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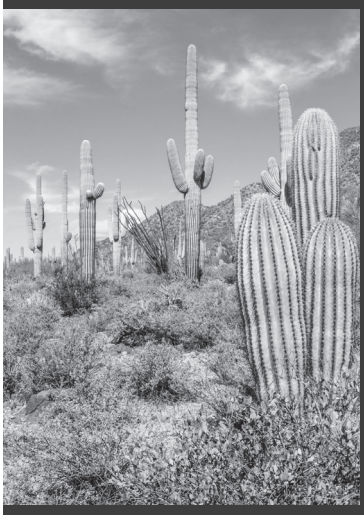

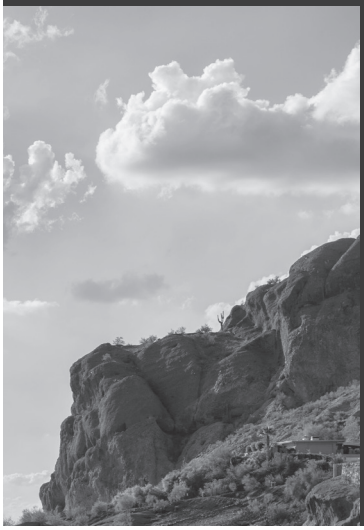

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July 18–21

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ABSTRACTS

This is a collection of the abstracts from the IAFP 2021 Annual Meeting.

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Scope of the Journal. The *Journal of Food Protection*® (*JFP*) is an international monthly scientific journal in the English language published by the International Association for Food Protection (IAFP). *JFP* is intended for publication of research and review articles on all aspects of food protection and safety. Major emphases of *JFP* are placed on studies dealing with (1) Tracking, detecting (including traditional, molecular, and real-time), inactivating, and controlling food-related hazards including microorganisms (including antibiotic resistance), microbial (mycotoxins, seafood toxins) and non-microbial toxins (heavy metals, pesticides, veterinary drug residues, migrants from food packaging, and processing contaminants), allergens and pests (insects, rodents) in human food, pet food and animal feed throughout the food chain; (2) Microbiological food quality and traditional/novel methods to assay microbiological food quality; (3) Prevention of food-related hazards and food spoilage through food preservatives and thermal/non-thermal processes, including process validation; (4) Food fermentations and food-related probiotics; (5) Safe food handling practices during pre-harvest, harvest, post-harvest, distribution and consumption, including food safety education for retailers, foodservice, and consumers; (6) Risk assessments for food-related hazards; (7) Economic impact of food-related hazards, foodborne illness, food loss, food spoilage, and adulterated foods; (8) Food fraud, food authentication, food defense, and foodborne disease outbreak investigations.

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IVAN PARKIN LECTURE

It's All About People, Isn't It?

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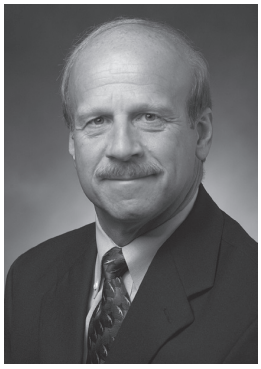

In recent years, there have been some major and notable foodborne outbreaks and product defects resulting in national recalls of a wide variety of food products that were contaminated with biological, chemical or physical hazards and affected many people. When these incidents were analyzed, they were often found to be caused by failures of Good Manufacturing Practices (GMPs) and were rarely caused by true food system failures. Yes, GMPs, problems with people performing their tasks correctly. Consider some high profile events such as a major foodborne outbreak where *Salmonella*-contaminated product was shipped despite knowing that it was contaminated, food allergen recalls where product and package mismatches occurred, or recalls related to physical hazards like plastic, glass or metal pieces being found in processed food products. Recalls that are easily preventable by empowered employees taking action when a potential problem might threaten the safety of a product.

Organizations normally address these, as well as other challenging situations, by allocating a variety of resources, including monetary resources, technological innovations and personnel. Of these, personnel is often the one resource that may not receive as much attention as the others, since a company workforce is already in place. So let's talk about people. People are the major asset of any organization. Think about it…how can a company or organization prosper and advance without a well-trained, strong, knowledgeable workforce, dedicated to company values and mission, working toward a common goal of producing, processing, transporting, distributing, preparing or merchandising safe foods? Those employees need to perform their job responsibilities in food safety and quality with consistency, each and every time they perform them. Many companies have on-boarding training, refresher training, on-the-job training and many other kinds of programs designed to provide employees with more information about their job tasks. But, are these programs working and are they effective?

The answer to this question begins with thoughts about the common characteristics (core values) of world-class food companies. These core values frequently set the organization apart from the competition and give employees an understanding of the fundamental beliefs of the company. One of these core values, a culture of learning, can and does have a profound influence on the behaviors of employees throughout every department and job function within the company. People are unique and different from each other, so their perceptions, beliefs, attitudes, values, principles, practices and behaviors toward food safety and quality in an organization are crucial to them performing their tasks correctly, competently and consistently. Think about the evolution of a learning culture within an organization…from the very traditional, "basic" training (the minimum amount of information needed to do one's job), to actually creating a learning environment that empowers people to build upon their knowledge and skills and gives them new, updated information and practices to do their work at a higher level of proficiency.

It's about providing new ideas, innovative thinking, exciting and meaningful engagement in teaching and learning, to create behavioral changes in the workforce. A culture of learning is learner centered, performance based and focused on results. It is creating an organization workforce of individuals who have reached the stage where they are "unconsciously competent." Employees who perform their tasks regularly, routinely, capably, knowledgeably and proficiently! Each and every time! Several companies that have a learning culture and achieved success in the marketplace will be highlighted.

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JOHN H. SILLIKER LECTURE

We All are Working on the Same Puzzle

Barbara J. Masters, DVM

Vice President, Regulatory Policy, Food and Agriculture
Tyson Foods
Washington, D.C., USA

I am honored and humbled to present the John H. Silliker Lecture. Dr. Silliker was a pioneer and a visionary when it comes to *Salmonella,* so it seems fitting to present on the pathogen we are all still at war with. Each year we attend the IAFP Annual Meeting to learn the latest and greatest scientific information on food safety. I have personally attended many presentations on *Salmonella*, focusing on poultry to produce – on-farm to processing. There are many sessions this year dedicated to the topic. Yet the CDC's Surveillance for Foodborne Disease Outbreaks United States, 2017: Annual Report reports: *The pathogen-food category pairs responsible for the most illnesses in outbreaks with a single confirmed etiologic agent were* Salmonella *in turkey (580 illnesses),* Salmonella *in fruits (421), and* Salmonella *in chicken (299).*[1]

Why are the pieces to this particular puzzle so hard to put together?

Having had the opportunity to wear the hat of a regulator, consultant, industry representative and member of a consumer education organization, I will attempt to examine the efforts taken to reduce *Salmonella* contamination and to ponder what the next steps may be.

The Food Safety and Inspection Service (FSIS) implemented performance standards for raw meat and poultry products in 1996. The performance standards have continued to evolve over the past twenty plus years. FSIS data demonstrates that establishments have met the standards and reduced *Salmonella* contamination in products over time.[2]

FSIS, CDC, and industry are all using newer laboratory methods. We have genetic sequencing, quantification and enumeration and other laboratory tools being developed.

Industry (beef, pork, and poultry alike) has been working through their respective trade organizations. I am speaking broadly for industry, not for any one company, plant or commodity. My observations have been that there has been sharing of best practices, research projects, and even multi-species task force formation. The trade groups have worked closely with FSIS, the Agricultural Research Service and the Centers for Diseases Control to exchange information and ideas. The poultry industry has shared learnings with the beef and pork industry and vice-versa. The industry is working aggressively to address this as a holistic concern.

The food safety education community has made efforts to continue to improve their tools. There has been information added to recipe cards to educate on handwashing and thermometer usage. There are biennial food safety education conferences to help participants gain insights into how to change consumer behavior to improve food safety practices. FSIS hosted their own food safety education conference in 2020.

The research community is actively engaged in *Salmonella* research. There are projects taking place on quantification methodology and highly pathogenic serotypes. There are on-going projects attempting to compare what comes from a farm or ranch to what is observed at a processing plant.

Having worn all these different hats, I feel I can speak with confidence when I say that when it comes to looking for the solution and fighting this pathogen – everyone is "all in." I used to think that each group was engaged in working on their own puzzle. I have come to realize we are all working on a different part of the same puzzle, but perhaps sometimes we are working too far apart to see how the pieces fit together. Perhaps this has prevented us from successfully completing the entirety of the complex puzzle laid before us.

Attending conferences like IAFP where we take off our "day job hat" and listen to the latest science – each through our own lens – provides one of the greatest opportunities for us to work together to find answers. I feel strongly there are answers to this challenge, and if we all look together using science-based, data-driven approaches, we are most likely to complete this frustrating puzzle that has long been challenging us all.

1. https://www.cdc.gov/fdoss/pdf/2017_FoodBorneOutbreaks_508.pdf
2. https://www.fsis.usda.gov/wps/portal/fsis/topics/data-collection-and-reports/microbiology/quarterly-reports-salmonella

Symposium Abstracts

S1 Foodborne Disease Outbreak Update

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ANDREA COTE: *United States Department of Agriculture Food Safety Inspection Service (USDA – FSIS), Atlanta, GA, USA*
AMANDA CONRAD: *Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA*
CLAUDIA RUSCHER: *State Office for Health and Social Affairs, Berlin, Germany, Germany*
ASMA MADAD: *U.S. Food and Drug Administration, College Park, MD, USA*
MICHAEL VASSER: *CDC, Atlanta, GA, USA*
LINDSAY WALERSTEIN: *U.S. Food and Drug Administration, College Park, MD, USA*
KARI IRVIN: *U.S. Food and Drug Administration, College Park, MD, USA*

This symposium covers five different topics surrounding specific outbreak investigations and outbreak investigation mechanics. Additionally, a slot will be held open for a late-breaking outbreak highlighting a recent illness outbreak investigation. An outbreak of *Salmonella* Newport linked to onions in the United States and Canada sickened more than 1,000 people. Canadian officials will review the epidemiology and traceback of the outbreak and the FDA will discuss findings from the farm investigation. CDC and FSIS will discuss an outbreak of 10 *Listeria monocytogenes* infections in three states linked to deli meats. In interviews with nine ill people, all reported eating Italian-style meats, such as salami, mortadella, and prosciutto. They reported purchasing prepackaged deli meats and meats sliced at deli counters at various locations. A specific type of deli meat and common supplier have not yet been identified. Two waves of hepatitis A outbreaks occurred in Sweden/Austria in 2018 and Germany in late 2019 and early 2020. Tracebacks revealed that a Polish producer had received frozen strawberries from Egypt where phylogenetic analyses linked the outbreak strain to similar strains formerly isolated from sewage, stool, and strawberries. An outbreak of *Salmonella* Enteritidis in peaches sickened more than 100 people in the United States. CDC will provide details on the epidemiological investigation, and FDA will discuss traceback activities as well as details from the orchard investigations. CDC, FDA, and FSIS will present the public-facing outbreak investigation tables. The outbreak investigations are a new tool to increase transparency on foodborne illness outbreaks under investigation.

S2 Physiological State of Mind: Detection Challenges for Stressed/Sub-lethally Injured Pathogens

YI CHEN: *U.S. Food and Drug Administration, College Park, MD, USA*
HALEY OLIVER: *Purdue University, West Lafayette, IN, USA*
MATHEW HENDERSON: *Land O'Frost, Inc, Chicago, IL, USA*

Microorganisms in food are frequently subjected to varying level of stress due to constant changes in the food production environment. Foodborne pathogens have adapted different defense mechanisms and physiological states to survive in these environments. Major stress factors include physical stresses, such as heat, high pressure, desiccation, and irradiation or chemical stresses, such as acids, salts, and oxidants, and biological stresses, such as microbial competition for nutritional resources. Responses to different stress factors can be a serious food safety concern, as the adapted pathogenic cells in foods surviving during processing steps may pose potential health risks to consumers.

This symposium will focus on gaining a better understanding of the effect of types of stress factors on major foodborne pathogens and the challenges it imposes on detection strategies and methodology. It will also focus on why it is important to use stressed cells rather than freshly harvested cells when performing challenge studies and validating food processing procedures. Different processing environments and food categories pose variable levels of risk for common pathogens like *Salmonella*, *Listeria,* and *E. coli*. Microbial cells adapted to a sub-lethal stress in one environment may exhibit enhanced survival and tolerance on subsequent exposure to a different stress. Injury to microbial cells may have two major consequences during sampling and testing: higher sensitivity to selective agents in the growth medium and a longer lag phase during revival and growth to include repair time. Resultantly, a true risk of not reaching the bacterial concentration necessary for the pathogen detection platforms. This results in challenges with different diagnostic platforms, either over-reporting with nucleic acid detection technologies or underreporting in cell culture-based traditional methods. How to strike a balance?

S3 New and Innovative Technologies for Sanitation in Dry Processing Environments

ABIGAIL B. SNYDER: *Cornell University, Ithaca, NY, USA*
VM BALASUBRAMANIAM: *The Ohio State University, Columbus, OH, USA*
STEVE LOMBARDO: *McCormick & Company, Baltimore, MD, USA*

Sanitation of food processing equipment and environmental surfaces reduces microbial harborage and removes food allergens. Sanitation in dry food processing environments where water is not used in cleaning programs represents a challenge for the industry. However, many new and innovative sanitation technologies are emerging to address this challenge. Conventional dry sanitation programs vary across industry but have included physical removal strategies (brushing and scraping, vacuuming and compressed air, flushing with an inert ingredient) as well as spot application of sanitizers applied with single-use clothes or reusable rags. Increasingly, novel technologies that leverage the basic principles of physical removal of food soils and microbial inactivation are being developed and applied by industry to increase sanitation efficiency, reduce downtime and the use of expensive reagents, further limit moisture introduction, and improve the elimination of microbial pathogens, spoilage biota, and allergenic residues. Novel approaches include the use of superheated steam as a surface sanitizer, directed air streams and dry ice blasting, and the use of alternative chemical interventions. In this symposium, different novel and innovative methods for sanitation in dry processing environments will be explored. The symposium will start with a discussion of performance metrics and success criteria for novel technologies, followed by in-depth presentations on innovations in tools and approaches for physical cleaning and microbial inactivation. There will be a discussion of industry applications and improved methods for monitoring and verification.

S4 Advances in Powdered Food Safety and Quality Sampling Plans: Theory, Simulation, & Practice

ROGER KISSLING: *Fonterra, Waikato, New Zealand, New Zealand*
MATTHEW J. STASIEWICZ: *University of Illinois Urbana-Champaign, Champaign, IL, USA*
PAMELA WILGER: *Cargill, Inc., Wayzata, MN, USA*

Powdered products are burdened by low-prevalence, low-level contamination that is typically heterogeneously distributed. Multiple commodities and products could benefit from improved management of these risks as evidenced by outbreaks of foodborne disease linked to dairy powders, wheat and nut flours, and powdered infant formula. This session will focus on advances in powder sampling and provide depth in one well-studied commodity, dairy powder, upon which other commodities may draw analogous lessons.

As manufacturers supply domestic and global markets, they must continue to manage rare microbiological contamination with foodborne pathogens such as *Salmonella*, chemical contamination with mycotoxins such as aflatoxin, and meet increasingly stringent customer quality requirements for indicator and spore counts, to ensure product compliance with regulatory standards. While product testing is one fundamental component of food safety and quality management, traditional best practices like ICMSF-style manual N60 grab sampling is underpowered when those hazards are at low prevalence and level. Modern technologies like autosamplers provide opportunities to improve practice by automating the process of sample collection. This opens the possibility to take many smaller samples with complex stratification and true randomness.

Currently, the food industry is needing improvements in practices to meet safety and quality challenges. This session meets that need by addressing knowledge gaps around the benefits of improved sampling plans, technologies, and implementation strategies, using dairy powders as an example case. The session will start with a speaker presenting recent statistical theory modelling powder sampling to define the performance of traditional grab compared to auto sampling approaches. The next speaker will present work simulating dairy powder sampling to better define the variability of sampling plans when applied to specific production and hazard scenarios. Finally, we will hear from an industry speaker providing perspective on the value and practicality of getting this done in a multinational company.

S5 How Regulators are Integrating Food Safety Culture into Food Safety Performance and Assessment Strategies

ROUNAQ NAYAK: *Harper Adams University, Newport, United Kingdom, United Kingdom*
CONRAD CHOINIERE: *U.S. Food and Drug Administration, College Park, MD, USA*
STEVEN WEARNE: *VP Chair Codex, London, United Kingdom, United Kingdom*
AMANDA HILL: *Dairy Food Safety Victoria, Camberwell, Australia, Australia*

Organizations have long recognized the importance of an organizational safety culture to address health and safety risks. Over the last ten years, organizations have continued to evolve their understanding of their own food safety culture. In parallel to the food industry, global regulators are actively exploring ways to integrate food safety culture as an indicator of an organization's food safety proficiency and compliance. Recently, several key events have accelerated this food safety culture consideration including:

- 2020 – Publication of the FAO Codex Alimentarius update to the Food Hygiene principles
- 2020 – Publication of the GFSI Benchmarking Document
- 2020 – Announcement of the FDA "Food for Thought" initiative
- 2020 – Amendment of EC 852/2004 (in consultation)

In order to align industry and regulators on the role of food safety culture within their respective organizations, it's important to understand this evolution with respect to expected change management. Key questions include:

- How are regulators training their own staff on food safety culture?
- What is the potential role of food safety culture in respect to future regulators' food safety assessments and investigations?
- What actions can industry take to help prepare for potential changes in regulatory food safety assessment and investigations?

S6 To Disinfect or Not? How to Appropriately Use Disinfectants in Food Settings

KRISTIN WILLIS: *EPA, Washington, DC, USA*
CHARLES PETTIGREW: *Procter & Gamble, Mason, OH, USA*
ANGELA FRASER: *Clemson University, Clemson, SC, USA*

The food industry has relied on sanitizers as their primary antimicrobial product. Disinfectants were usually an afterthought and used only infrequently. Their primary purpose was to treat bathrooms and the occasional blood or bodily fluid spill. This is not the case anymore. The SARS-CoV-2 pandemic has caused an unprecedented increase in the use of disinfectants within the food industry which has put surface hygiene center stage. It is clear disinfectants are going to become a routinely used item in food settings. Disinfectants are more complicated to use than a sanitizer and can come at a premium. There are also different regulations that dictate when and how disinfectants are used as compared to sanitizers that food operators are familiar with. So, how do we appropriately adopt the right disinfectant into our current sanitation standard operating procedures and ensure that they are used correctly? Expert speakers from government, academia, and industry will tackle three broad topics. First, what are the regulatory differences between our familiar sanitizers and the newly relevant disinfectants? Second, how does the Food Code address sanitizers and disinfectants, and what changes will we need to make to cope with the new normal? Third, the food industry has a variety of complex hygiene needs outside of what a sanitizer can accomplish. How do we think about disinfectants beyond the basic bloodborne pathogen, norovirus, and SARS-CoV-2 claims to get the most from our disinfectants and use them safely and appropriately in a food environment? Symposium attendees will leave with a deeper understanding of how sanitizers and disinfectants are regulated and assessed for efficacy, how to best evaluate and adopt a disinfectant within a food facility, and how to better utilize a disinfectant to tackle challenging problems, such as biofilms and viruses.

S7 Log Reduction and Product Grouping Strategies for Validation - Does One Size Fit All?

