Introduction: Cutaneous pathogenic bacteria often causing *Acne vulgaris* due to the infection of skin tissue. In addition, some cutaneous pathogens are also associated with foodborne disease. Currently, antibiotics have been commonly used to treat bacterial infection. However, excessive use of antibiotics causes drug-resistant bacteria.

Purpose: The object of this study was to evaluate the antibacterial potential of *Ishige okamurae* extract against foodborne bacteria and cutaneous pathogenic bacteria.

Methods: The ethanolic extract of *I. okamurae* was fractioned into *n*-hexane (Hex), dichloromethane, ethyl acetate, and water. The antibacterial activity of *I. okamurae* fractions was evaluated by the MIC and minimum bacterial concentration (MBC). The antibacterial synergy effect was assessed by the fractional inhibitory concentration (FIC) assay. Bacterial strains used in this study were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Propionibacterium acnes*.

Results: Among the ethanol soluble fractions, the Hex-soluble fraction showed the highest antibacterial activity against the pathogens, with MIC values ranging from 64 to 512 µg/ml and MBC values ranging from 256 to 2,048 µg/ml. Interestingly, the MICs of antibiotics (ceftazidime, ciprofloxacin, and meropenem) were dramatically reduced in combination with the Hex fraction. In combination with the Hex and antibiotics, the median ∑FICs ranged from 0.50 to 0.75, suggesting the combination of Hex fraction and antibiotics resulted in a synergistic antibacterial effect.

Significance: *I. okamurae* extracts will be a promising alternative antibacterial therapeutic agent against antibiotic-resistant foodborne bacteria and cutaneous bacterial infections.

P3-210 *Lactobacillus* with Over-Production of Linoleic Acids in Combating against Enteric Bacterial Infections

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❖ ***Developing Scientist Competitor***

Introduction: Probiotics like *Lactobacillus* are known as potential biological agents to control enteric bacterial pathogen colonization through modulation of gut microflora. However, commonly used probiotics have limitations in the amount of bio-active metabolites they can produce. Conjugated linoleic acids (CLA) are recognized as one of the critical metabolites from probiotics, providing numerous beneficial effects.

Purpose: We aim to over-express the myosin cross-reactive antigen gene (*mcra*) in *Lactobacillus casei* for enhancing the production of CLA, and to investigate *in vitro* and *in vivo* its effects against enteric bacterial infections.

Methods: The *mcra* was ligated into pMSP3535 vector for expression and pDS132 suicide vector to be transformed into *E. coli* B2155 for bacterial mating. Final mutant (LC-CLA) was harvested through chromosomal integration. The *mcra* expression was evaluated by quantitative PCR, and the total linoleic acids production was measured with high-performance liquid chromatography-mass spectrometry. LC-CLA was examined against enterohemorrhagic *E. coli* (EHEC) and *Salmonella* Typhimurium via *in vitro* bacterial growth, *ex vivo* host cell infection, and *in vivo* mice gut colonization.

Results: LC-CLA showed longer survival ability (+0.8 log CFU/ml), increased (5.20%) adhesion activity, and up-regulated (6.62-fold) *mcra* expression. The total CLA production was increased by 21-fold. LC-CLA competitively excluded (100%) *S.* Typhimurium/EHEC within 48 h. Its cell-free cultural supernatant down-regulated (2- to 5-fold) inflammatory cytokine/chemokine (IL-1β, IL-6, TNFα, CCL3, CXCL-8) genes and up-regulated (>3-fold) anti-inflammatory cytokine (IL-10) gene expression. Furthermore, LC-CLA significantly reduced adhesive/invasive abilities of both EHEC (78.47 and 97.44%, respectively) and *S.* Typhimurium (66.64 and 95.11%, respectively). Additionally, LC-CLA pre-treatment in three-week-old mice reduced the *S.* Typhimurium/EHEC cecal colonization by 1 to 2 log CFU/ml.

Significance: CLA over-production contributes to both anti-inflammation and reduction of pathogenic bacterial colonization. This novel probiotic might serve as efficient prevention against enteric bacterial infections.