JULIANY RIVERA CALO: *Ardent Mills, Denver, CO, USA*
IAN HILDEBRANDT: *Michigan State University, East Lansing, MI, USA*
NATHAN ANDERSON: *U.S. Food and Drug Administration, Bedford Park, IL, USA*

Control of pathogens in low water activity (aw) products is very challenging and may require a pathogen reduction step. FDA's Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food rule generally requires that pathogen reduction treatments be validated. Low aw foods comprise of a broad range of products including spices, flour, flour products, grains or grain products, nuts, nut butters, chocolate, pet foods and feed, etc. Manufacturers are tasked with validating dozens to hundreds of related but not identical products or ingredients. The ability to validate each of these individual products is operationally daunting. The products have different physical and chemical

characteristics which may impact the efficacy of the process and thus making validation challenging. Validation of each product is an enormous task that requires time and resources not often readily available to all food companies. Therefore, a good approach is to categorize or group products that have sufficiently similar intrinsic properties. The speakers will identify some of the challenges in product grouping and surrogate selection for process validations and discuss approaches/considerations.

A 5-log pathogen reduction is optimal for certain products (e.g., juice). However, 5-log reduction may not be necessary for certain products and other factors like risk assessment, pathogen prevalence, and product characteristics which should be taken into consideration. The speakers will discuss current data, different approaches, practical challenges, and applied knowledge to address these topics.

S8 Reducing Food Safety Risks of Pork Products: Science-based and Data-driven Steps to Reduce *Salmonella*

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TYLER STEPHENS: *Hygiena, Marion, TX, USA*
TOMMY WHEELER: *U.S. Department of Agriculture-ARS, Clay Center, NE, USA*
DAYNA HARHAY: *USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA*
JOSEPH BOSILEVAC: *USDA/ARS, Clay Center, NE, USA*
TERRANCE ARTHUR: *USDA/ARS, Clay Center, NE, USA*
JOHN SCHMIDT: *U.S. Department of Agriculture – ARS, Clay Center, NE, USA*

Performance standards related to *Salmonella* prevalence within pork processing systems are presenting new challenges to the industry. Although these standards are new to this industry, other sectors of the poultry and meat industry have had similar surveillance programs and standards applied for several years. These performance standards are proposed by regulatory bodies (USDA-FSIS) in order to drive down food safety risks associated with these products. For a processing system to meet and/or exceed these standards, various controls will be applied to pre-harvest, harvest, and fabrication steps. Understanding the food safety risk that is attributable to each of these processing steps, as well as what interventions or controls can be most effective, will require extensive sample collections of affected products. Along with sound study designs and sample plans, adequate microbiological testing methodologies will be important to ascertain, so that decisions to control or mitigate these risks can be based on sound, science-based data.

This symposium will dive into the academia, industry and government perspectives on control of Salmonella in pork products, including quantitative analysis for Salmonella in different pork matrices as well as pathogenicity of serotypes commonly associated with swine.

S9 Tracking and Combating Spoilage Microorganisms and Pathogens in Food Processing: Biosensing, Interventions, and Active Packaging

JUSTIN PAHARA: *Agriculture and Agri-Food Canada, Lethbridge, AB, Canada, Canada*
SAMPATHKUMAR BALAMURUGAN: *Agriculture and Agri-Food Canada, Guelph, ON, Canada, Canada*
XIANQIN YANG: *Agriculture and Agri-Food Canada, Lacombe, AB, Canada, Canada*
YIXIANG WANG: *McGill University, Ste-Anne-de-Bellevue, QC, Canada, Canada*

Increased demand in domestic and international trade and public concerns over foodborne pathogens have driven the food industry to tackle challenges in the control of spoilage microorganisms and pathogens during processing and transportation. Despite interventions, there are still complaints or returns from time to time on spoiled products or even recalls. The question is, how much do we know about interventions and their effectiveness, is there any other measure to control spoilage and pathogens?

While interventions such as carcass spray by hot water and acids in meat processing or washing/fungicide treatment of fresh produce are used, product spoilage still occurs and pathogens may not be fully removed, leading to product waste or recalls. Challenges include but are not limited to 1) one intervention can be more effective to control specific group(s) of microorganisms but not others, there is always a chance that different groups of microorganisms present on products from time to time; 2) microorganisms can develop resistance to interventions; and 3) no prolonged release of antimicrobial substance throughout the expected shelf life. To tackle some of these problems, there have been exciting recent developments in biosensing technology that allows real-time monitoring of pathogens and active packaging allows controlled release of antimicrobials for prolonged spoilage/pathogen control. While there are still technical challenges regarding these technologies, it is promising that combining conventional interventions and new technology would synergistically improve food safety and quality.

The objective of this session is to provide a forum to put into perspective the application, effectiveness, and future opportunities of post-harvest decontamination interventions or active packaging in the food industry, for the reduction of spoilage microorganisms or pathogens. How do different interventions contribute to the quality or palatability of the products or the emergence of resistant strains? Also included are recent advancements in active packaging technology and the environmental impact of such technology.

S10 FSMA Turns 10! Achievements, Compliance, and the Future of Food Safety

MIKE TAYLOR: *Former Deputy Commissioner, U.S. Food and Drug Administration, Washington, D.C., USA*
GLENN BASS: *U.S. Food & Drug Administration, White Oak, MD, USA*
KELLY STEVENS: *General Mills, Minneapolis, MN, USA*
BARBARA KOWALCYK, *The Ohio State University, Columbus, OH*
ESKINDANIEL BURGOYNE, *Canandian Food Inspection Agency, Ottawa, ON, Canada*
FRANK YIANNAS: *U.S. Food & Drug Administration (FDA), Silver Spring, MD, USA*

The FDA Food Safety Modernization Act (FSMA) was signed into law on January 4, 2011, with the purpose of enabling the FDA to better protect public health by strengthening the food safety system. Over the last ten years, FDA has finalized seven foundational rules that, collectively, require comprehensive, science-based preventive approaches to ensure food safety across the United States' food supply. The Preventive Controls for Human Food rule has had a particular impact not only on U.S. food businesses but also on overseas facilities that produce food for consumption in the U.S. This symposium looks at major achievements and challenges with FSMA implementation, including an update on compliance and enforcement activities, industry perspectives on the impact of the new regulations on their operations, and the future of food safety.

S11 Tracing Back to the Source: Challenges to Link Parasite and Viral Genotypes between Outbreak Clinical Samples and On-farm Environmental Sources of Contamination

ALEXANDRE DA SILVA: *U.S. Food and Drug Administration – CFSAN, Office of Applied Research and Safety Assessment, Laurel, MD, USA*
LEE-ANN JAYKUS, *North Carolina State University, Department of Food, Bioprocessing and Nutrition Sciences, Raleigh, NC*
RACHEL CHALMERS: *Public Health Wales, Microbiology and Health Protection, Singleton Hospital, Swansea, United Kingdom, United Kingdom*

The contamination of agricultural water may become an increasing concern in produce safety. Significant efforts are currently focused on water sampling and testing for the detection of waterborne viruses and parasites in an attempt to identify potential sources of produce contamination. Molecular epidemiology tools can be used to link cases of illnesses that have common food exposures, but challenges exist for environmental sampling supported by tracebacks during epidemiological investigations. This symposium will discuss the difficulty involved in source tracking on-farm environmental contamination to clinical samples derived from viral and parasitic infections. In addition, we will discuss how emerging technologies are being utilized for the genotyping of foodborne viruses and parasites detected in environmental samples.

S12 Food Safety Protection during Rendering of Animal Offal for Manufacturing Human and Animal Food/Feed: Needs and Opportunities

ANSEN POND: *Pilgrim's Pride, Mt. Pleasant, TX, USA*
THOMAS TAYLOR: *Texas A&M University, College Station, TX, USA*
JENNIFER ERICKSON: *U.S. Food and Drug Administration, Silver Spring, MD, USA*

The rendering of animal carcass components and materials provides for sustainable recycling of resources and a nutritious feed/food stream for consumers, livestock, and companion animals. Nevertheless, raw animal carcass materials and food waste scraps present a food safety hazard risk through contamination with microbial human and/or animal pathogens, such as *Salmonella* spp., *Clostridium perfringens,* and *Listeria monocytogenes*. Multiple studies in recent years have reported the presence of biological food safety hazards on finished rendered products, including blood and bone meals, from commercial U.S. renderers. Additionally, research has indicated a wide variation in the prevalence of microbial food safety hazards on differing commercial rendered products.

Throughout 2019 and the first half of 2020 alone, the U.S. FDA acted in eight disease outbreaks or incidents of products testing positive for bacterial foodborne pathogens. The U.S. FDA Food Safety Modernization Act (FDA-FSMA) and its implementing final rule mandating food safety preventive controls during the manufacture of animal foods (Title 21, U.S. Code of Federal Regulations §507) requires covered establishments to scientifically validate process preventive controls for food safety hazards. Scientific validation of food safety hazards control may be accomplished by various means but must be undergirded by rigorous design and implementation of sanitary design and sanitary operations within the rendering establishment to prevent food safety hazard contamination. This session will report on the current state of scientific knowledge surrounding the prevalence of human/animal microbial pathogens, the capacity of thermal rendering systems to inactivate such food safety hazards, and sanitation technologies capable of aiding sanitary operations in the manufacture of high protein and/or high fat, low-moisture foodstuffs, and components for further processing into animal food/feed. Experts from academia, government, and U.S. industry will present perspectives on research needs, opportunities, and where the greatest evolutions have and must still occur in the coming years.

S13 Balancing Food Safety and Soil Health through the Use of Biological Soil Amendments

ALDA PIRES: *Department of Population Health and Reproduction, School of Veterinary Medicine, University of California-Davis, Davis, CA, USA*
MICHELE JAY-RUSSELL: *Western Center for Food Safety, School of Veterinary Medicine, University of California-Davis, Davis, CA, USA*
PATRICIA MILLNER: *USDA–ARS, EMFSL, Beltsville, MD, USA*

Managing the health and fertility of soils is a priority for produce growers, and they often rely on biological soil amendments of animal origin (BSAAOs) to improve or maintain soil structure and fertility. In addition to improving the health of the soil, these practices directly benefit growers through improved soil productivity and increased crop yields. However, common practices such as applying untreated BSAAOs and using integrated crop-livestock systems can present a food safety risk by introducing human pathogens into the soil as well as the broader farm environment.

Although there is a growing interest in using soil amendments to improve soil health, there is limited data to demonstrate how these practices impact produce safety. The Food Safety Modernization Act Produce Safety Rule (FSMA PSR) and Good Agricultural Practices (GAPs) audits have set required practices for minimizing the risks of using BSAAOs on the farm. The FSMA PSR currently has reserved the establishment of an application interval for untreated BSAAOs within Subpart F until additional research and risk analysis is conducted to address the variability of pathogen die-off rates. Current research has examined the relationship between pathogen die-off rates in untreated BSAAOs and how the presence of human pathogens interacts with soil in diversified farming systems.

Growers must weigh more than microbiological risk when making production decisions, so their decisions on use of BSAAOs and integrated crop-livestock systems involve balancing multiple factors within their operation. This symposium will address how soil amendment practices may impact produce safety, factors that impact human pathogen die-off, and how this information aligns with existing food safety standards. The first two talks will present an overview of food safety considerations in integrated crop and livestock systems (such as rotational grazing), followed by a discussion of ecological and agricultural factors influencing pathogen die-off in vegetable fields. The last presentation will speak about the interface between soil health and food safety in produce production.

S14 Dust Off That Data! - Transform Testing Results into Meaningful Food Safety Improvements

SARAH I. MURPHY: *Cornell University, Ithaca, NY, USA*
STEPHANIE MAGGIO: *North Carolina State University, Raleigh, NC, USA*
MEHRDAD TAJKARIMI: *EAS Consulting Group, Los Angeles, CA, USA*

Now more than ever, food companies are conducting testing and collecting data. Processors and manufacturers accumulate an array of data, from environmental swab data to finished product data to critical control point data. The key to continuous improvement of a food safety program may lie with the data in that binder or spreadsheet. Expert speakers will dive into three ways to combine data and technology and use it to your advantage. First, learning how to develop statistically sound control charts utilizing process control data. Second, exploring ways to create heat maps using environmental monitoring results and how to visualize areas of concern or opportunity. Third, discovering the uses of artificial intelligence in food safety and quality management to track key performance indicators. The combination of these topics will assist in the effective use of data to see bigger pictures and to help elevate food safety programs to the next level.

S15 How COVID-19 Has Altered Consumers' Food Choices and Preferences and Their Hygienic Practices

MARGARET PERSON: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
DIMA FAOUR-KLINGBEIL: *School of Biological and Marine Sciences, University of Plymouth, Devon, United Kingdom, United Kingdom*
AMIN ELEIMAT (OLAIMAT): *Department of Clinical Nutrition and Dietetics Faculty of Applied Medical Sciences, The Hashemite University, Zarqa, MB, Jordan, Jordan*

The impact of the novel coronavirus pandemic (COVID-19) has spanned across various aspects of life globally. Compounded with the uncertainties within the scientific communities, the rapid spread of the novel coronavirus sparked a wave of public fears which affected their food choices and hygiene practices and how they perceive food safety. Although there is no evidence that COVID-19 can be transmitted through food, the public has been misguided by misleading information for consumers to be engaged in high-risk practices with the intent to eliminate the virus from food and for self-protection by using cleaning agents for washing fresh fruits and vegetables or inhaling or ingesting cleaners and disinfectants. Public concerns about contracting COVID-19 from food also led to a significant reduction in their consumption and ordering of ready-to-eat foods in some countries, whereas, in others, a substantial decline in food shopping and a rise in online food ordering services were reported.

Margaret Person will speak on a CDC survey that identified respondents engaged in non-recommended high-risk practices with the intent of preventing SARS-CoV-2 transmission, including using bleach on food products, applying household cleaning and disinfectant products to the skin, and inhaling or ingesting cleaners and disinfectants. Public messaging should continue to emphasize evidence-based safe cleaning and disinfection practices to prevent SARS-CoV-2 transmission in households, including hand hygiene and cleaning and disinfecting high-touch surfaces.

Dima Faour-Klingbeil will cover two surveys of food-handling and hygienic practices in Arab countries, where government scientific advice is limited and social media fill the knowledge gaps for food-handling and hygienic requirements for minimizing consumer risks.

Amin Eleimat will review the potential survival and transmission of SARS-CoV-2 on different food packaging materials and other food safety issues. The risk of virus survival on these packaging materials from farm to fork strengthens the need for promotion of HACCP and GMPs with targeted cleaning, sanitation, good hygienic practices, and use of effective packaging.

S16 If You Want to Go Fast, Go Alone; But If You Want to Go Far, Go Together: Collaborating with Historically Black Colleges and Universities, Hispanic-serving Institutions, Non-Governmental Organizations, and Community-based Organizations on Produce Safety Education and Training

ARMITRA JACKSON-DAVIS: *Alabama A&M University, Madison, AL, USA*
BILLY MITCHELL: *Local Food Safety Collaborative, Jeffersonville, GA, USA*
VEERACHANDRA YEMMIREDDY: *University of Texas Rio Grande Valley, Edinburg, TX, USA*

Under the Produce Safety Rule (PSR), the U.S. Food and Drug Administration (FDA) has the authority to regulate on-farm produce safety. Non-Governmental Organizations (NGOs), Historically Black Colleges and Universities (HBCUs), Hispanic Serving Institutions (HSIs), and Community-based Organizations (CBOs) all play an important role in produce safety education and outreach. These educators work alongside communities who have often been left out or sidelined by previous educational opportunities and may be reticent to re-engage, especially if it is with an unknown agency or source material such as the FDA or the PSR. Because of this, there is a need to work collaboratively to ensure that these audiences are reached with the training materials and support needed to be successful. In addition, the development of trainers at each level is essential for success. This ensures that the communities being served see themselves reflected in the trainers serving them, and vice versa. These trainers are able to rely on shared community values and understanding to connect the importance of and increase the understanding of produce safety education. Therefore, this symposium will provide an overview of the work that has been done by NGOs, CBOs, HBCUs, and HSIs while highlighting the work that needs to be done to ensure each group's food safety training needs are met. This symposium uniquely brings together three speakers who have experience serving with NGOs, HBCUs, HSIs, and CBOs. To date, a symposium that brings all groups in one session has not been presented at an IAFP meeting. In addition, this symposium aligns well with the submitted roundtable proposal entitled "Diversifying the Pipeline in Food Safety Education: Engaging Historically Black Colleges and Universities (HBCUs)".

S17 Can *Enterobacteriaceae* Testing Provide a Better Indicator of *Salmonella* Risk on Zone 1 Surfaces in Dry Processed Foods?

WARREN STONE: *Zone One Consulting LLC, Napa, CA, USA*
RICK KANABY: *Neogen Corporation, Lansing, MI, USA*
JOSEPH MEYER: *Kerry, Waunakee, WI, USA*

It is widely recognized that, if present, the level of *Salmonella* in dry processed foods is low, and contamination in foods can be sporadic in nature. Manufacturers, therefore, rely on the implementation of validated intervention steps, good manufacturing practices, and microbial monitoring of the plant environment to mitigate the risk of *Salmonella* in dry processed foods. Due to severe logistical concerns in holding and segregating the finished product representative of a food contact surface sample while awaiting the outcome of the *Salmonella* testing results, such evaluations are often on an infrequent schedule, i.e., annual/bi-annual/quarterly scheduled downtime. Taking preemptive remedial measures, such as wet cleaning, due to a presumptive *Salmonella* result, also needs careful thought and consideration, as it can potentially spread contamination throughout the plant environment. Hence, the benefit of consumer protection from increased testing for appropriate indicator organisms such as *Enterobacteriaceae* (EB) should not be underestimated. EB testing has a valuable role as a potential indicator organism in low-moisture processed foods, particularly those subjected to environmental exposure after a validated heat treatment. Depending on the initial contamination level and treatment, EB test results can provide a reliable indication of potential process failure, under-processing, or post-process contamination. Where a validated 5-log reduction for *Salmonella* spp. is applied, inactivation of *Enterobacteriaceae* spp. can undoubtedly be expected. Detection of *Enterobacteriaceae*, however, does not imply that there is a public health risk. Testing for *Enterobacteriaceae* in conjunction with next-generation sequencing (NGS) technology can help quickly assess the pathogen risk in a Zone 1 environment. This symposium aims to present information on the utility of testing for *Enterobacteriaceae* under appropriate circumstances and to encourage food processors to conduct more frequent Zone 1 testing as an additional verification tool in a *Salmonella* environmental program. Examples utilizing data generated in a plant environment will be presented.

S18 Recent State and Local Outbreak Investigations

IRINA CODY: *Texas Department of Health and Human Services, Austin, TX, USA*
D.J. IRVING: *Tennessee Department of Health, Nashville, TN, USA*
THAO SCHLICHTE: *Iowa Department of Inspections and Appeals, Des Moines, IA, USA*
BRENDALEE VIVEIROS: *Rhode Island Department of Health, Providence, RI, USA*
LAUREN EDWARDS: *Michigan Department of Agriculture and Rural Development, Lansing, MI, USA*
KATIE GARMAN: *Tennessee Department of Health, Nashville, TN, 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 food regulatory agencies in the investigation of foodborne illnesses including the U.S. Food and Drug Administration-funded Rapid Response Teams (RRT). The session will focus on the use of techniques such as environmental assessments, environmental sampling, and whole-genome sequencing to solve outbreaks at the state and local levels. The session will also discuss lessons learned and contributing factors identified during the investigations.

S19 One Size Does Not Fit All: Advancing Surrogate Science and Collaboration to Enable Pathogen Reduction Technologies in a Variety of Matrices

BRADLEY TAYLOR: *Brigham Young University, Provo, UT, USA*
JAMES DICKSON: *Iowa State University, Ames, IA, USA*
ERDOGAN CEYLAN: *Mérieux NutriSciences, Crete, IL, USA*

With increased numbers of outbreaks and recalls due to contamination of foodborne pathogens in low-water activity foods, such as nuts, spices, flour, and milk powders, the food industry is challenged with validating innovative processing technologies to mitigate this risk. Novel food processes require in-plant validation studies to evaluate whether the target pathogen is properly controlled, yet pathogen assessment in a food processing facility is not feasible. Laboratory-scale investigations may not replicate actual processing parameters, so validations using an appropriate surrogate are of vital importance. Selecting an appropriate surrogate depends largely on the pathogen it is meant to physiologically mimic, considering inactivation kinetics, processing parameters, and the characteristics of the food matrix. Identifying a conservative surrogate for use in process validations while avoiding over-processing of foods is critical yet challenging. Can one surrogate be widely used across all low-water activity foods and processing parameters to represent multiple pathogens' reductions? Evidence suggests this is not a likely possibility. While *Enterococcus faecium* NRRL B-2354 has been successfully used for some studies, it has not been validated for use across all matrices and processing conditions. This symposium will discuss the identification and characterization of various surrogates for *Salmonella* spp. and other pathogens using thermal inactivation studies in low-water activity foods and ingredients including flour, spices, dried fruits, and nuts. Furthermore, how factors such as food composition, processing parameters, and target pathogens influence the surrogate selection will also be presented. Speakers will focus on how to interpret studies using surrogates to validate thermal processes to inactivate pathogens and how these results are applied in practical applications. Overall, this symposium seeks to provide insight and address food industry concerns encountered during the selection, characterization, and adoption of the use of surrogates in low-water activity foods by industry and regulatory agencies.

S20 Novel and Emerging Technologies for Food Processing Facility Environmental Control

KATHY KNUTSON: *Kornacki Microbiology Solutions, Green Bay, WI, USA*
YALE LARY: *Holmes Smokehouse - RR Brand Foods, Lufkin, TX, USA*
BRIAN TAYLOR: *AirROS by SAGE Industrial, Fresno, CA, USA*
MICHELE SAYLES: *Diamond Pet Food, Topeka, KS, USA*

Preventive Controls for the Food Safety Modernization Act require that cleaning and sanitizing approaches be effective and verified. Validation of sanitation is strongly encouraged but not required. Included in verification are documentation of records related to approaches taken, chemical concentrations, CIP temperatures, etc. But what about verification and validation of their efficacy? Newer approaches to cleaning, sanitization, and environmental control and monitoring these actions for their efficacy will be presented including fluorescent-based cleaning verification systems for large surfaces, reactive oxygen specifies, and probiotic control.

S21 No Silver Bullet in Sight: How to Achieve Continuous Improvement in Fresh Produce Safety with Existing Knowledge and Tools

DREW MCDONALD: *Taylor Farms, Salinas, CA, USA*
TREVOR SUSLOW: *Product Marketing Association, Davis, CA, USA*
YAGUANG LUO: *USDA–ARS, EMFSL, Beltsville, MD, USA*

The fresh produce industry has made tremendous improvements to its food safety best practices over the last decade, which have complemented a growing demand for fresh produce by the consumer. However, foodborne pathogen recalls and outbreaks have touched widely-diverse fresh produce commodities and categories. Several have repeatedly been implicated in nationwide outbreaks, which clearly requires additional intervention. In particular, leafy greens implicated in annual *Escherichia coli* O157:H7 outbreaks must become a catalyst for commitment to continuous improvements in food safety systems. Researchers, industry leaders and practitioners, and government officials have been investing significant amounts of time and resources into investigating these patterns of occurrence. However, no specific trigger for causing this level of leafy greens contamination has been identified and no "silver bullet" appears imminent.

While we continue researching for a "silver bullet," the industry needs immediate and near-term practical solutions. The purpose of this symposium is to highlight how these solutions will emerge from existing knowledge and tools towards more effective and systemic improvement of produce safety. This includes opportunities across the farm-to-fork spectrum, as the cumulative impact of many small changes can result in an appreciable improvement in safety. This requires close collaboration and partnerships between researchers, industry, and government officials. It is difficult to predict where we can find a significant improvement in food safety for produce if we do not consolidate what we already know. This symposium is an opportunity to help the industry by highlighting specific existing knowledge, including specific metrics, and to help motivate growers and producers to adopt best practice programs. Presenters for this symposium will represent the diverse perspectives of industry personnel, researchers from academia, and government agencies. The dissemination of key learnings from previous and ongoing research is critical to the team goal of enhanced food safety.

S22 Identifying, Tracking, and Controlling Spoilage: "Toolbox" for Dairy Processing

NICOLE MARTIN: *Cornell University, Ithaca, NY, USA*
MICHELE GORMAN: *Chobani, LLC, Manlius, NY, USA*
NEIL BOGART: *Ecolab, Alabaster, AL, USA*

Dairy products, including pasteurized fluid milk, yogurt, and cheese, are very susceptible to quality issues that lead to spoilage, representing a major challenge for the dairy industry. Considering the dairy supply chain continuum, the dairy processing facility plays a critical role in minimizing and preventing spoilage.

In order to minimize and prevent spoilage, manufacturers must develop and implement programs that are focused not only on the prevention of contamination with spoilage organisms, but also on monitoring, identifying, and tracking for potential points of ingress of such organisms into product and/or process.

This symposium will describe a "dairy quality toolbox" by reviewing available practices, technologies, and strategies for identifying, tracking, and controlling spoilage risks. Specifically, this symposium will include an introduction to dairy product spoilage, highlighting the major groups of spoilage microorganisms as well as factors to consider when setting limits, followed by an overview of approaches and "tools" that are currently being applied or are in development for 1) identifying, 2) tracking and trending, and 3) controlling spoilage in dairy processing facilities.

Topics will be addressed from a holistic perspective of managing spoilage in a range of dairy processing facilities, in order to foster discussion that will drive the dairy industry toward new strategies for quality improvement.

S23 Your Significant Other: Using Statistics to Interpret Microbiological Data

SHARON BRUNELLE: *Brunelle Biotech Consulting, Corvallis, OR, USA*
ALEX BRANDT: *Food Safety Net Services, San Antonio, TX, USA*
MERYL SILVERMAN: *USDA FSIS, Washington, DC, USA*

A method validation is an experimental process of determining whether a test is fit for its intended purpose. The method under evaluation is compared to the recognized reference method and measured for performance characteristics such as sensitivity, specificity, accuracy, and recovery. These values provide the user with confidence in the method's ability to produce accurate and reliable results. If one of these methods (test or reference) has seemingly better performance, how do we determine if the difference in obtained results are significant? Testing for significance is determined statistically, although these measurements and interpretations are not well understood.

In industry, statistics are used to assess the quality of microbiological data, perform risk analysis and risk evaluations, understand the distributions of pathogens in products, and more. The objective of this symposium is to discuss the various performance characteristics used to measure method performance with a special focus on the probability of detection (POD) and significance. The speaker's purpose will be to provide knowledge on these measurements, how they are calculated, and what the corresponding values signify so that producers can make informed decisions on the methods used for product release.

S24 Latest Developments in Food Safety Standards for Water Reuse in Food Production and Processing

DON STOECKEL: *California Department of Food and Agriculture, Sacramento, CA, USA*
DIMA FAOUR-KLINGBEIN: *DFK for Safe Food Environment, Hannover, Germany, Germany*
KANG ZHOU: *Food and Agriculture Organization of the United Nations, Rome, Italy, Italy*

The pressure is on! Water scarcity is a globally rising problem mainly caused by overuse of water, population growth, economic development, urbanization, and changing food consumption patterns. The diminishing availability of water is expected to exacerbate global warming and climate change effects; hence, wastewater reuse is increasingly viewed as an alternative solution to meet the rising demands for water.

However, current default water standards for food use are high, with potable water being the gold standard for food use. While the use of reused (for instance re-cycled) water is becoming more practiced, no international standards have been established, and there are potential food safety hazards (e.g., microbial pathogens and chemical contaminants) associated with used water sources that need to be adequately managed to avoid food safety risks at the consumer phase.

The necessary scaling-up of water reuse may be possible given that significant volumes of used water are currently not utilized. However, these sources vary a lot in the possible presence of hazards and other quality parameters. For effective utility, used water from different sources of quality will need to be treated to a quality level that is acceptable and fit for the intended food use purpose, yet safe to consumers.

To provide guidance on consumer food safety expectations of water reuse, regulatory or other authoritative standards are urgently required. After all, agriculture/food production, processing, and preparation all use a lot of water but also come with large volumes of used water!

Codex Alimentarius, ISO, and country governments have started developing their own standards and establishing them as foundations for regulatory acceptance of fit-for-purpose water approaches by their member countries.

This symposium will feature key stakeholder views on the importance of developing standards for context-specific water reuse for food production and processing.

S25 Decoding Codex Alimentarius - Not a Secret Society

SARAH CAHILL: *Joint FAO/WHO Food Standards Programme, Rome, Italy, Italy*
JENNY SCOTT: *U.S. Food and Drug Administration – CFSAN, College Park, MD, USA*
MARTIN SLAYNE: *Slayne Consulting LLC, New York, NY, USA*

The Codex Alimentarius, literally "Food Code," is a collection of standards, guidelines, and codes of practice developed by the Joint FAO/WHO Food Standards Program to protect consumer health and promote international food trade. Members of the United Nations can participate in the process of Codex standards development through a number of committees focused on aspects of food safety, including chemical and microbiological contaminants. The Codex standards are science-based recommendations in all areas related to food safety and quality and, while these standards are not themselves regulations, they often serve as a basis for regulations by many countries. As such, the development of Codex standards has global implications for food regulations as well as global best practices. In addition, Codex is the relevant standard-setting organization for food safety with respect to dispute resolution in the WTO. In this session, speakers will lay the framework of Codex, share emerging topics and key activities within the committees, and provide insights as to how harmonized standards create a safer global food supply and eliminate barriers to trade.

S26 Shelf-life Testing: Problems, Pitfalls, and Promise

NICOLE MARTIN: *Cornell University, Ithaca, NY, USA*
BRADLEY STAWICK: *Stawick Laboratory Managment, Rutherford, NJ, USA*
JEFFREY KORNACKI: *Kornacki Microbiology Solutions, Inc., Madison, WI, USA*

In the food processing industry, there is little guidance on the definitions, interpretation, methodologies, troubleshooting, or criteria pertaining to shelf-life determination and testing. As a result, there is a lot of misinformation or misunderstanding of the information which is available. This can often lead to incorrect determination of shelf life and difficulty in root cause analysis when failures arise. The consequences of this lack of knowledge and guidance contribute to food waste, wasted resources and time, improper product formulation, and inappropriate testing.

In this symposium, the presenters will begin by providing a detailed overview of the definitions and methodologies surrounding shelf-life determination. Included in the lab method overview will be the process to identify potential risks associated with product shelf life and how to interpret and utilize the data of a shelf-life study. The symposium will also look toward the latest research and methods in accelerated methods to aid in shelf-life determination. Finally, a discussion on environmental monitoring programs will provide a holistic view of factors that can influence product shelf life and safety. Each presentation will include application of the concepts of real-life situations, provide prospective of areas in which problems may arise, and how a proper shelf-life determination can lead to success.

S27 Use of Novel and Alternate Processing Technologies for Dairy Products

TIMOTHY STUBBS: *National Dairy Council, Rosemont, IL, USA*
DEON MAHONEY: *DeonMahoneyConsulting, Melbourne, Australia, Australia*
STEPHEN WALKER: *U.S. Food and Drug Administration, Bedford Park, IL, USA*

Milk and dairy products have long been processed using thermal pasteurization process, and pasteurization parameters have not changed since the 1960s. However, thermal processed products can lose heat-sensitive compounds and affect nutritional qualities. Non-thermal processes such as High Pressure Processing (HPP) and Ultraviolet Light (UV light) have been used successfully for a number of foods, juice, and beverage products, and their applications have been mostly acidic or acidified foods, juices, and beverages. More recently, HPP has been applied to milk in lieu of pasteurization and approved for retail sale in Australia; UV light has also been used in South Africa for milk processing. Although there are regulatory differences in various countries, e.g., Pasteurized Milk Ordinance in the U.S., the regulations were built on thermal inactivation of the pertinent microorganisms. The food industry is interested in technologies that make milk and dairy products safe, e.g., applying HPP to milk and dairy products, but it is not clear how the technological hurdles such as validation studies to demonstrate equivalence to thermal pasteurization conditions and if the target microorganisms used in thermal inactivation are appropriate target microorganisms for HPP. Similarly, there are regulatory hurdles that currently exist. The session speakers will discuss the feasibility of HPP-treated milk, validation, and verification processes, discuss pertinent microorganisms, and potential pathways for regulatory acceptance. Panelists will come from diverse backgrounds, including experts with knowledge of HPP milk in Australia, regulator representatives (TBD), and technical experts.

S28 C is for Cyclospora: A Crash Course in the Emerging Pathogen from Farm to Table

YNES ORTEGA: *University of Georgia, Griffin, GA, USA*
ASMA MADAD: *U.S. Food and Drug Administration, College Park, MD, USA*
KALMIA KNIEL: *University of Delaware, Newark, DE, USA*
JENNIFER MCENTIRE: *United Fresh Produce Association, Washington, DC, USA*
TIMOTHY JACKSON: *Driscoll's of the Americas, Watsonville, CA, USA*

Despite its recent moments of fame and recognition, Cyclospora spp. are emerging as pathogens of great concern for the food industry. With its first case in the United States occurring approximately 25 years ago, seasonal outbreaks in recent years have highlighted the burden that Cyclospora places on consumers and producers. Although the mortality rates associated with Cyclospora spp. are not high, challenges lie with controlling and preventing contamination and spread. Outbreaks of Cyclospora spp. most commonly occurring in the spring and summer or in tropical areas affect multiple food products, such as leafy greens and fresh produce, and cause thousands of illnesses annually. Leaps and bounds are being made in an effort to curb the public health toll this pathogen imposes, but there are still many food safety professionals that know relatively little about Cyclospora spp. and the state of the field. This session will serve as a crash course on the emerging pathogen Cyclospora spp. across the food supply chain, from farm to fork, and provide an update on methodology, interventions to control and prevent contamination, and consumer knowledge about the pathogen in general. We will explore how Cyclospora has impacted food safety in several ways, including through historic outbreaks and the need to develop a subtyping system, sources of and strategies to mitigate contamination of the food, potential issues in processing environments, and challenges at the retail level with consideration of consumer attitudes and awareness. A panel discussion will take place after speakers present to serve as a chance to discuss current events involving Cyclospora spp. including outbreaks, novel molecular techniques, and upcoming risk-reduction and intervention strategies. The session discussion will serve as a way for audience members to engage with Cyclospora experts from various aspects of the food safety industry.

S29 What to Decide? Making Informed Decisions for Process Validation and Food Safety Legislation Using Bayesian Risk Models

ALBERTO GARRE: *Wageningen University, Wageningen, Netherlands, Netherlands*
DEANN AKINS-LEWENTHAL: *Conagra Brands, Omaha, NE, USA*
SOFIA SANTILLANA FARAKOS: *U.S. Food and Drug Administration–CFSAN, College Park, MD, USA*

Food safety management usually involves dichotomous decisions: What is an acceptable microbial concentration in a food? What is the target reduction of a critical control step? However, food safety is not black and white because microbial behavior is variable. Also, critical parameters like temperature and time vary along the food supply chain and significantly amplify the variability. Because variability is part of the system, it should be included in risk assessments that are used to come to informed decisions. In this symposium, we will show how this can be done using stochastic models built following a Bayesian approach. We will illustrate the application of these models to aid in food safety management and give examples of how industry uses it to validate processes and how government includes it to optimize quantitative microbial risk assessment.

S30 To Verify or Validate a Rapid Pathogen Method: What about the Matrix?

STEPHEN BURBICK: *The Kraft Heinz Company, Glenview, IL, USA*
WENDY MCMAHON: *Mérieux NutriSciences, Crete, IL, USA*
JEFFREY KORNACKI: *Kornacki Microbiology Solutions, Inc., Madison, WI, USA*

Rapid methods are often validated with one matrix to represent a category of foods. Due to this, the data may not correlate to other matrices within the same product category. Some may not even work with every matrix due to the microbial load of the product, differences in composition, or other ingredients added. It is often unclear whether a matrix needs to be validated or whether a matrix verification study can be completed. Some matrices have limited validation data or none at all. Many companies have completed their own studies for various methods; however, this information is not widely shared. There is a risk to the industry when a method is not suitable for a specific product matrix: both false negatives and false positives may occur, resulting in unnecessary product disposal and costs, or risks to the consumer. This symposium will address how industry can evaluate high-risk raw materials for pathogens when validated methods may not exist; what alternative options there are when a matrix cannot be validated; and what risks are being posed to the industry. It will cover the background for method validation studies including guidance for design and acceptance criteria and what a verification study looks like. There are also method or matrix complications including certain ingredients in the formulations. Case studies and suggestions for detecting pathogens in various matrices will be discussed.

S31 To Be Acid or To Be Acidified, That is the Question

FRED BREIDT: *USDA/ARS, Raleigh, NC, USA*
DAVID BRESNAHAN: *Bresnahan TPC, Inc, Kenmore, WA, USA*
ERDOGAN CEYLAN: *Mérieux NutriSciences, Crete, IL, USA*

The withdrawal of "Draft Guidance for Industry: Acidified Foods" in 2015 has prompted different points of views between manufacturers, academia, Food Process Authorities, inspectors, and regulatory bodies in interpreting the acidified foods criteria, such as pre-acidified ingredients added to the product, the status of water, low-acid powders and liquids, the definition of a small amount of low-acid ingredients, and the definition of a significant shift in pH. Regardless of how one would classify the product, the focus should be science-based when evaluating the microbiological stability and safety of the products. This session is targeted toward federal and academic researchers, industry professionals, and regulatory personnel, with the goal of achieving science-based regulations for safety and clarifying the difference between acid and acidified foods. This is an important and controversial issue affecting many acidic food products on the U.S. market, from salsas and salad dressings to beverages and pickled vegetable products. Recent research data, including buffer modeling, may allow new science-based insights on how low-acid ingredients influence the safety and classification of foods as acid or acidified food products.

S32 Communication for Risk Management: What, When, How, and Who?

KARIN HOELZER: *Maximus, Washington, DC, USA*
JOHN PETIE: *WellPet, Boston, MA, USA*
JEN PINO-GALLAGHER: *M3 Insurance, Madison, WI, USA*

Effective risk management requires that those involved in it understand and can effectively communicate the risks to be managed and the consequences for failure. This is true whether the risk is operator safety, food safety, facility security, financial risk, reputational risk, or legal exposure. Communicating risk is difficult for many reasons, not least that people process risks differently based upon many factors including their ability to understand probability, their prior experience, the potential for conflicting incentives, and the type of risk under discussion. Risk communication is a discipline in its own right, but we all have to understand and communicate risks on a regular basis. While there has been a great deal of research on how to communicate with consumers, there has been very little study on how to communicate upwards to senior management. What challenges do we face, and what techniques can help us to do this better? Key questions include:

- What risks concern decision-makers?
- What information do decision-makers need?
- How can we structure information to encourage appropriate action?
- How does communication style need to change for different categories of risks or different audiences?
- What skills do food safety professionals need to communicate risks appropriately?
- Where do we learn those skills?

The answer to the last question is "attend this symposium," which will look at risk communication from several angles giving new perspectives and providing communication techniques from different disciplines.

S33 Allergen Management at Retail in the New Era of Smarter Food Safety: From the Front Lines of Restaurants, Food Service, and Retail Grocery

STEVE OSWALD: *Wakefern Food Corp., Elizabeth, NJ, USA*
AL BAROUDI: *The Cheesecake Factory, Calabasas, CA, USA*
GLENDA LEWIS: *Food & Drug Administration, CFSAN, College Park, MD, USA*

The food industry strives to provide customers safe quality foods every day. However, there are so many challenges to maintain food safety, especially with allergen control. When the FDA introduced The New Era of Smarter Food Safety, it was evident the agency wanted to focus on new business models and retail modernization. If retailers focused solely on labeling as mandated with the Food Allergen Labeling and Consumer Protection Act of 2004, compliance would be pretty black and white. Unfortunately, retail resides in a world of significant grey, and food safety professionals know they have a significant responsibility to help consumers better understand cross-contact risks associated with food allergens. This symposium addresses the current regulatory compliance expectations, concerns, and perspectives of the food allergen community, and what retailers are doing with new and emerging retail business models and possible additions to "The Big Eight" by levering technology and new tools to help raise food allergy awareness with the consumers we serve.