P3-211 Antimicrobial Potential of Chinese Cabbage Using Different Solvents

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Introduction: Chinese cabbage (*Brassica campestris L.* ssp. *Peckinensis*) is the most widely consumed *Brassica* vegetable in Asian countries, including Korea, both as a raw vegetable and in processed form. Chinese cabbage is a rich source of nutrients and phytochemicals such as flavonoids and glucosinolate, as well as their breakdown products, which may have antibacterial, antioxidant, and anticancer properties.

Purpose: The purpose of this study was to investigate the antimicrobial potential of Chinese cabbage against foodborne pathogens.

Methods: The antimicrobial activity of Chinese cabbage was determined using different solvents and a computer-based docking method. The total phenol (TP) and total flavonoid (TF) contents were monitored, as well as antioxidant activity. In addition, gas chromatography–mass spectrometry analysis was also carried out to examine the variety of volatile compounds responsible for the cabbage's antimicrobial and antioxidant activity. The binding affinity of molecules derived from different cabbages towards lipopolysaccride (LPS) was determined theoretically by a computer-based molecular docking method.

Results: The results showed that remarkable inhibition of the bacterial growth was shown against the tested organisms. A MIC of 33 mg/ml was shown against *Escherichia coli*, and a MIC of 66 mg/ml was determined against all organisms tested in this study. The TP and TF in Chinese cabbage chloroform extract was determined as 32.6±0.05 mg GAE/g and 28.1±0.04 mg QE/g, respectively. A total of eight compounds were detected from the Chinese cabbage crude extract. Through the computer-based docking method, 1,2-Benzenedicarboxylic acid and 2,3-Dicyanopropionamide exhibited a strong binding affinity towards the lipopolysaccride, which is a highly charged outer membrane that is crucial for survival and pathogenicity of the bacteria.

Significance: All these findings together indicate that Chinese cabbage has potential for antimicrobial activity and could be a good source of natural antimicrobial agents.

P3-212 Antimicrobial Activity of Pecan Shell Extracts against Various Foodborne Bacterial Pathogens

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Introduction: Pecan nut shells are rich source of bioactive compounds, having natural inhibitory activity against various microorganisms. However, their efficacy against various foodborne pathogens under several influencing factors need to be studied.

Purpose: This study investigated the effect of pecan variety and the method of extraction on the content of bioactives and the antimicrobial activity of pecan shell extracts against foodborne pathogens.

Methods: Twelve different varieties of defatted pecan nut shells (50 to 100 µm) were subjected to aqueous infusion and ethanol extraction (1:20 weight/volume) procedures, respectively. The MIC of lyophilized shell extracts (from 10 to 0.0625 mg/ml) dissolved in deionized water containing 5% dimethyl sulfoxide (volume/volume) were tested against *Escherichia coli* O157:H7 (three-strain), *Salmonella* spp. (three-strain), and *Listeria monocytogenes* (four-strain), each at 10^5 CFU/ml. The MIC results were confirmed by a colorimetric microbial viability assay based on the reduction of Iodonitrotetrazolium chloride dye and compared with controls. Further, the minimum bactericidal concentrations (MBC) of the test organisms were determined by plating on selective media. Chemical analysis of shell extracts was also performed simultaneously. The data were analyzed by analysis of variance using SAS software.

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Results: Pecan variety and extraction methodology significantly affected (P<0.05) the total phenolics content and antimicrobial property of shell extracts. Ethanolic extractions contained higher levels of total phenolics (330.34 [var. Gloria Grande] to 1061.59 [var. Sumner] mg GAE/g) compared to aqueous infusions (61.8 [var. Elliot] to 969.55 [var. Sumner] mg GAE/g). However, higher phenolic content does not translate to higher antimicrobial activity. Aqueous infusions of certain varieties (i.e., Curtis, P-Cou2, and Sumner) showed lower MICs (2.5 mg/ml) against *Salmonella* spp. compared to others. The MIC and MBC values of the shell extracts ranged from 2.5 to 5 mg/ml.