S34 Risk Ranking Approaches to Inform Diverse Decisions in Government and Industry

MYRIAM MERAD: *Université Paris Dauphine-Cnrs, Paris, France, France*
DANE BERNARD: *Bold Bear Food Safety, Arnold, MD, USA*
YUHUAN CHEN: *U.S. Food and Drug Administration – CFSAN, College Park, MD, USA*

How to use risk ranking approaches to inform risk management decisions is of increasing interest to regulatory agencies and industry sectors. This symposium will present current thinking on risk ranking with a focus on multiple foods and multiple hazards. Different points in the food supply chain

(raw materials vs. finished products) may be the focus of risk ranking. Depending on the risk management questions posed and the availability of data and resources, risk ranking may involve a quantitative and semi-quantitative model or a simplistic risk matrix. The proposed speakers, who are from academia, industry, and government, will present current thinking and recent advancements in risk ranking methodology, and real-world examples of how risk ranking and risk evaluation (quantitative or qualitative) have been used to inform decisions in diverse regulatory and industry settings. They will also share some of the lessons learned and their perspectives on future directions.

S35 We Quantified, Now What? Actual *Salmonella* Quantification Approaches Utilized in the Protein Industry Today

JACQUELYN ADAMS: *Tyson Foods, Inc., Springdale, AR, USA*
MELODY THOMPSON: *Cargill Meat Solutions, Wichita, KS, USA*
SHERRI WILLIAMS: *JBS, Greeley, CO, USA*

USDA's FSIS has placed an emphasis on *Salmonella* performance standards to reduce the risk of foodborne illnesses associated with raw protein products. Multiple stakeholders in the protein industries have adopted programs that provide *Salmonella* quantification data to assess risks and implement corrective actions utilizing enrichment processes with faster time to results. Therefore, the goal of this symposium is to take the previous theorized methods and walk-through validated and verified data applications that are currently being utilized to make decisions in the protein industries. Industry leaders will share their processes of implementation and data analysis to highlight how quantification justifies their food safety decisions. Methods for rapid quantification with data options such as limits or threshold testing and quantitative result interpretations will be explained. Each of the speakers has a unique perspective to share from the various proteins, food safety plans, and current applications utilized to make data-driven decisions through *Salmonella* quantification. Attendees of this symposium will learn why and how to add *Salmonella* quantification into their food safety plans from the different approaches currently available to the protein industries. Overall, this symposium will provide insight into the forefront of method improvements and showcase how quantification data can improve food safety and help the industry and regulatory agencies understand pathogen contamination risks to consumers.

S36 The Forgotten Option: Formulation-based Preventive Controls for Human Foods

LORALYN LEDENBACH: *Kraft Heinz Company, Glenview, IL, USA*
DERRICK BAUTISTA: *Del Monte Foods, Inc., Walnut Creek, CA, USA*
HEIDY DEN BESTEN: *Wageningen University and Research, Wageningen, Netherlands, Netherlands*

This session addresses establishing formulation-based preventive controls for microbiological safety, including those for foods historically believed to be safe but lacking in scientifically valid controls that are also feasible for manufacturing. Example foods include soy sauce, certain brined vegetables, cold-blended barbecue sauces, and other foods where an antimicrobial treatment such as thermal processing or irradiation is either not employed or rendered ineffective due to further processing and packaging after the lethal step. While formulation safe harbors for the prevention of growth of toxin-forming pathogens are well-known, it is often the validation of the destruction of infectious vegetative pathogens that is of primary concern for such products. A vast array of intrinsic formulation parameters and variable extrinsic storage conditions may result in the need for a host of microbial inactivation validation studies that can be lengthy and expensive. In this symposium, we will discuss the current guidance for formulation-based preventive controls as well as an industry perspective on validating these controls and an academic approach to validation using different options such as microbiological modeling. Particular attention will be paid to real-world scenarios where the manufacturing, distribution, and shelf life of foods necessitate diverse approaches for validating the lethality of foodborne pathogens.

S37 U.S. Army-Funded Research of Novel Food Safety Technologies

JOSHUA MAGNONE: *U.S. Army DEVCOM Soldier Center, Natick, MA, USA*
GENEVIEVE FLOCK: *U.S. Army DEVCOM Soldier Center, Natick, MA, USA*
BOCE ZHANG: *University of Massachusetts, Lowell, Lowell, MA, USA*
SAM NUGEN: *Cornell University, Ithaca, NY, USA*
GIANNA AHNRUHD: *U.S. Army DEVCOM Soldier Center, Natick, MA, USA*
ANN NELSON: *Creative LIBS Solutions, Bernalillo, NM, USA*
DAVID CREMERS: *Creative LIBS Solutions, Bernalillo, NM, USA*
ROSALIE MULTARI: *Creative LIBS Solutions, Bernalillo, NM, USA*
MELISSA BOSTIAN: *Applied Research Associates, Inc., Albuquerque, NM, USA*
GREGORY RULE: *Applied Research Associates, Inc., Albuquerque, NM, USA*
ANDRE SENECAL: *U.S. Army DEVCOM Soldier Center, Natick, MA, USA*
PATRICK MAREK: *U.S. Army DEVCOM Soldier Center, Natick, MA, USA*
MICHAEL WIEDERODER: *U.S. Army DEVCOM Soldier Center, Natick, MA, USA*
PRADEEP KURUP: *University of Massachusetts Lowell, Lowell, MA, USA*
JARED CARSON: *U.S. Army DEVCOM Soldier Center, Natick, MA, USA*
MICHELLE RICHARDSON: *U.S. Army NSRDEC, Natick, MA, USA*
ANN BARRETT: *U.S. Army DEVCOM Soldier Center, Natick, MA, USA*
DANIELLE FROIO-BLUMSACK: *U.S. Army DEVCOM Soldier Center, Natick, MA, USA*

The U.S. Army Combat Feeding Division's mission is to provide operationally relevant research and development to deliver solutions for evolving field feeding challenges for the American Warfighter. Within that division, the Food Protection and Innovative Packaging Team (FPIPT) is responsible for protecting warfighter health by the prevention, detection, and elimination of food contaminants through the development of procedures, validation of novel diagnostics, analysis of risk assessment tools, and validation of novel pathogen reduction methodologies. They also conduct research to provide advanced materials, polymer processing technologies, and novel packaging concepts. These efforts maintain, sustain, and provide the Warfighter with rations that will ensure high levels of performance and quality, nutrient retention, and safety.

Current efforts include collaborations with academia, industry, and other government organizations to develop and transfer technology that will enhance food safety and protect the health of the deployed Warfighter. These food safety efforts include studying the efficacy of dry bacteriophage preparations; rapid identification technology for food and water pathogen viability; enhanced materials to extend shelf life; analyzing the efficacy of spectral imaging to detect ration quality degradation and chemical/biological contaminants; and the development of cost-effective sensors for the rapid screening of complex food and water matrices as a presumptive test to justify confirmatory testing.

The purpose of this symposium is to introduce the IAFP community to these research efforts as well as the U.S. Army's short- and long-term goals for food safety in field feeding. This session can serve as an opportunity to expand outreach and foster increased collaboration in trying to meet the unique food safety capability requirements needed for the modern Warfighter and the rest of the food safety community.

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S38 Flour Safety: Challenges and Lessons Learned from the Recent Outbreaks and Sampling Study

APARNA TATAVARTHY: *U.S. Food and Drug Administration, College Park, MD, USA*
ALEXANDER GILL: *Health Canada, Ottawa, ON, Canada, Canada*
JULIANY RIVERA CALO: *Ardent Mills, Denver, CO, USA*

Foodborne outbreaks associated with the consumption of low-water activity (aw) foods (aw < 0.80) including flour, have been historically less frequent than those associated with foods with water activity at levels allowing growth of many microorganisms, including *Salmonella* and *E. coli*. However, recent outbreaks of Shiga-toxin producing *E. coli* (STEC) associated with the consumption of raw flour (2016), and flour-based products including raw cookie dough (2009), pizza products (2013), and dry dough mix (2016) have raised concerns that the consumption of raw flour represents significant public health risks as vehicles of foodborne pathogens. These outbreaks emphasize the need to assess food safety risks associated with raw or improperly cooked flour intended for human consumption, and to evaluate the appropriate risk-based preventive controls needed to reduce the risk of STEC infections to an acceptable level.

To understand the prevalence of STEC in flour, FDA conducted a limited baseline retail flour sampling assignment in 2018–2019. To understand consumer knowledge and behavior, FDA's Food Safety and Nutrition Survey (2019) included questions on raw flour consumption.

In this symposium, the results of the FDA study to understand prevalence of STEC in flour through a retail flour sampling assignment (2018–2019) will be shared and discussed with the audience. Consumer knowledge and perceptions on raw flour consumption will be briefly shared. In addition, data on persistence of STEC and relative survival of different STEC strains in flour will be discussed. Finally, FDA's vision of 'farm to table' approach for flour safety, the collaboration between FDA and the North American Millers Association (NAMA), and industry's perspective on mitigation and related challenges will be highlighted.

S39 Root Cause Analysis: Approaches for Investigating Contamination Incidents and Preventing Recurrence

JACK GUZEWICH: *Retired, Albany, NY, USA*
MARK MOORMAN: *U.S. Food and Drug Administration, College Park, MD, USA*
ANGIE SIEMENS: *Cargill, Inc., Wichita, KS, USA*

This symposium will provide an overview of root cause analysis and how it is being used to improve food safety. It will first describe a guide for conducting a food safety root cause analysis. It will then describe how root cause analysis is being used by government during foodborne disease outbreak investigations. Next, it will describe how the food industry can use root cause analysis as a prevention tool during investigations of near-miss events. The history of root cause analysis begins with Taiichi Ohno, former executive vice president of the Toyota Motor Corporation and developer of the Toyota Production System. Today, root cause analysis is used by numerous governmental and quasi-governmental agencies, such as the National Transportation Safety Board (NTSB), the Chemical Safety Board, the Nuclear Regulatory Commission (NRC), National Aeronautics and Space Administration (NASA), and Centers for Disease Control and Prevention's (CDC's) National Center of Environmental Health (NCEH). The ultimate goal of RCA is to uncover the systemic weaknesses in the food system that permitted its breakdown so the system can be redesigned in a way that prevents recurrence. Analysis of results can be highly informative for many stakeholders, including food safety professionals in the same or related industries, personnel in regulatory agencies, educators, academic researchers, and consultants. Presenters will also describe challenges in implementing root cause analysis, including information sharing during and after the investigation, drilling down to people's knowledge and behaviors/food safety culture, and even agreeing on what a root cause analysis investigation should identify, analyze and report.

S40 Cannabis and Your Supply Chain - How to Protect Yourself and Your Customers

REND AL-MONDHIRY: *Amin Talati Wasserman, Washington, DC, USA*
LARISA PAVLICK: *United Natural Products Alliance, Salt Lake City, UT, USA*
ELAN SUDBERG: *Alkemist Labs, Garden Grove, CA, USA*

Cannabis is a growing food ingredient, and with increased legalization occurring across many states (and potentially federally), the opportunity to add Cannabis to food continues to grow. In this symposium, we will discuss current legal trends that apply to Cannabis in food, common challenges to watch out for when processing Cannabis into food and dietary supplements, and finally what types of testing and labeling requirements are necessary to place your product in the marketplace.

S41 Every Flush Has Data: The Role or Wastewater Epidemiology in Improving Food Safety with Lessons Learned from COVID-19

KALMIA KNIEL: *University of Delaware, Newark, DE, USA*
LAWRENCE GOODRIDGE: *University of Guelph, Guelph, ON, Canada, Canada*
GREGORY SIRAGUSA: *Scout Microbiology LLC, Waukesha, WI, USA*

Wastewater-based analyses for pathogens have provided unique public health tools for the detection of many infectious diseases since the 20th century and have expanded rapidly during the current COVID-19 global pandemic. Wastewater detection of SARS-CoV-2 has aided epidemiologists to predict and track COVID-19 outbreaks. The goal of this symposium is to increase the awareness of food safety professionals about the role that wastewater epidemiology can potentially play in detecting enteric foodborne bacterial, parasitic, and viral pathogens in wastewater as an early warning or sentinel system to predict outbreaks. The first speaker will address the evolution of wastewater analysis from detecting polio to modern-day SARS-CoV-2 monitoring and how previous investigations have influenced methodology, techniques, and data analysis for foodborne pathogens. The second speaker will address the advantages and challenges of using wastewater as a matrix to assay for various foodborne pathogens, and how recent technological and analytical advances in sample recovery, along with data analysis, can be harnessed in a manner to detect foodborne pathogens. Finally, the third speaker will describe if detection and recovery of enteric foodborne pathogens from wastewater can be improved by co-targeting or recognizing other members of the microbial community (through whole genome sequencing and metagenomic approaches) that can

provide early warnings or novel signals to protect public health in a local community or to provide an alert of an outbreak. Overall, the symposium addresses wastewater analysis for pathogens to discuss how current techniques and procedures used for SARS-CoV-2 detection can inform and improve the detection of foodborne pathogens in wastewater. Speakers from the U.S. and Canada, along with those from private industry, will combine their regional and scientific expertise in this emerging food safety arena.

S42 Managing Meat and Poultry Safety: Uniting Food Safety Regulations and Industry Efforts for Process Control

MANPREET SINGH: *University of Georgia, Athens, GA, USA*
THOMAS TAYLOR: *Texas A&M University, College Station, TX, USA*
JAMES DICKSON: *Iowa State University, Ames, IA, USA*

Salmonella remains a major cause of foodborne illness in the U.S. regardless of tremendous efforts by the meat and poultry industry incorporation antimicrobial interventions. With the revised food safety performance standards for *Salmonella* for all meat species and *Campylobacter* in poultry, there is increased scrutiny by the regulatory agencies and also efforts by the meat and poultry industry. The USDA FSIS published a "Roadmap to Reducing *Salmonella* – Driving Change through Science-based Policy" and outlined its efforts to modernize inspection systems. Currently, the USDA FSIS has implemented the New Poultry Inspection System (NPIS) for the poultry industry and has proposed the same for the swine industry as well.

Controlling and monitoring these pathogens is critically important to meet the revised performance standards. The goal of this symposium is to address challenges in controlling these pathogens and how the changing regulations for poultry, beef, and pork processing may impact sampling and testing methods. Each presentation will include a review of the current and proposed USDA FSIS regulations impacting the industries.

S43 Making Donations Count: Reducing Waste in Hunger Relief Organizations

ELLEN THOMAS SHUMAKER: *RTI International, Research Triangle Park, NC, USA*
LAUREN SASTRE: *East Carolina University, Greenville, NC, USA*
H. LESTER SCHONBERGER: *Virginia Tech, Blacksburg, VA, USA*

Hunger relief organizations and initiatives have become increasingly important mechanisms to address food insecurity, which has significantly risen due to the impacts of the COVID-19 pandemic. The logistics of donating food prove challenging, partly due to confusion surrounding product dating terminology (e.g., sell-by, use-by, expiration date) that are based on quality rather than food safety concerns. As a result, people and organizations may discard food that is safe to eat. Alternately, businesses may be donating unsafe food, i.e., through allergen cross-contact or food that is of questionable quality that ultimately contributes to food waste because it is unusable.

Despite the U.S. federal Bill Emerson Good Samaritan Food Donation Act and varying state regulations that permit food donation and redistribution with limited liability and regulatory oversight, perceived liability and public relations concerns limit food donations by potential donors, e.g., manufacturers, restaurants, caterers, farms, especially for perishable and prepared foods. While there are food safety resources for hunger relief organizations, they do not effectively represent the variety of programs seeking to support their local community, e.g., gleaning, university-based food recovery programs. There is a need to improve current recommendations and practices for organizations that donate or could donate safe, nutritious foods, as well as for entities that distribute donations.

This symposium will a) explore the intersection of food safety, food waste, and food recovery by discussing research on the factors that contribute to foods being successfully donated to a hunger relief organization, e.g., facilitated pick-up of unused food from food retail locations; b) discuss factors that hinder donation, e.g., misunderstanding of "best by" labels, lack of knowledge about donation sites, or what foods should be donated; c) discuss trends and novel initiatives, e.g., farm-to-clinic participating in hunger relief; and d) identify challenges related to managing food quality and safety concerns.

S44 Beyond Metagenomic Sequencing: Metadata, Ontologies, and Big Data

CHRISTOPHER GRIM: *Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Laurel, MD, USA*
ABIGAIL B. SNYDER: *Cornell University, Ithaca, NY, USA*
EMMA GRIFFITHS: *Department of Molecular Biology and Biochemistry, Simon Fraser University, Vancouver, BC, Canada, Canada*

A rapidly-growing metagenomics database has demonstrated its potential to improve understanding of food microbiomes and their role in pathogen dispersion in a food production environment or on food safety and quality. Several studies have reported microbiomes of human food, animal food and feed, food production environment, and farm production environment. However, this ever-expanding metagenomics database has created a "Big Data" challenge of integrating these metagenomes. The integrative approach is necessary to tackle this challenge which might require working with artificial intelligence and deep learning applications.

One way to assimilate metadata is by employing shared vocabularies to maximize the interoperability of metagenomes from multiple sources. Standardizing vocabularies and ontologies would allow integration of datasets from within and across complex food environments and may answer critical data gaps. This is critical to generate machine-readable and searchable datasets, vital to the next decade of AI and machine-empowered epidemiology and surveillance. The goal of this symposium is to provide a framework for utilizing standardized or harmonized vocabularies and ontologies to generate a machine-readable dataset and to integrate metadata from varied sources with shared features.

Presenters will begin by providing background on the FDA's effort to integrate metagenomes for improved food safety; continues with an introduction to building a controlled vocabulary database; and finishes with insights on using food ontology – from farm to fork. Food safety specialists must become more familiar with metadata standards to improve the utility and interoperability of food metagenomes.

S45 Failure to Launch - Learn to Live with Your Food Safety Plan Year Round

JORGE HERNANDEZ: *Wendy's, Dublin, OH, USA*
LONE JESPERSEN: *Cultivate, Hauterive, Switzerland, Switzerland*
NEIL BOGART: *Ecolab, Alabaster, AL, USA*

The pressures to keep corporate food safety plans relevant as the global food industry faces new scientific discoveries, innovative technology, upgraded/modernized regulations, and private standards, are at an all-time high. Companies are facing such disruptions every day. The Corporate Food Safety plan is updated and passes the next government inspection and the GFSI audit, but are they adopted as updated at all levels? Disruptions and change are difficult and, in the scramble to update food safety plans, it is not unusual to miss the change management step to ensure these are adopted and followed as updated, sometimes failing to incorporate the realities of field operations into the plan. This failure to launch the plan as updated limits the impact food companies can make to reduce recalls and foodborne illnesses, increasing external threats on the C-Suite and their

brand. Speakers will discuss how you live the plan, get to the plan, position the plan, and do not position the plan. First, a review will take place of how third-party food safety audits can be better used to support the food safety plan and updates. Second, speakers will challenge session participants to look at external threats and pressures affecting the implementation of a food safety system. Third, session attendees will be provided with ideas on how to get buy-in from the C-Suite and functional ownership year-round. The combination of these topics will help attendees return with a practical hands-on approach to improve their companies' food safety performance.

S46 Progressing Allergen Risk Management: Thresholds and Quantitative Risk Assessment

LYNNE HABER: *University of Cincinnati, Cincinnatti, OH, USA*
SAMUEL GODEFROY: *Université Laval, Department of Food Science, Faculty of Agriculture and Food Sciences, Quebec City, QC, Canada, Canada*
BENJAMIN REMINGTON: *University of Nebraska, Lincoln, NE, USA*

Food allergies constitute a significant public health issue that affects approximately 32 million Americans. The Food Allergy Research and Education (FARE) reports a 377% increase in the diagnosis of anaphylactic food reactions between 2007 and 2016. Although research is ongoing for therapeutics, the primary management for food-allergic consumers is strict dietary avoidance. Food choices, however, may be limited for these consumers because of the widespread and inconsistent use of precautionary allergen labeling (e.g., statements such as may contain). The concept of reference doses based on a threshold effect is routinely used in public health risk assessment to inform the approach to risk management. For food allergens, dose-response modeling of clinical data from oral food challenges in food-allergic individuals has the potential to inform allergen risk management decisions and drive consistency in allergen labeling. This session will explore the current understanding and application of reference doses for food allergens (derived from the modeling of individual thresholds) and consider what information and tools are needed to progress toward reliable allergen risk assessment and management decisions.

S47 WGS Quality and Quantity - Can You Have It All?

PETER EVANS: *USDA, Washington, DC, USA*
ANGELA NGUYEN: *Mérieux NutriSciences, Crete, IL, USA*
JEROME COMBRISSON: *Mars Global Services, Aimargues, France, France*

Whole genome sequencing (WGS) is no longer considered new technology, yet it has not become commonplace in the food industry due to a lack of standardization, expertise, regulatory concerns, and monetary constraints. Despite all of these hurdles, WGS can provide a level of resolution that is unmatched. WGS is one of many increasingly prevalent technologies where generation of the data in the laboratory is only the beginning, as this generated data can be endlessly mined for information. Considering countless distinct analyses can be applied to sequencing data from a single experiment, this highlights the importance of the initial data quality itself. The initial data quality can impact how the result is used or the interpretation that can be gleaned from each applied analysis. This symposium will explore the recent updates to WGS standardization, discuss how to ensure quality of data routinely for a non-routine technology, and how the quality of data impacts the practical outcomes of using WGS in the food industry.

S48 General Update on *Bacillus* and Overview of Available Tools to Identify, Distinguish, and Trace *B. cereus* Microbial Hazard

FLORENCE POSTOLLEC: *ADRIA Food Technology Institute - UMT ACTIA 19.03 ALTER'iX, France, Quimper, France, France*
BRIAN FEDERICI: *University of California Riverside, Riverside, CA, USA*
MARIEM ELLOUZE: *Nestlé Research, Lausanne, Switzerland, Switzerland*

Bacillus cereus sensu lato, also known as the *B. cereus* group, includes closely related Gram-positive, spore-forming, and aerobic bacilli, widely distributed in the environment and food matrices. Besides characteristic colonies on Mossel agar, these species exhibit highly divergent properties and their distinction remains challenging. Presently their classification relies mainly on distinctive phenotypic traits, such as pathogenic potential to mammals *(B. anthracis*, *B. cytotoxicus,* emetic/diarrheic strains of *B. cereus*) and insects (*B. thuringiensis*), enzymatic ability causing food spoilage (*B. weihenstephanesis*, *B. wiedmannii*), thermotypes, as well as colony morphology (*B. (pseudo)mycoides*). Recently, *Bacillus toyonensis, Bacillus manliponensis, Bacillus gaemokensis,* and *Bacillus bingmayongensis* have been recognized as plausible members of this group.

While food and raw material generally show low spore contamination, food poisoning outbreaks are mainly due to improper conditions of use and storage of food after cooking. Careless food-handling, especially time and temperature abuse, of products such as cooked rice, sauces, soups, and ready-to-eat products allows the production of bacterial toxins associated with *B. cereus* foodborne illnesses.

The issue that continuously exasperates the food industry and food safety officials is how to separate the bacteria that can cause food spoilage from the strains that can cause human illness. This is a complete nightmare for food business operators and public health agencies. This session will give an update on available knowledge and tools related to *B. cereus* issue.

S49 After 2020, Where Do We Go Next in Enhancing Consumer Food Safety Education?

SHARMI DAS: *U.S. Food and Drug Administration, Washington, DC, USA*
SHELLEY FEIST: *Partnership for Food Safety Education, Arlington, VA, USA*
MICHAEL ROBERSON: *Publix Super Markets, Inc., Lakeland, FL, USA*

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S50 The Impact of Foodborne Disease: Emerging Research on Disease Outcomes and Economic Burden

ERIKA AUSTHOF: *University of Arizona, Tucson, AZ, USA*
ERIKA AUSTHOF: *University of Arizona, Tucson, AZ, USA*
KRISTEN POGREBA-BROWN: *University of Arizona, Tucson, AZ, USA*
SANDRA HOFFMANN: *USDA, Economic Research Service, Washington, DC, USA*

Quantitative assessments of the impacts of foodborne illnesses are an integral part of analysis used to manage food safety risks across the food system. The purpose of this session is to share results from a program of current research designed to develop new estimates of the health and economic impacts of foodborne disease in the U.S. The first presentation provides an overview of a set of systematic reviews conducted to assess the literature on complications and long-term outcomes from gastroenteric illness, toxoplasmosis, listeriosis, and vibriosis. Outcomes examined include sepsis, joint outcomes, meningitis, pregnancy outcomes, blindness, hearing loss, and long-term cogitative and physical disabilities. The second presentation does a deeper dive looking at the results from a meta-analysis of long-term outcomes of listeriosis. These review efforts are used to inform the assessment of the impacts of foodborne illnesses in the U.S. The final presentation presents preliminary results on new estimates of the cost of foodborne illness in the U.S. These new estimates make use of results from the systematic reviews presented here as well as new analysis of a broad range of data and literature to model costs of treatment of foodborne illness and earnings loss. This is new research funded by the USDA Economic Research Service and the Food and Drug Administration and conducted in collaboration with the Colorado School of Public Health at the University of Colorado and Mel and Enid Zuckerman College of Public Health at the University of Arizona.

S51 A Growing Concern for Marine Biotoxins

EWEN TODD: *Ewen Todd Consulting, Okemos, MI, USA*
DANA DVORACEK-DRIKSNA: *Neogen Corporation, Lansing, MI, USA*
TIM HARWOOD: *New Zealand Food Safety Science & Research Centre, Palmerston North, New Zealand, New Zealand*

The symposium will first look at an overview of seafood toxins and their sources around the world to show how widespread they are, with some regions more prone to toxicity than others. With fisheries looking to exploit new stocks, both wild-harvested and aquacultured, the risks of harmful algal blooms and their toxins can increase. There are serious challenges to prediction where dinoflagellate and other blooms might occur since they are frequently not visible to those who harvest; actions taken to limit their contact with the food species; and detection methods for the toxins in the shellfish and fish. Climatic and nutritional conditions, exacerbated by global warming of the waters, also pose research questions. Although paralytic shellfish poisonings (PSP) have been known for centuries in North America, the interactions between the causative dinoflagellates, the water conditions, and the shellfish species still require up-to-date research. There is now collaboration between researchers in New Zealand, Australia, UK, and French Polynesia on PSP issues. The discovery of amnesic shellfish poisonings (ASP) in 1987 opened up a new area of global concern for domoic acid. What are the impacts of hurricanes and heavy rain on neurologic shellfish poisonings (NSP) caused by Karenia in the Gulf of Mexico? What is the cause of green sea turtle poisonings, often fatal in Southeast Asia? Seafood toxins not only affect our food supply but also wildlife such as crustaceans, sea lions, whales, and sea birds, which may be visible only to local communities when there are high mortalities. Rapid detection methodologies for toxins replacing the mouse bioassay is a rapidly expanding field but who sets the standards? All three speakers will give an overview of historical and current information and shed light on some of these types of questions.

S52 Paradigm Shifting Foodborne Outbreaks and Their Impact on Food Safety

DOUG KARAS: *U.S. Food and Drug Administration, College Park, MD, USA*
STELIOS VIAZIS: *U.S. Food and Drug Administration, Portland, OR, USA*
MICHAEL BAZACO: *U.S. Food and Drug Administration, College Park, MD, USA*
DAVID ACHESON: *The Acheson Group, Bigfork, MT, USA*
KATHLEEN GLASS: *Food Research Institute, University of Wisconsin-Madison, Madison, WI, USA*
KIS HALE: *USDA, Washington, DC, USA*

Foodborne outbreaks are highly visible and highly scrutinized by the public, the media, and food safety professionals alike. Most outbreaks tend to fall in well-characterized food/pathogen pairs, such as Listeria monocytogenes/deli meat, Escherichia coli/leafy greens, and Campylobacter/chicken. Every so often though, an outbreak occurs that challenges established food contamination paradigms. These outbreaks may be considered atypical in context but can uncover previously unknown issues that may be widespread. Foodborne outbreaks that deviate from current paradigms have revealed processing flaws, emerging diseases, new consumer behaviors (i.e., handling, treating, or consuming foods outside of its intended use), and less understood food/pathogen pairs. Many of these "unexpected" outbreaks become landmark outbreaks that are integrated into food safety curriculums and can ultimately overhaul an entire industry. For example, E. coli outbreaks in raw flour have initiated a food safety paradigm shift, catalyzing a wave of changes – from new labeling practices, increased interest in irradiation as a processing technique, and new consumer outreach programs. A thorough review of paradigm-shifting outbreaks is imperative, allowing the food safety community to reflect on the lessons learned and the broader impacts on the world of food safety. This symposium will explore several notable outbreaks that challenged conventions by 1) reviewing the cause of each outbreak, including the circumstances that made each unusual or unexpected in context; 2) reflecting on changes in industry, regulation, and education resulting from the outbreak; and 3) evaluating how the broader lessons from each outbreak impacted the long term goals and operations of food safety professionals.

S53 Safeguarding Food Security and Food Industry Workforce in Pandemic Times Using Breakthroughs in Molecular Diagnostics and Advances in Genomic Epidemiology

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DOUGLAS MARSHALL: *Eurofins Scientific Inc., Fort Collins, CO, USA*
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Since its first outbreak in Wuhan, China in December 2019, the SARS-CoV-2 virus has spread rapidly across six continents, infecting millions of people and causing an economic recession. To limit the spread of the virus, countries worldwide enforced stay-at-home orders for non-essential businesses. In order to maintain necessities, essential businesses maintained operations. Unfortunately, many essential businesses, such as food processing facilities, experienced severe outbreaks of COVID-19. These outbreaks endangered the lives of the workforce and threatened the stability of the food supply chain.

There are some critical factors why meatpacking and poultry facilities and dairy farms emerged as hotspots across the country – densely packed work environments, suboptimal sanitary conditions, limited access to testing in these rural areas, low-wage workers with no paid sick days, etc. While some companies were able to learn these risk factors and address some of them quickly, many others were not able to act swiftly. While this COVID-19 pandemic caught us off guard, we should be well prepared for a future one, if it arises.

Access to rapid and reliable diagnostic testing and methods such as contact tracing and genomic epidemiology to quickly catch a budding outbreak in real-time can help to safeguard the wellbeing of the food industry workforce and ensure the stability of the food supply chain. To achieve these, strong collaboration across the food industry, government, testing labs, and diagnostic assay and device manufacturers is paramount. We should be able to leverage breakthroughs including advances in molecular assays, rapidly deployable testing, big data analytics, and cloud infrastructure.

This symposium will feature insights from the thought leaders in the food industry, government, centralized and distributed testing lab directors, and molecular diagnostic platform developers.

S54 Defining Criteria for Assessment and Execution of Gluten-Free and Allergen-Free Claims

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Many current foods on the market contain allergen-free or gluten-free claims on labels or for marketing on websites. Even without such "free" claims, the absence of particular allergens in the ingredient or the "contains" statement drives allergic consumers to purchase those foods. Good manufacturing practices can help minimize the allergen cross-contact for safe consumption of packaged foods by allergic consumers. Gluten-free claims have a regulatory basis in many countries with a limit set by CODEX to ensure the safety of the patient and are based on relevant intake date on the dose at which a patient with celiac disease will not see adverse effects. Alternatively, there are currently very few regulations globally or audits for other allergen-free claims. This session will summarize relevant human dose-response data for gluten/allergen-free claims, provide background on audits to assess facility practices and programs for gluten-free claims, summarize global regulations on gluten/allergen-free claims, and provide industry best practices on testing programs to support gluten/allergen-free claims.

S55 Environmental Transmission, Detection, and Molecular Characterization of Foodborne and Waterborne Parasites

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MAURICIO DURIGAN: *U.S. Food and Drug Administration – CFSAN, Office of Applied Research and Safety Assessment, Laurel, MD, USA*
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Waterborne and foodborne parasites represent a serious threat to human health. The parasitic forms in the environment are ubiquitous and resistant to environmental conditions, various disinfectants, and many treatment practices. Detection and proper identification of parasites are challenging. An important limitation when using traditional microscopy-based detection tools is their inability to differentiate morphologically identical species, genotypes, or subtypes for important water and foodborne parasites, such *Cryptosporidium* spp., *Giardia duodenalis*, or *Cyclospora* spp. The contamination with both human-pathogenic and nonpathogenic species, genotypes, or subtypes makes sensitive molecular detection methods necessary for a correct identification of those environmental parasitic forms to the species/genotype/subtype level. In addition, different species or genetic variants may co-exist in an environmental sample; failure to recognize this diversity hampers our understanding of food- and waterborne parasite transmission and source tracking. The use of new methods, such as next generation sequencing to identify all genetic variants present in environmental samples, can underpin a better understanding of the complexity of food- and waterborne parasite composition on those samples that so far have been largely overlooked. Another challenge of the molecular identification of parasites in environmental samples is that they are usually present in very low numbers in samples with high concentrations of PCR inhibitors and non-target organisms. In this symposium, we will discuss the use of novel molecular detection and genotyping methods that have been developed for water- and foodborne parasites.

S56 Recent Advances in Understanding Phage Applications to Mitigate Food Safety Risk

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JOËL VAN MIERLO: *Micreos Food Safety, Wageningen, Netherlands, Netherlands*
HANY ANANY: *Agriculture and Agri-Food Canada, Guelph Research and Development Center, Guelph, ON, Canada, Canada*
LONE BRONDSTED: *University of Copenhagen, Copenhagen, Denmark, Denmark*

Numerous advances have been made in the understanding and application of phage technology by the food industry for the mitigation of food safety risks. As phages are becoming a more mainstream intervention, the objective of this session is to provide an update on the understanding and dynamics of phages to combat foodborne pathogens. For this purpose, practical examples of phage applications in an industrial setting will be given. Furthermore, advances in the understanding of the mechanics, as well as challenges and opportunities in the application of phages for mitigation of pathogens on various products will be explored. In all, the symposium will provide a full update on advances in phage research from academicians, practical applications from industry personnel as well as explore some of the triumphs and lessons learned in the utilization of phages to reduce the risk of foodborne illness.

S57 Developing Atmospheric Cold Plasma as a Nonthermal Food Safety Tool

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KEVIN KEENER: *University of Guelph, Guelph, ON, Canada, Canada*
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Cold plasma is an emerging technology proposed as a nonthermal process to reduce food safety risks on a variety of foods. Atmospheric cold plasma has a benefit in that it can be applied to packaged foods to reduce surface contaminants such as *Salmonella* or STEC on fresh fruits and vegetables or *L. monocytogenes* on high-moisture cheeses. This symposium will present an overview of the technology, examples of inactivating microbes as well as demonstrating it as a novel approach to reduce the presence of mycotoxins on grains. Results from validation studies will be presented and challenges to commercialization will be addressed.

S58 Lessons Learned from Consumer Food Safety Initiatives Related to the COVID-19 Pandemic to Guide Future Outreach and Communication Practices

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WENQING (WENNIE) XU: *LSU AgCenter, Baton Rouge, LA, USA*
SILVIA DUMITRESCU: *IFIC, Washington, DC, USA*
ALI WEBSTER: *IFIC, Washington, DC, USA*
ZHINONG YAN: *Walmart, Bentonville, AR, USA*

Consumer food safety education campaigns have long been utilized by academia, retail, and government institutions to communicate foodborne disease risks and best risk-mitigation practices to reduce public health impacts. Although current evidence suggests that COVID-19 is not likely to be transferred via food matrices, consumers' implementation of health and hygiene practices in food-related contexts (grocery stores, dining out, at-home) are instrumental in ensuring personal food safety practices. Additionally, consumers have altered food purchasing behaviors to reduce risks (purchasing from E-commerce food platforms, different interactions at farmers markets, and U-pick farms). The COVID-19 pandemic necessitated additional consumer food safety outreach and education during a dynamic period of scientific discovery, risk evaluation, and recommendation development for consumer practices. Rapid and adaptive education outreach was essential to convey the nature of risk to instill consumer confidence and encourage adopting safe practices. Research even suggests that, due to targeted and frequent messaging, consumers now wash their hands 41% more frequently than pre-pandemic.

This symposium will address the collaborative and multi-pronged consumer outreach efforts initiated during the pandemic that resulted in rapid and collaborative responses among consumer-facing bodies to develop, share, and distribute culturally adaptive and consistently updated consumer resources to "meet people where they are." Speakers who spearheaded timely media/public-facing initiatives and campaigns from academia, retail, and consumer research sectors will share their collaborative experiences to rapidly create new educational resources, strategies for dissemination, and partnerships for expanded and adaptive outreach to consumers.

Symposium attendees will learn: 1) about food safety resources and educational approaches during COVID-19 which, due to collaborations, were re-branded and utilized throughout various academic and public health institutions; 2) strategies employed to expand outreach through technology and partnerships within both existing networks and new municipality partnerships; 3) measured impacts of initiatives; and 4) lessons learned from these educational campaigns through consumer information accession, feedback, and practices in the retail space. This session will inform practices for ongoing collaborative consumer food safety initiatives.

S59 Where's the Beef? Grinding Recordkeeping and Intended Use at Retail

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THOMAS COLLARO: *U.S. Department of Agriculture – FSIS, Waltham, MA, USA*
TODD ROSSOW: *Publix Super Markets, Inc., Lakeland, FL, USA*
SHERRI WILLIAMS-TRUJILO: *JBS/USA, Greeley, CO, USA*

Recently, Shiga toxin-producing *Escherichia coli* (STEC) has been linked to consumption of beef ground at retail. Outbreak investigations have shown that insufficient sanitation between lots of beef products and inadequate grinding records at retail have hindered investigators' ability to determine the source of contaminated products. In addition, retailers have produced raw ground beef using whole cuts of beef that were intended for intact use and trim from those products. This practice poses a food safety concern because beef-producing establishments typically apply more stringent methods to control STEC in beef intended for non-intact use (e.g., ground beef and mechanically tenderized steaks) than in beef intended for intact use (e.g., steaks). STEC may be present on the surface of raw intact beef that the supplier intends solely for intact use and is killed through normal cooking processes (e.g., grilling). However, when intact beef is ground, a practice common at retail, STEC can be moved to the interior and not be killed by customary cooking (i.e., rare or medium). This symposium will bring together representatives from federal and state governments and the beef processing and retail industries to discuss how grinding recordkeeping and intended use can be applied to help ensure the safety of beef products that are ground at retail.