Significance: Pecan shell extracts obtained from different varieties using different extraction protocols show promise for use as natural antimicrobial agents.

P3-213 Identification and Heterologous Expression of Novel Antimicrobial Bacteriocins from a Soil Metagenome

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❖ ***Developing Scientist Competitor***

Introduction: Antibiotic-resistant foodborne pathogens in food-producing animals such as poultry, cows, swine and dairy animals cause important economic and human losses. To preserve foods and ensure safety, synthetic preservatives are usually used. Development of novel natural antimicrobials can be an alternative strategy to replace those conventional antibiotics and preservatives. Bacteriocins are small ribosomally synthesized natural products that can have inhibitory activity against foodborne pathogens.

Purpose: The objective of the study was to express and purify metagenome-derived bacteriocins in an *Escherichia coli* expression system.

Methods: A metagenomic approach was used to discover bacteriocins encoded by soil microorganisms sampled from soil at a long-term agricultural rotation plot (Cullars Rotation) at Auburn University. Metagenomic clones were sequenced in column, plate and row pools, and contig sequences from each respective pool, and they were analyzed using BAGEL3 and antiSMASH3.0 to predict bacteriocins. Class III bacteriocins, lasso peptides, lanthipeptides, sactipeptides, and bottromycin-like bacteriocins were identified from the library. Among the identified bacteriocins, five predicted gene sequences were PCR amplified, subcloned, and expressed as a fusion protein partner with the solubility enhancer SUMO and purified by affinity chromatography.

Results: The predicted bacteriocin gene sequences were highly divergent as compared to known bacteriocins in the GenBank nr/nt database, ranging from 34 to 89% shared identity to their top BLAST hit. The phylogenetic origin of those predicted bacateriocins were *Acidobacteria*, *Actinobacteria*, *Proteobacteria*, and *Cyanobacteria*. Out of those selected five clones, only three expressed bacteriocins as determined by polyacrylamide gel electrophoresis analysis. A class I bacteriocin (Sactipeptide) was highly expressed at 30 and 37°C, whereas the other two class II bacteriocins were highly expressed at 37°C, but the expression was low at 20 and 25°C for both classes of bacteriocins.

Significance: The novel bacteriocins identified could be used to create an attractive antimicrobial agent in the meat and dairy production industry.

P3-214 Antibacterial Activity of *Carnobacterium* spp. Isolated from Vacuum-packaged Meats under Chilled Anaerobic Conditions

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Introduction: *Carnobacterium maltaromaticum* and *Carnobacterium divergens* are frequently predominant among microbiota on chilled vacuum-packaged (VP) meats. Due to the potential of producing antibacterial compounds, the two species may be used as protective cultures to extend the storage life of VP meat and improve meat safety.

Purpose: To investigate the inhibitory activity of 15 strains of *Carnobacterium* spp. obtained from chilled VP meat on the growth of spoilage-associated bacteria and pathogenic bacteria under chilled anaerobic conditions.

Methods: The inhibitory activity of three strains of *C. maltaromaticum* and 12 strains of *C. divergens* which had been identified by whole genome sequencing analysis from previous studies were selected. The target bacteria included both spoilage-associated bacteria and pathogenic bacteria, i.e., the same 15 strains of *Carnobacterium* spp., four *Hafnia,* four *Rhanella*, six *Serratia*, three *Yersinia*, 13 pathogenic *Escherichia coli* of different serotypes, and three *Salmonella* spp. Inhibitory activity was tested on tryptone soya agar incubated in an anaerobic jar at 10°C for 5 days, and was determined as positive when an inhibition zone was present.

Results: Two *C. maltaromaticum* and three *C. divergens* strains showed inhibitory activity. Target bacteria sensitive to the inhibitory strains were mostly *C. maltaromaticum* or *C. divergens,* two out of four *Hafnia*, and one out of four *Rhanella*; none of the *Serratia* or pathogenic bacteria were inhibited. *C. maltaromaticum* A5 showed the widest inhibition spectrum by inhibiting 13 *Carnobacterium* spp., two *Hafnia*, and one *Rhanella*, followed by *C. maltaromaticum* A7, which showed inhibition on the growth of seven *Carnobacterium* spp. and one *Rhanella* spp.

Significance: The five strains of *Carnobacterium* spp., *C. maltaromaticum* A5 and A7 in particular, can be explored as protective cultures in the future.

P3-215 Evaluation of Antimicrobial Activities of Plant Aqueous Extracts against Different Strains of *Salmonella* Typhimurium and Their Application to Improve Safety of Pork Meat

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❖ ***Developing Scientist Competitor***

Introduction: The utilization of plant aqueous extracts as natural antimicrobial preservatives in foods has not been extensively studied.

Purpose: To examine the antimicrobial activity of eleven plant aqueous extracts against three strains of *Salmonella* Typhimurium and evaluate their application in the food industry.

Methods: Oregano, centrifuged oregano, thyme, calendula, basil, laurel, rosemary, spearmint, corn silk, and garlic aqueous extracts collected by hydrodistillation and a commercially acquired oregano extract were tested for their *in vitro* antimicrobial efficiency against three *Salmonella* Typhimurium strains (4/74, FS8, FS115) at 4 and 37°C. Subsequently, pork meat was inoculated with FS8 strain (~6 log CFU/g), and covered with sodium alginate edible coatings made of aqueous or commercial oregano extract (OAE or OCE) with (0.5% volume/volume) or without oregano essential oil and stored at 4°C for 6 days.

Results: The antimicrobial effect of extracts was mainly depended on the plant and storage temperature. At 4°C, pathogen populations were reduced for all extracts apart from garlic. Oregano extract exhibited the strongest antimicrobial activity, followed by thyme, calendula, and centrifuged oregano. Higher populations were enumerated for FS8 strain (P<0.05) during the 7th and 8th days of storage for calendula extract stored at 4°C. At 37°C, oregano, calendula, thyme, centrifuged oregano, and basil displayed a bactericidal effect, eliminating or reducing enumerated populations by approximately 4.0 log CFU/ml within 9 to 24 h. Garlic, spearmint, rosemary, and corn silk permitted growth. Contrary to the above tested plants, laurel had a strain-dependent effect on *Salmonella* Typhimurium at 37°C; FS8 increased by 1.3 log CFU/ml, while 4/74 and FS115 were reduced by 2.2 and 1.5 log CFU/ml, respectively. Storage of pork meat with OAE and OCE reduced FS8 populations by approximately 2 log CFU/g until the end of the storage.

Significance: Plant aqueous extracts, derived as by-products of essential oil production, could serve as alternative natural food preservatives.

P3-216 Validation of Novel Cultured Cane Sugar and Vinegar Powder Solution to Provide Double Shelf Life from *Listeria monocytogenes* Inhibition in Uncured Deli Sliced Chicken Stored at 40°F in Comparison with Vinegar Solution

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Introduction: Consumer demand for clean label products and simple ingredients has pushed the meat industry to develop more natural meat products. *Listeria monocytogenes* is a gram positive bacteria that can contaminate RTE meat products. The shelf life requirement for uncured poultry products is becoming difficult to achieve due to their high moisture, low salt content, and higher pH values. Verdad® Opti Powder N70 is a fermentation product containing multiple benefits. It provides the cleanest flavor, with an extended shelf life and *L. monocytogenes* control in these difficult formulations. It provides twice the shelf life of a vinegar solution. Verdad® Powder N6 assessed, along with a dried vinegar solution.

Purpose: To evaluate the antimicrobial performance of cultured cane sugar and vinegar powder product (CSV-P) for controlling *L. monocytogenes* outgrowth in uncured deli sliced chicken, which was vacuum packaged and stored at 40°F for 100 days.