Roundtable Abstracts

RT1 Improving the Food Recall Effectiveness of Regulatory Agencies

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ARMANDO ZAMORA: *U.S. Food and Drug Administration, Rockville, MD, USA*
NANCY BEYER: *Missouri Department of Health and Senior Services, Jefferson City, MO, USA*
JESSICA BADOUR: *Georgia Department of Agriculture, Atlanta, GA, USA*

One of the fundamental duties of government regulatory agencies is ensuring the safety of food marketed to consumers. When these agencies become aware of unsafe or potentially unsafe food in the marketplace, they must act quickly to keep people from becoming ill or being harmed. If an illness has already occurred, the agencies need to respond quickly to prevent more people from becoming ill. In addition, food recalls can assist regulatory agencies in pinpointing the source of the problem within the food production system so corrective action can be taken. These efforts are an important duty of federal, state, and local government agencies, who have not always worked collaboratively in the past when food recalls occur.

These agencies, however, now plan to take additional steps as part of a broader action plan to improve their oversight of food safety and the recall process. In particular, improvements with recall timeliness and the scope of information provided to the public are important areas where improvements can be made. In addition, FDA's proposed traceability rule may soon improve food traceability and have a huge impact on improving how food recalls are completed.

These steps tie into the FDA's *New Era of Smarter Food Safety* where innovation and a simpler, more efficient approach are taken to achieve change. The recall process is a focal point in the FDA's *Blueprint for the Future,* and this panel will highlight the new proposals and methods that could be used to improve recalls through technology applications that streamline communication and share real-time data working toward harmonizing language between federal agencies. This group will take the audience on a modernizing recall journey while discussing how federal, state, and local government agencies are improving their policies and practices to ensure that recalls are initiated, overseen, and completed promptly and effectively to protect consumers.

RT2 Don't You Forget about Me! Educating Underrepresented Growers on Produce Safety

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JOSIAH GRIFFIN: *Indigenous Food & Agriculture Initiative, Fayetteville, AR, USA*
ANDREW WILLIAMS: *The United Christian Community Association, Safford, AL, USA*
ANNALISA HULTBERG: *University of Minnesota, St. Paul, MN, USA*
ELIZABETH BIHN: *Cornell University, Geneva, NY, USA*
ARMITRA JACKSON-DAVIS: *Alabama A&M University, Madison, AL, USA*

Many educational resources that produce safety for educators' use are designed for a general audience. Generalized materials can often lack cultural relevance or competency by some populations, assuming instead that the learner is fluent and literate in English. Farmers from historically and traditionally underrepresented populations (e.g., small farmers, new farmers, Plain growers, Asian growers, Black growers, Native American growers, Latinx growers) have become increasingly involved in farm operations in the United States according to the 2007, 2012, and 2017 Census of Agriculture. Due to the inaccessibility and lack of adaptations, underrepresented growers do not receive suitable training for their needs. These growers may require programming tailored toward different languages and literacy levels as well as the time, money, and material resources they have available. Additionally, some of these populations may choose not to engage with available educational programming and content due to potential distrust of educators' intervention by viewing educators as harmful to their business.

This roundtable will a) discuss specific produce safety approaches for educating underrepresented growers about Produce Safety Rule practices that they can easily implement on their farms, given their needs and resources; b) address trust-building among underrepresented groups of growers to open opportunities for produce safety education; c) highlight ways educators have modified outreach and educational materials to overcome challenges or to meet specific needs; d) share experiences working with various underrepresented populations to discuss methods of better serving these groups; and e) address the successes, failures, and challenges working with these groups. The lessons learned from this roundtable will allow attendees to more effectively work with diversified produce growers to address their needs and implement more progressive food safety practices to meet buyer and federal regulatory expectations.

RT3 Opportunities and Challenges: Developments in *Clostridium botulinum* Challenge Studies

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SABINE PELLETT: *University of Wisconsin-Madison, Madison, WI, USA*
MAXINE ROMAN: *Kraft Heinz, Glenview, IL, USA*
STEPHEN GROVE: *Nestlé Development Centre – Solon, Solon, OH, USA*
MICHAEL W. PECK: *QIB Extra Ltd., Norwich, United Kingdom, United Kingdom*

Consumer demand for minimally processed, wholesome, yet safe food commodities, especially sous-vide products, refrigerated processed foods of extended durability (REPFED), and low-acid canned foods continues to grow. Key target pathogens for these foods are heat-resistant Group I proteolytic *Clostridium botulinum*, and the less heat-resistant, but psychrotrophic Group II nonproteolytic *Clostridium botulinum*. Inoculated challenge studies are often required to validate processing technologies to inactivate *C. botulinum* spores, or to ensure that food formulations, which can include using conventional and clean label antimicrobials, inhibit spore germination, cell multiplication, and production of neuroparalytic botulinum neurotoxins (BoNTs). These challenge studies are costly because *C. botulinum* is a highly-regulated pathogen, and the mouse bioassay is the gold standard assay for BoNT detection in foods. The nontoxigenic *C. sporogenes* strain PA 3679 has historically been used as a surrogate for group I *C. botulinum* for thermal processing validations due to its increased heat resistance. Although toxin gene inactivation and deletion mutants have been reported in Group I and Group II *C. botulinum*, these nontoxigenic potential surrogates for inactivation studies have not been validated. Furthermore, using genetically modified nontoxigenic strains has raised biosafety and biosecurity concerns over the stability of the mutated toxin gene. Growth inhibition challenge studies pose a different problem in which use of a nontoxigenic strain is impractical since the requirement for challenge testing

is to measure BoNT formation unless the nontoxigenic strain is engineered with a reporter system, which would require further validation. The goal of this symposium is to discuss these challenges with international experts in academia, the food industry, and regulatory agencies and consider whether there is a path forward on developing new solutions and alternatives for validating the safety of food processing technologies and novel food formulations.

RT4 Informal Markets: Building Consumer Demand for Food Safety in Low-Resource Environments

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AUGUSTINE OKORUWA: *GAIN – Global Alliance for Improved Nutrition, Abuja, Nigeria, Nigeria*
STELLA NORDHAGEN: *GAIN – Global Alliance for Improved Nutrition, Geneva, Switzerland, Switzerland*
ELISABETTA LAMBERTINI: *GAIN – Global Alliance for Improved Nutrition, Rockville, MD, USA*

In 2014, the World Health Organization estimated that unsafe food causes one in every 10 people to fall ill each year, leading to 600 million cases of illness and 420,000 deaths each year. Children under five are most vulnerable, and consumers in sub-Saharan Africa face the greatest disease burden. Many consumers in low- and middle-income countries (LMIC), including those in Africa, buy their food at traditional markets. These markets often lack essential infrastructures, like clean water, safe storage, a cold chain, sanitary facilities, and other essential food service equipment. How can food safety be improved and foodborne illnesses be reduced in these environments?

The Global Alliance for Improved Nutrition (GAIN) is leading a program with USAID/Feed the Future called EatSafe, to identify consumer- and vendor-led interventions to improve food safety at traditional/informal markets. Prevention is key. Training a cadre of well-informed consumers in informal markets can help create consumer demand for safe, nutritious food and assist food suppliers and vendors to improve food safety practices. GAIN has also been conducting consumer and vendor surveys on the impacts of COVID-19 on informal markets. This roundtable will discuss the evidence on how to impact consumer demand and food safety in informal markets and explore:

- COVID-19 impacts on informal markets
- The links between foodborne illness and malnutrition in LMICs
- The interplay of individual behavior, socio-economic drivers, associations, technology, and local government in their impact on food safety improvements
- Efforts by USAID to improve food safety and nutrition with an emphasis on informal markets

RT5 Are All *Salmonella* Equal? Genomic Approach for Risk Ranking *Salmonella* Strains

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NOELLE NOYES: *Food-Centric Corridor, Infectious Disease Laboratory, Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN, USA*
DAYNA HARHAY: *USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA*
MARTIN WIEDMANN: *Cornell University, Ithaca, NY, USA*

Salmonella is a leading cause of foodborne illness globally. Different serotypes have different levels of pathogenicity. For example, *Salmonella Cerro* and *Salmonella Kentucky* are frequently found in meat and poultry but rarely cause illness. Whole genomic sequencing (WGS) is an important tool used to detect *Salmonella* outbreaks and to help identify possible sources of contamination. WGS combined with machine learning techniques may enable us to identify genetic factors associated with pathogenicity and virulence in different serotypes. Defining a standard risk ranking approach would allow for industry and regulatory agencies to design appropriate risk management strategies that could focus resources on public health relevant strains. In this roundtable, researchers will share information on genomic approaches to identifying virulence factors. This may enable strains to be classified based on their virulence and pathogenicity, rather than serotype. Potential practical applications of this approach will be discussed.

RT6 Strengthening Food Safety Risk Management on the African Continent through International Collaboration

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JOYCE THAIYA: *Ministry of AgricultureNairobi,Kenya*
KEBEDE AMENU: *Addis Ababa University, Bishoftu, Ethiopia, Ethiopia*
ADEWALE OLUSEGUN OBADINA: *Federal University of Agriculture Abeokuta, Abeokuta, Nigeria, Nigeria*
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BARBARA KOWALCYK: *The Ohio State University, Columbus, OH, USA*

Following up from a very successful roundtable event at IAFP 2020 on food safety challenges and opportunities on the African continent, a second roundtable is proposed to explore concrete opportunities within the global IAFP community to join forces to address the management of key African continent food safety risks associated with chemical, microbial, and physical hazards.

A number of international collaborations regarding epidemiology, in particular foodborne hazards and standards development, already exist within and between African countries and countries outside Africa. These add tremendous value to African food safety, but there are many more African food safety needs. Importantly, many such needs are similar for other low- and middle-income countries around the globe.

Some of the key needs expressed during the RT discussion at IAFP 2020 were the desire to build capabilities in food safety risk assessment, developing microbiological criteria for managing intra- and inter-regional food trade based on risk, and risk analysis as an overarching framework.

Aiming for a very interactive session with the audience, the following topics are illustrative for the scope of the RT:

- Regional and Local Food Safety Challenges in Africa
- Governmental Risk Management Challenges and Opportunities in Africa
- Formal and Informal Industry Risk Management Challenges and Opportunities in Africa
- Food Safety Capability-Building Challenges and Opportunities in Africa
- Examples of Appropriate International Food Safety Collaboration Success Stories
- Exploring Opportunities for Collaborations within the IAFP Community

This RT is part of an effort across the global IAFP community to foster more international collaboration and sharing of expertise to strengthen food safety risk management in regions where needed.

RT7 The Drive for Better Sanitation and Food Safety Compliance through Measurements, Management, and Culture

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PAULA HERALD: *Steritech, Prospect, KY, USA*
CHARLES SEAMAN: *Hy-Vee, Ankeny, IA, USA*
BARBARA CHAMBERLIN: *New Mexico State University, Las Cruces, NM, USA*
LILLIAN HSU: *U.S. Food and Drug Administration-CFSAN, Laurel, MD, USA*
LONE JESPERSEN: *Cultivate, Hauterive, Switzerland, Switzerland*
HALEY OLIVER, *Purdue University, West Lafayette, IN, USA*

The most important part of any food safety process or practice is the person executing those practices. Food safety and sanitation programs frequently fail when personnel neglect to comply with company procedures. This fact makes it critical for us to look for ways to evaluate and improve process compliance where it is critical to preventing foodborne illnesses. A strong sanitation program, with practices that are performed properly and consistently, is a prerequisite for any HACCP plan. This roundtable session will focus on how to gain better compliance with a sanitation program in various food settings (e.g., manufacturing and retail). The panel will discuss new programs that use digital resources to measure compliance and allow a company to better manage food safety and sanitation practices utilizing data. The panel will also discuss the elements of food safety culture that impact gaining better compliance, including establishing standards and measurements, auditing, building leadership buy-in, and upholding accountability. The panel, comprised of experts from multiple sectors, will share various viewpoints, trouble spots, and success stories of how to achieve higher levels of food safety and sanitation compliance. Attendees will gain a greater understanding of data collection, process evaluation, and how to focus on elements of culture building to drive food safety process compliance in their own facilities.

RT8 When Crime Threatens Food Safety

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JENNIFER VAN DE LIGT: *Food Protection and Defense Institute, Saint Paul, MN, USA*
CLARE MENEZES: *McCormick UK Ltd., Haddenham, United Kingdom, United Kingdom*
NEAL FREDRICKSON: *Cargill, Wayzata, MN, USA*
ROY FENOFF: *The Citadel, Charleston, SC, USA*
ELISE FORWARD: *Forward Food Solutions, LLC, River Falls, WI, USA*

Food defense and food fraud prevention continue to be important areas of focus for industry and regulators. Significant supply chain disruptions, such as those caused by the COVID-19 pandemic, affect both the opportunity and motivation for food crime. This roundtable session will provide attendees with the opportunity to hear first-hand experience and advice for reducing the risk of food crime that threatens food safety. The session will include brief presentations by panelists, time for open discussion, and solicitation of audience questions for the panel, which is comprised of experts with decades of experience in a variety of roles focused on the prevention of intentional adulteration in all forms.

The panelists represent academia, government, a consulting practice, and two industry-leading food companies. They will address a variety of challenges in this area, including "What scenarios provide the opportunity for crime in the food system?"; "How do we incorporate crime prevention into a food safety system?"; "How has the COVID-19 pandemic affected food defense and food fraud prevention efforts?"; "How does industry implement cost-effective and valuable testing programs?"; and "How has auditing to food defense and food fraud/EMA requirements evolved over the past five years?"

RT9 NGS Case Study - The Challenges and Solutions to Implementing Genomics in a Live Factory Environment

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MARTIN WEIDMANN: *Cornell University, Ithaca, NY, USA*
SÉAMUS FANNING: *University College Dublin, Dublin, Ireland, Ireland*
JOHN DONAGHY: *Nestlé S.A., Vevey, Switzerland, Switzerland*
PABLO CARRION: *Nestle Purina, St. Louis, MO, USA*

This topic is a case study on the challenges and solutions to implementing next-generation sequencing (NGS) in a live factory environment. It will cover the technical and regulatory concerns for a food company and how these were resolved in order to enable the extraordinary benefits of NGS to be achieved.

A better approach is required that combines traditional, tried, and tested approaches with new scientific tools to deepen insights and knowledge of microbial ecology in the production environment in a cost-effective way. Applied effectively, such methods can move responses from a reactive footing to a proactive approach. This is done by using data science and computer machine learning to generate early warning signals that have the potential to prevent incidents and non-conformities.

However, implementing these solutions requires multi-stakeholder buy-in and ensuring the structures and mechanisms are in place for a smooth rollout. From why, how, when, and where to swab, right through to reviewing the data for insight and analysis – these steps are considered and discussed with the panel of experts.

The expectation is that this will be highly informative for the food industry and will support the uptake of next-generation approaches to food safety.

RT10 Changing Lanes in the Middle of a Pandemic: Challenges and Lessons Learned from Managing SARS-CoV-2 in the Food Sector

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DONALD W. SCHAFFNER: *Rutgers, The State University of New Jersey, New Brunswick, NJ, USA*
ERIC MOORE: *Testo Solutions USA, Inc., West Chester, PA, USA*
KATIEROSE MCCULLOUGH: *North American Meat Institute, Washington, DC, USA*
STEVEN MANDERNACH: *Association of Food and Drug Officials, New York, NY, USA*
DONNA GARREN: *American Frozen Food Institute, Arlington, VA, USA*

The world has changed dramatically in the time since the start of the COVID-19 pandemic. Many areas of the food sector have dealt with changes related to being essential services and a required part of the food infrastructure. COVID-19 positive individuals (symptomatic, presymptomatic, and asymptomatic) work alongside others daily. Some food businesses like retail and foodservice outlets have members of the public interacting with employees as well. While food safety professionals have experience in managing foodborne pathogens (both emerging and known), the COVID-19 pandemic has provided challenges in investigating a new respiratory pathogen and its impacts on food safety. While SARS-CoV-2 is not identified as a foodborne pathogen, the pandemic has impacted traditional food safety activities such as inspections, supplier verification, and environmental sampling and has led to supply chain issues. In addition, new areas of management like evaluating ventilation systems, physical distancing, disinfection of common touch surfaces, and respiratory illness declarations (and resulting test/trace/isolate steps) often lie with food safety professionals. As restrictions lift and are levied (and will likely ebb and flow over the next 12-18 months) there is a need to discuss lessons learned and perspectives on what could have been managed better. In this roundtable we will assemble a series of panelists who were involved in various aspects of decision-making around best management practices; supporting the food sector in evaluating technologies; defining and filling data gaps; and communicating risks to various audiences. This session will feature insights from various sector stakeholders with an eye on providing real-world examples of how the food sector remained resilient and responded.

RT11 Diversity in Food Culture from Sushi to Steak Tartare: An Interdisciplinary Approach to Understanding Roots of Food Safety Behaviors

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JOE MAC REGENSTEIN: *Cornell University, Ithaca, NY, USA*
AMARAT (AMY) SIMONNE: *University of Florida, Gainesville, FL, USA*
DIMA FAOUR-KLINGBEIN: *DFK for Safe Food Environment, Hannover, Germany, Germany*
BOBBY KRISHNA: *Dubai Municipality, Dubai, United Arab Emirates, United Arab Emirates*
CAROLINE SMITH DEWAAL: *Global Alliance for Improved Nutrition, Washington, DC, USA*

Food safety is a complex global health issue and to achieve successful food safety interventions, different research disciplines and perspectives need to be brought to the table. In an era of globalization, the cultural and behavioral aspects of food consumption and food preparation, as well as gender and the religious/spiritual aspects of food, must be considered. Views toward raw foods vary widely across different cultures. For example, raw fish, raw meat, and raw vegetables are widely accepted in some cultures, while in others they are considered unsafe.

In high-income developed countries such as the U.S., foodborne illness attribution studies provide insight into various food/pathogen combinations, but don't illuminate underlying factors, such as how different traditions might dictate or influence preparation methods or consumption choices. In low- and middle-income countries, lack of money and technology may limit availability of a "cold chain" or drive reliance on street markets that promote other methods (i.e., curing, fermentation, and natural herbal extracts) as techniques of choice for food safety and food preservation.

This roundtable session will explore perspectives of researchers and experts from different disciplines on culinary cultural diversities from around the globe. The panel will address questions like: How safe is food sold and prepared at street markets? How do food handlers and processors manage food safety without a cold chain? What is the role of curing, fermentation, and natural herbal extracts in ensuring food safety and food preservation? What is the role of religious and cultural food systems? How can consumers balance food safety and sustainability? Panelists will bring their knowledge of food cultures from around the world to compare trends and explain the diversity of food customs.

RT12 Food Safety Interventions in Low- and Middle-Income Countries: How Can QMRA be Used Effectively?

SINH DANG: *CGIAR, Hanoi, Viet Nam, Viet Nam*
PETER BEN EMBAREK: *World Health Organization, Geneva, Switzerland, Switzerland*
KEBEDE AMENU: *Addis Ababa University, Bishoftu, Ethiopia, Ethiopia*
DELIA GRACE: *International Livestock Research Institute, Nairobi, Kenya, Kenya*

Within different countries in Africa (and Southeast Asia), several multidisciplinary projects are carried out to investigate the public health impact of various foodborne diseases attributed to selected food commodities and how this impact can be reduced. Public health estimations, value chain analysis, microbial sampling, quantitative microbiological risk assessments, and cost-effectiveness assessments of interventions are conducted. Studies are also done on how to influence governance and value chain actors and the consumer.

In this roundtable, experts from governments, academia, and non-governmental organizations are invited to give their view on the food safety management situation in LMIC and how QMRA can be helpful in determining the optimal way forward.