Methods: Ground chicken breast, salt, sugar, and spices were mixed and vacuum tumbled before stuffing in cellulose casings, and then cooked and smoked. Slices of 25 g each were surface inoculated with a five-strain *L. monocytogenes* cocktail and were individually packaged to be incubated at 40°F. Three sets of samples were split for each treatment and plated individually. Biweekly analyses for *L. monocytogenes* counts (plated on Modified Oxford Media & incubated at 35°C for 24 h) was done on for 100 days.

Results: Inoculation level of 2.74 Log CFU/g of pathogen was achieved on Day 0 for all the treatments. No antimicrobial treatment exhibited fastest growth and reached 4.74 and 8.34 log CFU/g counts on day 14 and 21, respectively. Dried vinegar treatment (0.7%) delayed the growth of *L. monocytogenes* to 4.74 log CFU/g for 44 days. CSV-P treatment (1.88%) suppressed the growth of *L. monocytogenes* throughout the 100 days at 40°F.

Significance: This research validates the efficacy of the CSV-P over vinegar powder solution in controlling the outgrowth of *L. monocytogenes* in uncured deli sliced chicken for 100 days.

P3-217 Addition of Oregano Extract to a Cranberry Marinade Enhances Inhibition of *Listeria* on Chicken

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Introduction: The antimicrobial properties of cranberry extracts are well-documented. We have previously demonstrated that foodborne microorganisms are inhibited by cranberry extracts in broth and liquid food systems, as well as on unprocessed chicken. Analogous to cranberry, herbal extracts also inhibit foodborne microorganisms. Previous studies have shown that oregano extract exerts a synergistic effect on the antimicrobial properties of cranberry.

Purpose: The goal of this study was to assess the potential of oregano extract to enhance the inhibition of *Listeria* on chicken, applied via a cranberry-based marinade.

Methods: Unprocessed chicken slices were inoculated with 5 log CFU *Listeria innocua* (a surrogate for *L. monocytogenes*) and air-dried before exposure to marinades containing cranberry extract, oregano extract, or combinations of the two in various ratios. Untreated but inoculated chicken, as well as water-treated chicken slices, were included as controls. A minimum of three replicates were analyzed for each treatment. Chicken slices were marinated at 4°C for 1 h before they were neutralized with D/E broth and processed for recovery and enumeration of *L. innocua* on standard methods agar. Efficacy of microbial inhibition was statistically compared using analysis of variance.

Results: Exposure to a marinade containing a 0.25 X cranberry extract combined with 1% oregano extract resulted in a 2-log reduction (P< 0.05) of *L. innocua* on chicken slices. These results were similar to the effect of marinade containing 0.5 X cranberry extract. Exposure to marinades containing either 0.25 X cranberry extract or 1% oregano extract resulted in a ~1-log reduction of *L. innocua.*

Significance: Previous studies have demonstrated that pre-treatment of beef and fish with a cranberry-oregano mixture prevented growth of *Listeria*. In comparison, we demonstrate that a marinade containing cranberry and oregano extracts inhibits *Listeria* after it has contaminated unprocessed chicken.

P3-218 Cranberry Extract Inhibits Foodborne Bacteria without Detectable Resistance

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Introduction: In recent decades, bacterial resistance to antibiotics has been increasingly reported, creating an urgent need for alternative antimicrobials. Natural compounds such as cranberry extract (CE) may present a unique opportunity to address the growth of antibiotic resistance.

Purpose: This study evaluated the ability of foodborne organisms, *Listeria innocua* and *Escherichia coli*, to develop resistance to CE.

Methods: MICs of chloramphenicol, ofloxacin, and CE against 5 log CFU/ml of *L. innocua* and *E. coli* were determined by broth dilution assay at pH 6.0, 35°C. Bacteria at 5 log CFU/ml were then exposed to a series of antibiotics or CE concentrations (0, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, and 256X MIC) and serially passaged every 24±2 h from the second highest concentration, allowing bacterial growth into the series of fresh media containing antibiotics or CE. Changes in MICs were determined. All experiments were carried out for up to 30 days at pH 6.0, 35°C and replicated at least twice.