RT13 Frozen and Fresh Produce: Enteric Viruses Contamination, Detection and Public Health Impact

SOPHIE BUTOT: *Nestlé Research Center, Lausanne, Switzerland, Switzerland*
JAN VINJÉ: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
SABAH BIDAWID: *Health Canada, Ottawa, ON, Canada, Canada*
LEE-ANN JAYKUS: *North Carolina State University, Department of Food, Bioprocessing and Nutrition Sciences, Raleigh, NC, USA*

Foodborne outbreaks resulting from consumption of produce due to consumption of produce contaminated with human enteric viruses, namely Norovirus and Hepatitis A, is an emerging worldwide challenge. The global supply chain of frozen and fresh produce includes many growing regions endemic for enteric virus contamination. As efforts to implement agricultural practices, worker hygiene, and water management programs, there is interest among industry and national regulatory bodies to understand the prevalence of enteric viruses in these products. However, since these

viruses are not culturable, standardized methodologies only test for evidence of viral nucleic acid, which does not reflect the infectious potential of the contaminant. In this roundtable, a panel of global viral experts will discuss the current science of enteric virus detection and public health implications during routine and surveillance testing. The experts will share new approaches to tackle sampling and testing of produce associated with a low and heterogeneously distributed level of enteric viruses.

RT14 Incentives for Preharvest Control of Zoonoses in Food Animals

TODD CALLAWAY: *University of Georgia, Athens, GA, USA*
BILL MARLER: *Marler Clark, The Food Safety Law Firm, Seattle, WA, USA*
RAFAEL RIVERA: *U.S. Poultry and Egg Association, Tucker, GA, USA*
TANYA ROBERTS: *Center for Foodborne Illness Research & Prevention, Vashon, WA, USA*
CRAIG WILSON: *Costco Wholesale, Issaquah, WA, USA*
J. EMILIO ESTEBAN: *United States Department of Agriculture, Food Safety and Inspection Service, Washington, DC, USA*

In addition to the USDA's One Health approach, there are two primary needs for control of zoonotic pathogen carriage in preharvest: 1) Animal feeding operations contaminate the environment including produce via manure, runoff, air, and wild animals. The Environmental Protection Agency published a review in 2013 of water contamination by animal feeding operations but has not taken additional action. 2) Slaughter dressing procedures do not and cannot eliminate zoonoses in animal products because of lymph nodes containing zoonoses, fecal leakage entering follicles during defeathering or dehairing, and aerosols generated during hide pulling and other operations. The scientific literature has described these problems for over a half-century. For preharvest control, scientists have published numerous innovations in mitigating zoonotic carriage in food animals in the 48 years since Nurmi and Rantala demonstrated the effect of competitive exclusion on *Salmonella* carriage in broilers and the 43 years since Pomeroy demonstrated biosecurity could produce *Salmonella*-free turkeys. Those innovations include bacteriophages, pre-probiotics, and vaccines that also improve feed conversion efficiency. Incentives for implementing controls have been few. The panel will discuss the options for promoting preharvest interventions including, economic, regulatory, and litigation.

RT15 Agricultural Water Quality Management in a Changing Regulatory Landscape

LAURA STRAWN: *Virginia Tech, Blacksburg, VA, USA*
ROGER NOONAN: *New England Farmers Union, Boston, MA, USA*
DREW MCDONALD: *Taylor Farms, Sacramento, CA, USA*
FAITH CRITZER: *Washington State University, School of Food Science, Prosser, WA, USA*
CHANNAH ROCK: *University of Arizona, Maricopa, AZ, USA*
KRUTI RAVALIYA: *U.S. Food and Drug Administration, College Park, MD, USA*
HUGO POBLETE: *Sociedad Agricola La Rosa Sofruco, Peumo, Chile, Chile*

The FSMA Produce Safety Rule establishes science-based minimum standards for fresh produce growers and packers. Subpart E addresses agricultural water and includes microbial quality standards to prevent contamination to the harvestable portion of the fresh produce with pathogens. However, industry-wide acceptance of subpart E has been hard to achieve. Consequently, FDA pushed forward compliance dates and announced plans to re-evaluate subpart E.

FDA will likely provide guidance or rulemaking by early 2021 that is expected to bring fundamental changes to the produce industry. Revisions to the rule would result in challenges and opportunities to the fresh produce community including regulatory agencies, producers, educators, researchers, and industry organizations. Each group has the common goal of reducing risks to produce safety; as such, a multidisciplinary understanding and collaboration across industry sectors will be needed. Since produce-related outbreaks related to agricultural water have continued in the absence of water quality requirements, various groups have implemented voluntary requirements to reduce risk — including treatment. Panelists will be asked to discuss implications that revised requirements may have on those emerging industry practices.

This IAFP roundtable is a forum for leaders representing various stakeholder groups to discuss the implementation of subpart E of the Produce Safety Rule as it is currently understood. The pending guidance or rulemaking activity from FDA changes the overall landscape. As a result of these proposed agricultural water standards, various groups must work with produce growers and packers to adapt water-use practices, both pre- and post-harvest. Industry groups will continue coordinating with the regulatory agencies and growers. Educators will adapt training materials to help growers make on-farm decisions. Regulators will work through what compliance looks like. At the end of the day, growers will use the regulatory guidance as a primary input to decision-making processes that reduce risks to produce. This roundtable is intended to facilitate these goals.

RT16 Boo! Does That Delivery from a Ghost Kitchen Scare You?

ASHLEY RONDORF: *Chick-fil-A, Atlanta, GA, USA*
KATHLEEN ZINGSHEIM: *Maricopa County Arizona Environmental Services Department, Phoenix, AZ, USA*
JORGE HERNANDEZ: *Wendy's, Dublin, OH, USA*
HOWARD POPOOLA: *The Kroger Company, Cincinatti, OH, USA*
ELEANOR YOST: *Carlton Fields, Tampa, FL, USA*

Ghost kitchens, cloud kitchens, dark kitchens, virtual restaurants – no matter what they are called, these foodservice operations that are delivery and pick-up only operations are becoming more popular as restaurants attempt to minimize costs and capitalize upon the customer demands for delivery and other non-restaurant venues for food.

Several branded concepts may share space and facilities under the same roof, or there may be "virtual" brands of several ethnic cuisines prepared by a single chef in these kitchens. Due to the profitability of these operations compared to the traditional restaurant, these ghost kitchens are expanding not only in the U.S. but also in Europe, Asia, and the Middle East. These operations present challenges for liability, licensing, and assessment in regard to ensuring food safety.

This symposium will present success stories on why these kitchens are gaining in popularity and how they differ from other foodservice operations; the food safety issues these type of operations may face; challenges of regulating and inspecting; and what should be considered before launching into this type of venture while keeping food safety a priority.

RT17 A North American Perspective on Antimicrobial Resistance and Regulatory Action

RON PHILLIPS: *AHI, Washington, DC, USA*
ENRIQUE PEREZ-GUTIERREZ: *Pan American Health Organization, Washington, DC, USA*
MANISHA MEHROTRA: *Health Canada, Ottawa, ON, Canada, Canada*
UDAY DESSAI: *USDA Food Safety & Inspection Service, Washington, DC, USA*
JASON FOLSTER: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
HEATHER HARBOTTLE: *U.S. Food and Drug Administration, Rockville, MD, USA*

Stakeholders ask, "What actions are FDA/CVM and global governments taking in relation to antimicrobial resistance (AMR) in food-producing animals, and what future policies are being enacted to combat AMR?" This topic will be addressed from a multi-agency, multi-country perspective. FDA/CVM will cover pre-market regulatory requirements to address the potential for AMR development among foodborne pathogens of human health concern (GFI #152), changes enacted *via* GFI #213 for weight gain/feed efficiency indications, and current and future actions FDA/CVM has outlined in its five-year plan to combat AMR. The CDC will give a perspective on human consequences of AMR development, including detection of outbreaks using molecular methods (such as whole genome sequencing and the NARMS program) and the resistant illnesses on the horizon. The USDA will provide an update on resistant bacterial pathogens and indicators recovered at the point of slaughter. And international speakers from Canada and the Pan American Health Organization (PAHO) will give an update on the global perspective on recommendations to combat AMR under the Tripartite Collaboration (WHO, the Food and Agriculture Organization of the United Nations, and the World Organisation for Animal Health) using a One Health approach and the most current governmental actions and considerations. Representatives from industry will provide an industry perspective on how regulations impact the approval/sales of antimicrobials to food-animal producers considering the recent and future actions to combat AMR.

The objective will be to present current data on the detection of antimicrobial resistance in foodborne pathogen outbreaks and emerging global regulatory actions. As a result of the session, areas of current and future international harmonization to combat AMR will be identified and industry concerns can be highlighted.

RT18 Diversifying the Pipeline in Food Safety Education: Engaging Historically Black Colleges and Universities (HBCUs)

OLGA BOLDEN-TILLER: *Tuskegee University, Tuskegee, AL, USA*
ALIYAR FOULADKHAH: *Public Health Microbiology Laboratory, Tennessee State University, Nashville, TN, USA*
MICHELLE DANYLUK: *University of Florida CREC, Lake Alfred, FL, USA*
KRISTIN WOODS: *Alabama Cooperative Extension System, Grove Hill, AL, USA*
SHECOYA WHITE: *Mississippi State University, Mississippi State, MS, USA*
ARMITRA JACKSON-DAVIS: *Alabama A&M University, Madison, AL, USA*

Currently, there is a national focus on diversity, equity, and inclusion in organizations around the United States. This focus is crucial to the success of diversity and inclusion initiatives that seek to increase the participation from underrepresented populations. As educators, diversity enriches our perspectives and helps us reach stakeholders in more meaningful ways with more useful information. From the research perspective, the novel ideas that arise from diverse perspectives will help us use a complex systems approach to solve the world's most significant food safety challenges.

With the development of the newly-formed International Association for Food Protection (IAFP) Diversity, Equity and Inclusion Council, IAFP has formally made a step in the right direction to focus on these areas within the organization. As IAFP aims to increase diversity efforts, the proposed roundtable would serve as a mechanism for starting the discussion of how we can increase participation of 1890s and Historically Black Colleges and Universities (HBCUs) within the IAFP organization. The roundtable is unique in that it would serve to facilitate meaningful discussion on novel ways to increase active participation of 1890s/HBCUs within the IAFP organization. To date, a roundtable of this type has not been organized for IAFP.

This roundtable will consist of panelists who represent the following: 1) Individual from an 1862 that has worked collaboratively with 1890s/HBCUs; 2) Individuals who currently work at 1890s/HBCUs; 3) Representation from IAFP leadership; 4) Administrator from an 1890/HBCU; and 5) Individual who graduated from an 1890/HBCU and currently works at an 1862 institution and who is a member of IAFP.

Given that there is representation from the IAFP Diversity, Equity and Inclusion Council at the roundtable organizer level and also at the roundtable panelists level, this roundtable could be essential to the efforts of the council.

RT19 Emergency Use of Microbial Methods of Detection by Industry - Alternative Routes Proving Fit for Purpose

KELLY STEVENS: *General Mills, Minneapolis, MN, USA*
THOMAS HAMMACK: *U.S. Food and Drug Administration, Center for Food Safety & Applied Nutrition, College Park, MD, USA*
MEGAN BROWN: *Eurofins Microbiology Laboratories, Inc., Madison, WI, USA*
PATRICK BIRD: *AOAC International, Cincinnati, OH, USA*
DEANN AKINS-LEWENTHAL: *Conagra Brands, Omaha, NE, USA*

The food safety industry must ensure analytical methods designed to detect hazards are fit-for-purpose for their specific commodities. Meanwhile, the food industry is developing hundreds of new ingredients and products that will require screening for microbial hazards. With the ever-expanding diversity of these food products, what happens when a testing laboratory is presented with an urgent request for testing a matrix that was not included in the method's original validation study? The laboratory is asked to deviate from the intended use of the method by testing a different matrix or a different test portion size.

This situation can occur when a food manufacturer requires a faster turn-around time for product release than their standard method allows, when there is a sudden issue with the performance of a rapid test kit, or if test kits from the manufacturer are back-ordered. Do these situations constitute an emergency? Arguably, this upset in product distribution could constitute an emergency in terms of the supply chain which is already under stress due to pandemic disruptions – with real effects on consumer well-being and the economy.

This roundtable will consider the key components required for a matrix validation and acceptable strategies for matrix validation that can be used in emergency situations. What approaches will allow expediency while assuring that the method is fit for purpose?

RT20 FDA's New Era of Smarter Food Safety: One Year after the "Blueprint" Release, How is the Industry Embracing This Change?

MONISHA P CHAKRABORTY: *Taylor Farms Pacific, Tracy, CA, USA*
ANDREW KENNEDY: *USFDA, Silver Spring, MD, USA*
DERRICK BAUTISTA: *Del Monte Foods, Inc., Walnut Creek, CA, USA*
PAMELA WILGER: *Cargill, Inc., Wayzata, MN, USA*
AARON ASMUS: *Hormel Foods, Austin, MN, USA*
NATHAN ANDERSON: *U.S. Food and Drug Administration, Bedford Park, IL, USA*

The ultimate goal of the *Blueprint* is to use enhanced traceability and transparency to reduce the number of foodborne illnesses in the U.S. This new approach to food safety will leverage technology and tools to create a safer digital traceable food system. There are four foundational pillars that cover a range of technologies, analytics, business models, modernization, and values.

Digitalization is a critical step in improving traceability, food safety, and quality programs which will decrease the room for error. In July 2020, FDA released the *Smarter Food Safety ERA Blueprint*. FDA intends to help the food system to speak the same traceability language through the use of critical tracking events and key data elements, and encourage interoperable, low-cost solutions.

In July 2021, a year after the release of the *Blueprint*, many companies will be well-poised to adopt at least a few modules of digitalization, if not more, and companies will also be test driving the digitalization aspect. The goal will be to understand how companies are meeting regulatory compliance, adopting digitalization of one or more modules, including food safety, quality procedures, environmental monitoring, sanitation, recall, raw material, supplier management, kill step controls, and verification systems in terms of speed, efficiency, and compliance.

Discussions will revolve around the state of digitization efforts at food companies, components of the era people believe in the most, what makes the most sense, what will be harder to achieve, what tools there are, what tools are missing, how are the gaps being addressed, how much progress has been made by the industry and the agency, and what are the collaborative efforts.

Essentially how is the food industry embracing digitalization efforts? How are the regulatory agencies and auditing bodies viewing this, and how are they supporting this technology-based approach?

RT21 Riding the Tide of Multi-Disciplinary Approaches to Evaluate Behavior-Change Effectiveness of Food Safety Education

LAWRENCE GOODRIDGE: *University of Guelph, Guelph, ON, Canada, Canada*
JOHN BOYCE: *J.M. Boyce Consulting, LLC, Middletown, CT, USA*
AMY REIBMAN: *Purdue University, West Lafayette, IN, USA*
AARON LAVALLEE: *USDA FSIS OPACE, Washington, DC, USA*
YAOHUA (BETTY) FENG: *Purdue University, West Lafayette, IN, USA*

Improper food-handling practices continue to be one of the most significant factors contributing to foodborne illnesses. Therefore, food safety education and training are emphasized among both commercial and residential food handlers. Program evaluation is the key to document the impact and assess the effectiveness. Most of those evaluation measurements collect data from self-reported surveys. Previous studies reported discrepancies between self-reported and actual behaviors. Thus, it has been challenging to document the behavior-change effectiveness of food safety education without efficient measurements. This roundtable invited a multi-disciplinary panel to showcase novel approaches to evaluate behavior-change effectiveness of food safety education, to discuss the challenges and opportunities of observational approaches, and to shed light on future extension and training program evaluation.

The first speaker, Dr. Betty Feng, will share her experience with observational studies in food safety program evaluation and present the challenges of in-person and videotaped observation data analysis. The second speaker, Dr. Amy Reibman, will follow up with a video analytic approach to analyze videotaped observational data of handling washing. The third presentation by Mr. Aaron Lavallee will share USDA FSIS's unique collaborations on exploring the use of observation and microbial analysis to evaluate consumers' meat and poultry handling practices. The fourth speaker, Dr. John Joyce, will showcase a novel combination of electronic monitoring and observation to improve hand-washing among food handlers. Last but not least, Dr. Larry Goodridge will talk about a highly integrated project combining pathogen surveillance of wastewater with social media conversation analysis to look for early warning signals of outbreaks.

This session will be convened by Dr. Christine Bruhn, an advocate for food safety program evaluation and a renowned consumer behavior researcher, and a student representative, Minh Duong, from IAFP's Student PDG. At the end of the presentations, the convenors will facilitate a panel discussion to brainstorm ideas on how to ride the tide of multi-disciplinary food safety program evaluation for future food safety professionals.

RT22 Collaboration in the Desert - A Research Model for Advancing Fresh Produce Safety

JOHN BOELTS: *Desert Premium, Yuma, AZ, USA*
CHANNAH ROCK: *University of Arizona, Maricopa, AZ, USA*
ELSTON GRUBAUGH: *Wellton Mohawk Irrigation and Drainage District, Wellton, AZ, USA*
JULIE ANN KASE: *U.S. Food and Drug Administration, College Park, MD, USA*
TERESSA LOPEZ: *Arizona LGMA, Phoenix, AZ, USA*

Recent outbreaks in fresh produce coupled with heightened media coverage have elevated produce safety into the forefront of public attention. More specifically, the 2018 outbreak involving romaine lettuce grown in the Yuma region – a major growing region for leafy greens sold in the United States, was linked to Escherichia coli O157:H7. Yet the origin, environmental distribution, and potential reservoir(s) of the outbreak strain remains unknown. In response to the outbreak, the California and Arizona Leafy Greens Marketing agreements adopted revised metrics in an effort to address the possible role of atmospheric deposition and water quality on crop contamination. Exacerbating these concerns, findings included in the FDA Environmental Assessment (FDA 2018), have left industry with many unresolved questions as to how best to improve their food safety standards and practices in the desert growing region. In an effort to support fact-finding and ultimately public health protection, the FDA, Wellton-Mohawk Irrigation and Drainage District, University of Arizona Cooperative Extension, and local Arizona fresh produce industry came together to facilitate a collaborative research effort in 2019. The objective of this collaboration is to capitalize on the unique opportunity to evaluate real-world conditions within close proximity to potentially implicated fields from the 2018 outbreak. This venture has allowed the research and extension team to generate new knowledge that is useful to industry in order to bridge the gap created by recent outbreaks. With input from both Academic and Industry Advisory boards, the team focuses on areas of irrigation water, soil/sediment, atmospheric conditions, and animal impacts on the presence and persistence

of microbial contamination in the environment, as well as protocols appropriate for the detection of indicators/pathogens in commercial-scale agriculture. The goal of this roundtable is to share experiences of the FDA, LGMA, UA Cooperative Extension, and local industry on the successes and challenges of collaborative research with regulatory agencies and recommendations for other regions considering similar efforts to enhance food safety.

RT23 Novel Foods, Novel Challenges: Food Safety Concerns in Plant-Based, Cell-Cultured, and "Clean Label" Products

LILIA M. SANTIAGO-CONNOLLY: *Kellogg Company, Battle Creek, MI, USA*
DAVID RASMUSSEN: *KraftHeinz, Chicago, IL, USA*
JOCELYN ALFIERI: *Mérieux NutriSciences, Markham, ON, Canada, Canada*
DEANN AKINS-LEWENTHAL: *Conagra Brands, Omaha, NE, USA*
TRACIE SHEEHAN: *Mérieux NutriSciences, Chicago, IL, USA*

Booming demand for plant-based, cell-cultured, and "clean" foods is driving product development in our industry. At the same time, this increased focus on novel foods introduces novel challenges for food safety. Accordingly, this roundtable session will explore four facets of food safety pertaining to novel foods:

1. **HAACP Concerns**: How do novel products affect food safety plans? What are the unique challenges using crops, cell-cultured animal proteins, and "clean" inputs for illness and other hazards? In the absence of historical data, where do we get justification for plant-based food safety regarding procedures such as kill steps? Can we use the same validation data from non-plant studies?
2. **Food Safety in Plant-Based Foods**: Food safety programs must maintain rigorous standards for microbial threats such as *E. coli* that affect both plant- and animal-based products. At the same time, we must also account for threats and challenges associated with specific agricultural crops (e.g., mycotoxins and allergens).
3. **Food Safety in Cell-Cultured Animal Protein:** Similarly, cell-cultured animal protein is gaining momentum in the effort to develop sustainability and conscientious solutions for feeding the world. When Singapore's Food Agency recently approved cell-cultured chicken, it marked a global first that many countries will likely follow. But again, what unique food safety concerns apply to this nascent food science? How can we best develop a suitable scope?
4. **"Clean Label"… What's Missing?:** What is the impact of "clean label" formulations on food safety in general? Does formulating "clean" products mean removing steps or inputs that were originally included to bolster safety and mitigate risk?
5. **Regulation Propagation**: How might regulations increase, and what are the regulatory implications? How might current consumer and regulatory trends impact plant-based and "clean" foods? Particular emphasis will be on GMOs, allergens, and labeling.