Results: MICs of chloramphenicol, ofloxacin, and CE for *L. innocua* were 5 ppm, 5 ppm, and 1%, and for *E. coli* were 10 ppm, 0.5 ppm, and 1.5%, respectively. No change in CE MICs was found during the test period for both organisms. For *L. innocua*, ofloxacin MIC increased by 4-fold after Day 4, and chloramphenicol MIC increase by up to 128- and 256-fold after 6- and 13-day serial exposure, respectively. For *E. coli*, ofloxacin MIC increased by up to 8-fold after 4-day and 32-fold after 14-day serial exposure. Chloramphenicol MIC for *E. coli* increased 16-fold once the organism was serially exposed to the antibiotic for >14 days.

Significance: Our results showed that while bacteria developed antibiotic resistance, CE effectively inhibited foodborne organisms without detectable resistance.

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P3-219 Antibacterial and Antibiofilm Activities of Ginger (*Zingiber officinale)* Extracts against Some Isolates of *Escherichia coli* O157:H7 from Retailed Dispensed Powdered Milk in Ibadan, Nigeria

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❖ ***Developing Scientist Competitor***

Introduction: *Escherichia coli* O157:H7 is a pathotype known to cause food poisoning. Studies on its prevalence in retailed powered milk and control of biofilm formation by using natural spices are limited.

Purpose: This study determined the prevalence of *Escherichia coli* O157:H7 in retailed powdered milk in Ibadan, Nigeria and assessed the antibacterial and anti-biofilm activities of crude ginger (*Zingiber officinale*) extract and four other fractions using different solvents.

Methods: A total of 108 of milk samples were collected from seven markets. Enumeration of *Escherichia coli* O157:H7 was performed using standard methods. The antibiotic sensitivity pattern was determined using disc diffusion method. *Z. officinale* was obtained, identified, extracted, fractionated using four solvents (ethanol, ethylacetate, chloroform, and hexane), and concentrated. Phytochemical screening and thin layer chromatography was done. Antibacterial activity and MIC were evaluated by means of agar-well diffusion assay at 50, 25, 12.5, and 6.25 mg/ml concentrations. Biofilm inhibition and dispersion were assessed on microtitre plates and quantified using crystal violet binding assay.

Results: A prevalence of 2.7% was obtained, and all the isolates were resistant to one or multiple antibiotics; ciprofloxacin, nitrofurantoin, and gentamycin were the most effective. Phytochemical results showed the presence of alkaloids, anthraquinones, flavonoids, saponins, tannins, and terpenoids in all or some of the extract, while cardiac glycosides and steroids were absent. All the fractions had antibacterial effects, while the highest and lowest effects were yielded by the ethanolic (0.50±0.04) and chloroform fractions (0.25±0.07), respectively, at a MIC of 50 mg/ml. Biofilm inhibition and dispersion were exhibited with the crude extract and ethanol fractions having the highest effect, while the hexane fraction had the least effect at 50 mg/ml concentration.

Significance: The occurrence of *E. coli* O157:H7 in retailed powdered milk suggests pre- or post-production contamination. Ginger (*Zingiber officinale*) extract is useful for biofilm control.

P3-220 Use of Green-label Bacteriocin-containing Microbial Fermentates for Control of *Listeria monocytogenes* in RTE Meat Applications

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❖ ***Developing Scientist Competitor***

Introduction: Listeria monocytogenes has been a recurring problem associated with the manufacture of RTE meats. Consumer preference for natural products has resulted in companies switching to "celery nitrite" and using natural antimicrobials (e.g., fermentates and dried vinegar) instead of chemical preservatives.

Purpose: We have compiled multiple mode-of-action (MOA) bacteriocin cocktails for use as "green label" microbial fermentates against *L. monocytogenes* and examined these against several commercial antimicrobials.

Methods: Mixtures of bacteriocins produced by lactic acid bacteria (LAB) were used that represented four different MOAs against *L. monocytogenes*. These fermentate mixtures created a multi-MOA bacteriocin antimicrobial that was used as a post-process surface treatment or included in the meat matrix during the manufacture. Shelf life studies were conducted comparing two different mixtures of our preparation to two different commercial antimicrobials. Hot dogs were manufactured in our meat pilot plant and shelf life studies (n=45) were evaluated against high and low inoculums of a four-strain cocktail of *L. monocytogenes*. Treated hot dogs were stored at 5°C and plated on modified Oxford agar for *L. monocytogenes* and Man, Rogosa, and Sharpe agar (pH 5.5) for LAB.

Results: Bacteriocin added to hot dog surfaces was sufficient to maintain *Listeria* levels below 3.0-log for 6 and 8 weeks in high and low inoculum studies, respectively. The application of bacteriocin cocktail within the meat matrix during manufacture held *Listeria* levels at approximately 2.0-log after 6 weeks. Bacteriocins exhibited better inhibition than one commercial antimicrobial at both inoculation levels and better than the second one at high inoculation levels. All trials were performed in triplicate replication, and repeated measures one-way analysis of variance was carried out to see significant differences (P<0.05) in the response to different antimicrobial treatments.

Significance: The use of multiple MOA bacteriocin fermentates could serve as natural, green label antimicrobials to control levels of *Listeria* that may inadvertently contaminate RTE meat products.

P3-221 Antimicrobial Effectiveness of Iso-Eugenol against Human Enteric Pathogens in Refrigerated Raw Pineapple Juice with Added *Yucca schidigera* Extract

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❖ ***Developing Scientist Competitor***

Introduction: Increasing consumer demand for juices that are fresh, nutritious, devoid of synthetic preservatives, and safe has encouraged extensive research on novel food preservation methods. Plant essential oils (EOs) and their components are widely accepted by consumers as natural and have exhibited antimicrobial activity against human enteric pathogens. Iso-eugenol (ISO-EU), a component of clove EO, and Yucca extract (YEX), a natural emulsifier from the *Yucca schidigera* plant, have not been evaluated together for antimicrobial effectiveness against pathogens in juices.

Purpose: The purpose of this study was to investigate the antimicrobial efficacy of ISO-EU against *Escherichia coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes* in refrigerated (4°C) pineapple juice with added YEX.

Methods: Tyndallized pineapple juice (pH 3.5) with added 0.5% YEX and 0.50, 0.75, 1.0, or 1.5 µl/ml of ISO-EU was inoculated with a five-strain mixture of *E. coli* O157:H7, *L. monocytogenes*, or *S. enterica* to obtain an initial viable count of ~7.10 log CFU/ml and stored at 4°C. Inoculated juice with 0.5% YEX alone served as control. At set intervals during 70 days of storage, survivors were determined by surface plating diluted (10-fold) juice samples on tryptic soy agar with 0.6% yeast extract followed by counting bacterial colonies after incubation (35°C, 48 h).

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Results: None of the pathogens multiplied in the refrigerated (4°C) juice with or without added ISO-EU. All three pathogens survived up to 56 days in the control juice. All ISO-EU treatments achieved >5 log reduction of each pathogen within 24 h. ISO-EU (1.5 µl/ml) was most effective and completely inactivated *E. coli* O157:H7 and *L. monocytogenes* after 4 h and *S. enterica* after 6 h (P<0.05).

Significance: The results demonstrate that ISO-EU (0.5 to 1.5 µl/ml) combined with yucca extract has good potential for destroying human enteric pathogens in refrigerated pineapple juice.

P3-222 Isolation of Bacteriocin-producing Lactic Acid Bacteria from Fermented Foods Using Improved Deferred Antagonism Assay

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Introduction: Bacteriocins are antimicrobial peptides or proteins produced by certain bacteria. Lactic acid bacteria (LAB) are common bacteriocin-producers and are often found in fermented foods. These bacteria and their bacteriocins can inhibit other bacteria that cause food spoilage and foodborne disease.

Purpose: This study aimed to improve the bacteriocin-producer isolation method, deferred antagonism assay, by optimizing media composition, as well as isolating and identifying bacteriocin-producing LAB from fermented foods.

Methods: Three types of media (Man, Rogosa, and Sharpe [MRS] agar, M17 agar, and Elliker agar) with two types of buffering salts (disodium-β-glycerophosphate and a combination of Na_2HPO_4 and NaH_2PO_4) at different initial medium pH (5.5 to 6.9) were tested with three bacteriocin-producing LAB and three bacteriocin non-producing LAB via deferred antagonism assay. Tween 80 and ethanol were added at 1% to the isolation media to assess their effects on bacteriocin production. The improved deferred antagonism assay was employed to isolate bacteriocin-producing LAB from kimchee, sauerkraut, yogurt, and kefir. Bacteriocin-encoding genes in the isolated strains were amplified and sequenced. Bacteriocin-like compounds produced by the isolates were treated by heating at 100°C with proteinase K or pronase E prior to being tested for their antimicrobial effects.

Results: The three bacteriocin non-producing LAB formed inhibition zones against *Listeria monocytogenes* on MRS agar with either buffering salt. Elliker agar with the combination of Na_2HPO_4 and NaH_2PO_4 at pH 6.9 and 1% Tween 80 was identified as the most appropriate bottom media in deferred antagonism assay. With the improved assay, three types of *Lactococcus lactis* carrying nisin Z and lactococcin 972 genes and one type of *Lactobacillus plantarum* carrying plantaricin S gene were isolated from fermented food samples. Their bacteriocins were thermostable but inactivated by the two enzymes.

Significance: This study optimized the isolation of bacteriocin-producing LAB, which can potentially be applied in fermented foods to improve their quality and safety.

P3-223 Determining the Efficacy of Chemical and Bacteriophage Treatments to Disrupt *Escherichia coli* O157:H7 Biofilms

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✦ ***Undergraduate Student Award Competitor***

Introduction: *Escherichia coli* O157:H7 can produce strong biofilms on food contact surfaces that can be difficult to remove. Chlorine, a commonly used sanitizer in the food industry, could reduce this problem. However, chlorine is a cause for concern due to the health and environmental risks it poses. It is therefore necessary to evaluate effective alternatives that could also disrupt bacterial biofilms.

Purpose: Evaluate the biofilm-disrupting efficacy of chemical sanitizers and bacteriophages *in vitro* and on food contact surfaces.

Methods: Biofilm-disrupting capabilities of chlorine (200 ppm), peracetic-acid (200 ppm), sodium-acid-sulfate (0.1, 1, and 3%), water and *Escherichia coli* O157:H7-specific bacteriophage cocktail (9 log PFU/ml) were examined. A cocktail of three *E. coli* O157:H7 strains (~9 log CFU/ml) were used to form biofilms in microtiter plates and on stainless steel or high-density polyethylene coupons. Plates/coupons were then treated with respective treatments. Untreated positive and negative controls were also used. Biofilm disruption *in vitro* was measured as change in absorbance (A_{595}), whereas that on coupons was measured by enumerating *E. coli* O157:H7 population. Efficacy of sanitizers was tested at 0 min, 10 min, and 8 h, while that of bacteriophages was tested at 0 min, 3 h, and 6 h. Data from three replicates were analyzed using analysis of variance (P<0.05).

Results: Sodium acid sulfate (1 and 3%) was the most effective treatment *in vitro*, showing an immediate reduction in absorbance (0.230 nm) at 0 h, compared to the control (1.00 nm). Phage treatment was also able to disrupt biofilms, with reductions in absorbance from 2.250 (0 h) to 0.531 nm (6 h). All chemical treatments were extremely effective on the coupons, reducing pathogen populations to undetectable levels at 0 min. Bacteriophages also showed significant reductions (2.3 to 4.1 log) in pathogen populations on the coupons, compared to the control.

Significance: Alternatives to chlorine such as peracetic-acid, sodium acid sulfate, and bacteriophages could be used for disruption of biofilms on food contact surfaces.

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