Note on Format: Per Program Committee recommendations, please change to a symposium with two shared presentations followed by a 30-minute panel.

We have verbal commitments from Fortune 100 and Fortune 300 companies.

RT24 Operational Choices and Risk-based Decision Making Around Clean Breaks in Dry Environments

BENJAMIN WARREN: *Land O' Lakes, Arden Hills, MN, USA*
YVONNE MASTERS: *John B. Sanfilippo & Son, Inc., Chicago, IL, USA*
EDITH WILKIN: *Leprino Foods Co, Denver, CO, USA*
ELIZABETH GRASSO-KELLEY: *Illinois Institute of Technology, Department of Food Science and Nutrition / Institute for Food Safety and Health, Bedford Park, IL, USA*
NATHAN ANDERSON: *U.S. Food and Drug Administration, Bedford Park, IL, USA*
LESLIE HINTZ: *U.S. Food and Drug Administration, College Park, MD, USA*

Controlling environmental cross-contamination during food manufacturing is increasingly recognized as a significant factor in ensuring food safety. Food manufacturers rely on self-determined sanitation "clean breaks" between processing runs in the establishment of lot sizes for the purpose of traceability and limiting recalls. Sanitation clean breaks represent documented activities that separate the last time food contact surfaces or other nearby surfaces from which drainage or transfer onto food contact surfaces could occur during the normal course of operations were fully cleaned and sanitized. Sanitation clean breaks are commonly implemented after a pre-determined duration of production. Conceptually, application of sanitation clean breaks implies that contaminating pathogens on environmental surfaces have been eliminated through cleaning and sanitization, and therefore, no longer present a food safety risk to subsequent lots processed on the same equipment. However, this approach may be overly reductive and could be taken to imply zero risks following designated sanitation processes. These processes are inherently difficult to validate and effectively monitor without focused due diligence. The concept of sanitation clean breaks is further complicated in dry food manufacturing environments where conventional wet methods of sanitation (water spray, aqueous sanitizer application) are not used. In this roundtable, different approaches to implementing and evaluating sanitation clean breaks in dry food processing environments will be discussed. The issue of operational choices and their risk-based tradeoffs will be discussed in depth.

RT25 Food Irradiation: Where We've Been, Where We are Now, and What's Next

KATHLEEN O'DONNELL: *Wegmans Food Markets, Inc., Rochester, NY, USA*
BARBARA KOWALCYK: *The Ohio State University, Columbus, OH, USA*
UDIT MINOCHA: *USDA FSIS, Atlanta, GA, USA*
LANE HIGHBARGER: *U.S. Food and Drug Administration, College Park, MD, USA*
MISHA ROBYN: *Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA*

The incidence of most infections transmitted commonly through food has not declined for many years. Interventions targeting various points along the entire farm-to-fork spectrum should be considered to improve food safety and prevent illness. Irradiation is an effective food safety intervention, resulting in a 99%–99.99% reduction in pathogens. National and international health and regulatory organizations approve of irradiation, including the Centers for Disease Control and Prevention, the Food and Drug Administration, the U.S. Department of Agriculture, and the World Health Organization. Despite a large body of scientific evidence demonstrating safety and benefits, irradiation is largely underutilized for food safety. Consumer acceptability of irradiation as a food safety intervention and cost have been cited as notable barriers to its implementation. In the past decade, there have been multiple factors that may have influenced consumer acceptability toward irradiation, including several recent and historically large outbreaks of enteric illnesses linked to foods that could be treated with irradiation to reduce pathogens (e.g., *Salmonella* Newport outbreaks linked to ground beef). In addition, irradiation was discussed as a possible intervention during the AFDO Healthy People 2030 meeting and has been discussed in the 2020 USDA FSIS Roadmap to Reducing *Salmonella* Plan.

For this roundtable session, we will explore the effectiveness of irradiation on microorganisms and their different life stages (both vegetative and spore forms); the current use of irradiation within the industry for foods like spices, produce, and meat and poultry; the barriers to using irradiation for food safety; consumer opinions of irradiation, including recent focus groups conducted by CDC; the role of the FDA regarding approval of the use of food irradiation; regulations around irradiation; USDA APHIS' and FSIS' roles in regulating irradiation; and status of phytosanitary treatment by irradiation.

RT26 A Support Group for Difficult Matrices - You're Not Alone with Your Detection and Confirmation Problems

CÉCILE VADIER: *Barry-Callebaut France, Louviers, France, France*
ANDREA CIPRIANI: *Mérieux NutriSciences, Chicago, IL, USA*
DANIEL SMIESZEK: *Nestlé, Dublin, OH, USA*
BRADLEY ZIEBELL: *Conagra Brands, Omaha, NE, USA*
PAMELA WILGER: *Cargill, Inc., Wayzata, MN, USA*

Several low-water activity foods present unique challenges when it comes to accurate microbial detection. These challenges are caused by matrix inhibition (antioxidants and antimicrobials), heterogeneity, or sub-lethal injury due to processing. For example, some common spices possess antimicrobial properties, cocoa and chocolate contain antibacterial phytochemicals, and infant formulas undergo processing that makes it difficult to recover injured cells. Panelists in this roundtable session are subject matter experts in their respective areas and will provide guidance regarding challenges and identified approaches to dealing with intrinsically challenging matrices, including and beyond the given examples. The aim of this session is to share coping strategies to elevate the understanding for the entire supply chain, identify opportunities where different industries can learn from each other, and promote continual improvement in the areas of rapid detection and confirmation strategies.

RT27 Ever Thought of Being an Expert Witness?

LEE-ANN JAYKUS: *North Carolina State University, Department of Food, Bioprocessing and Nutrition Sciences, Raleigh, NC, USA*
DAVID SHARFSTEIN: *Hogan Lovells, Washington, DC, USA*
DAVID ACHESON: *The Acheson Group, Bigfork, MT, USA*
ROBERT BUCHANAN: *University of Maryland-College Park, College Park, MD, USA*
CHRISTOPHER GRIFFITH: *Professor Emeritus, Cardiff Metropolitan University, Cardiff, UK, Dorchester, United Kingdom, United Kingdom*
EWEN TODD: *Ewen Todd Consulting, Okemos, MI, USA*

This RT explores how lawyers and food safety experts intersect to demonstrate how science can clarify a lawsuit in favor of either a plaintiff or defendant. Although the expert is hired by a lawyer to support his/her case, the science has to be unbiased, based on peer-reviewed literature and the scientist's credibility and experience. Without the last two qualifications, a judge may throw out an expert's testimony and for not meeting the Daubert standard, which will be discussed at the RT. Although you as an expert are paid by one particular firm to produce the report, you are in reality working for the court, and your job is to analyze and interpret the various forms of evidence. Of course, experts can disagree on how they have interpreted the evidence, and careful preparation is required to support your case which is likely to be challenged by the opposing legal team and their expert witness.

The panel will present an international perspective and consists of food safety experts with many years of food safety experience. The panel will share how they prepare for what is asked of them and what advice they have given to lawyers who are not familiar with foodborne illness or food contamination. Experts will need to have a breadth of food safety skills and expertise relevant to an individual case and be able to give opinions on different types of cases where the evidence can be strong or weak, depending upon the contaminated food, the pathogen, the implicated establishment, its staff, and its inspection history. General outbreaks of foodborne disease acquired from different foods and premises and by different pathogens will be compared to the types of evidence required for so-called cases of travelers' diarrhea or individual cases. Sometimes, it is better to advise a lawyer and client not to proceed as the data to support their case is insufficient to meet their legal requirements. Cases vary in their degree of severity, both in terms of the number of people affected and the nature of any longer-term sequelae. This is likely to be linked to the amount of compensation or criminal sanctions requested. Unfortunately, case reports are rarely available in the published literature, although they can be used to identify failures in food safety management and food safety culture. This session should prove fascinating for the IAFP Membership and guests, especially with cautionary tales for those actually producing, serving, and selling food.

RT28 Fact or Fiction? How to Evaluate Antimicrobial Products for Your Sanitation Program

TAJAH BLACKBURN: *Environmental Protection Agency, District of Columbia, DC, USA*
ANGELA FRASER: *Clemson University, Clemson, SC, USA*
JEFF EDELEN: *Whole Foods Market, Austin, TX, USA*
ROSIMEIRE MIRANDA: *LSG Sky Chefs, Irving, TX, USA*
DAVID BUCKLEY: *Diversey, Charlotte, NC, USA*

SARS-CoV-2 has brought heightened hygiene awareness to the food industry. Although concerns over a specific virus will wane over time, we can hope that the increased focus on hygiene programs will endure. As a result, many food operations have been reevaluating their hygiene program. But reevaluation is a continuous cycle, not a one-time task. There are countless technologies, equipment, operations, and products that continually change and each of these changes can require reevaluation of hygiene programs. Food operators often have to evaluate a wide variety of hygiene options before selecting an appropriate program. The SARS-CoV-2 outbreak has clearly demonstrated how difficult such evaluations can be. Food operators have seen a variety of novel approaches or technologies to improve hygiene, such as electrostatic sprayers, UV-C light, steam cleaners, and nanotechnologies. But how do we cut through marketing claims and evaluate these new technologies alongside traditional products to determine the best hygiene options to address changing concerns and technology? How do industry professionals take into account the claims made by manufacturers of specific antimicrobial compounds? How do we determine the value of new technology that is crossing our desks on a regular basis and differentiate between fact and fiction among the plethora of options? This panel will discuss strategies that the food industry can use for evaluating microbial control systems. The panel will address ways that food operators can determine the safety, cost, perception, efficacy, and acceptance of these products. The panel will be comprised of experts from multiple sectors who will share their perspectives, pain points, and success stories on these topics and on how to appropriately evaluate and integrate antimicrobial systems in a constantly changing post-pandemic environment. Roundtable attendees will leave with a better understanding of how to assess microbial control programs, improve their own sanitation processes, and be better prepared to respond to technological changes in sanitation technology when they come.

RT29 Can You Trust Third-Party Certification?

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MITCH GILGOUR: *Sysco, Houston, TX, USA*
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It would be fair to say that the world of food safety certification has faced one of its biggest challenges over the past months, as the COVID-19 pandemic has led to sudden, significant disruptions in the traditional audit processes. Many certified sites faced the prospect of certificates lapsing as the pandemic spread globally.

However, out of every challenge comes opportunity and many believed that the pandemic served to simply highlight many significant and increasing threats third-party food safety certification has been facing for awhile: increasing difficulties in attracting and retaining auditors; the audit process keeping up to date with technological evolution; the slow pace of change in standards at odds with the needs of the food industry; and maintaining trust from the food industry itself in certification as an effective tool to manage risk in ever more complex supply chains.

As a benchmarking organization for food safety Certification Programmes (also commonly known as schemes), the Global Food Safety Initiative (GFSI) has found itself in the eye of this storm. In this session, we will share some of our experience and learnings as well as some of the steps that GFSI has taken to support the industry through this crisis and, most importantly, address sustainably the question of trust in third-party certification. Our panel will debate whether this is enough and what the true industry concerns are with third-party certification.

RT30 Microbial Resistance - Is It Related to Sanitation?

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There has been considerable debate and a large amount of opinion expressed regarding the impact of sanitation on microbial resistance or tolerance. However, there is little consensus of the nature and severity of this issue, its causes, and what responses, if any, are required to address it. In large part, this is because there is a paucity of data on the relationship between tolerance and sanitizer use. This is further complicated because tolerance to sanitizers is often conflated with resistance to antibiotics. But in many ways, sanitizer tolerance is even more complex than antibiotic resistance because sanitizer tolerance involves not just intrinsic tolerance and genotypically acquired tolerance, but phenotypically acquired tolerance as well. Add in frequent misuse of sanitizers that range from "shock" treatments at far higher levels than are recommended to use of sanitizers sub-lethal levels and this subject becomes even more complex. The roundtable will bring together experts from academia, industry, and government to discuss the latest data on resistance. They will try to make sense of the data and what that data means for food operators. Panelists will also discuss research gaps and even indulge in some informed opinions of their own on what food operators should do to address tolerance.

Technical Abstracts

T1-01 Precise Detection of Human Norovirus Based on Magnetic Separation and RT-qPCR

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Introduction: Human Norovirus (HuNoVs) is a leading cause of foodborne illness in the world. It's a challenging work to precisely detect HuNoVs in fruits and vegetables owing that the viruses were hard to be collected in these matrices.

Purpose: The aim of this study was to realize precise detection of HuNoVs in strawberries.

Methods: In this study, we prepared porcine gastric mucin (PGM)-labeled magnetic nanoparticles synthesized by ourselves for capturing HuNoVs. Then, we optimized the capture conditions for capturing HuNoVs and screen the elution buffer. Fifty microliters of buffer were used to elute viral particles from 25 g strawberries, then 50 μg magnetic probes was used to collect viral particles from the elution buffer. After collecting and heating the complex of magnetic probes and HuNovs, the supernatant solution was used as RNA template to perform RT-qPCR.

Results: For the magnetic separation for HuNoVs, the optimized buffer was Tris·HCl (pH9.5), which was also optimized buffer for eluting viruses from strawberries. This buffer also made the result of RT-qPCR, in which the heated solution containing magnetically captured HuNoVs was used as RNA template, more sensitive. Comparing with the traditional method, the sensitivity of our strategy was 3 orders of magnitudes higher in the artificially contaminated strawberries.

Significance: In short, our strategy has great potential for the precise detection of HuNoV in strawberries, which may provide ideas and reference for the development of detection methods for other fruits and vegetables.

T1-02 Replication of Infectious Human Norovirus Recovered from Common High-Touch Surfaces

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❖ ***Developing Scientist Entrant***

Introduction: Contamination of fomites by human norovirus (HuNoV) can initiate and prolong outbreaks in food handling environments. Fomite swabbing is necessary to predict HuNoV exposure and target interventions. Historically, swab-recovered HuNoV is measured by molecular methods that detect RNA but not infectious HuNoV. The recent development of HuNoV cultivation in human intestinal enteroids (HIEs) now enables detection of infectious HuNoV. It is unknown if the swabbing process and swab matrix will allow for cultivation of fomite-recovered HuNoV.

Purpose: We used the HIE HuNoV culture system to evaluate infectivity of HuNoV recovered via swabs from experimentally infected surfaces.

Methods: Three surfaces - a countertop (N = 32), door handle (N = 10), and sanitizer dispenser (N = 11) - were experimentally inoculated with HuNoV-positive stool suspension. Surfaces were swabbed with macrofoam swabs premoistened in PBS plus 0.02% Tween80. Swab eluate was tested for infectious HuNoV by cultivation in HIE monolayers.

Results: Infectious HuNoV can be recovered from surfaces inoculated with at least 10^5 HuNoV genome equivalents/3 cm^2. In total, 57% (N = 53) of recovered swabs contained infectious HuNoV detected by HIEs. No difference in percent positive swabs was observed between the three surfaces at P = 0.2.

Significance: We demonstrate that fomite swabbing can be combined with the HIE method to cultivate infectious HuNoV from the environment filling a significant gap in HuNoV detection. Identification of infectious HuNoVs from swabs can increase monitoring accuracy, enhance risk estimates, and help prevent outbreaks. Currently, high titers of HuNoV are required to measure HuNoV growth in HIEs and the HIE system precludes absolute quantification of infectious viruses. However, the HIE system is capable of providing a binary indication of infectious HuNoV which enhances existing detection methods.

T1-03 Persistence of Inactivated Hepatitis A Virus RNA in Water, on Food Contact Surfaces and on Blueberries

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❖ ***Developing Scientist Entrant***

Introduction: Enteric viruses, such as human norovirus and hepatitis A virus (HAV), are the leading cause of transmissible foodborne illness. The gold-standard method for virus detection throughout the food chain is RT-qPCR, which detects portions of genomes including non-infectious viral particles and naked viral RNA.

Purpose: The aim of this study was to evaluate the persistence of heat-inactivated HAV in water, in phosphate-buffered saline, on stainless steel, on polyvinyl chloride and on blueberries at different temperatures.

Methods: Inactivated HAV (2.5×10^2, 2.5×10^4 and 2.5×10^6 genome copies) was placed in microtubes containing molecular biology grade water (100 μL) or phosphate-buffered saline (100 μL), as well as on stainless steel and polyvinyl chloride coupons (1 cm^2), and on blueberries. Persistence of inactivated HAV RNA was evaluated by RT-qPCR up to 90 days under various temperatures (-80°C, -20°C, 4°C and 23°C) and incubation times. All experiments were replicated three times.

Results: In water and phosphate-buffered saline, large-level and medium-level load of inactivated HAV could be detected up to 90 days regardless of storage temperature. However, detectable RNA dropped significantly ($P < 0.05$, Tukey's multiple comparison test) in water at large-level load after only 24 h at 23°C compared to -20°C or -80°C. On polyvinyl chloride and blueberries, inactivated HAV was detectable under most conditions. On stainless steel, the large initial load persisted for 90 days while the medium-level load was detected only up to 16 days at 23°C or 60 days at 4°C.

Significance: The persistence of viral RNA from non-infectious virions should be a main issue when developing detection methods for enteric viruses in food matrices and surfaces. Pretreatments that discriminate between naked RNA, non-infectious virions and infectious virions need to be included prior to RT-qPCR in order to reduce the risk of positive results associated to non-infectious viral particles.

T1-04 Applicability of Pulsed Light Technology to Inactivate Foodborne Viruses and Impact on Sensory Properties of Berries

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Introduction: The global burden associated with viral foodborne diseases remains a major challenge. While several inactivation strategies are deployed in food industries, many of them are not well suited for several food matrices like delicate berries. Pulsed light is a green technology based on the emission of high-intensity light pulses that have been shown to inactivate several microorganisms.

Purpose: The goal of this study was to evaluate the potential of pulsed light technology to inactivate foodborne viruses and understand its impact on sensory properties on berries.

Methods: Sensory properties, such as color, texture (Kramer shear cell) and weight were assessed on blueberries, strawberries, raspberries, blackberries, and lettuce before and after the pulsed light inactivation treatment. The maximum fluence used was 11.45 J/cm^2. Hepatitis A virus (HAV) and murine

norovirus 1 (MNV-1) were used for experimental contamination of strawberries, raspberries, and blackberries at a concentration of $1x10^5$ PFU/mL followed by the pulsed light inactivation treatment. Viral infectivity was assessed using viral plaque assay.

Results: Our result shows that pulsed light does not affect the texture and weight of tested berries and lettuce. However, pulsed light treated blueberries (35.42 ± 1.52) and lettuce (55.82 ± 7.31) showed a significant reduction ($P < 0.0002$, ANOVA) in luminosity (darkening of the matrix) compared to untreated. Pulsed light was able to reduce the HAV and MNV-1 viral charge on strawberries (2.10 ± 0.08 log; 1.61 ± 0.38), raspberries (1.97 ± 0.16 log; 1.89 ± 0.30) and blackberries (1.25 ± 0.57 log; 1.37 ± 0.62), respectively.

Significance: These results will help food industries to better understand the potential, the strengths, and limits of pulsed light technology for viral inactivation. Most food industries already have automated technologies, therefore implanting the pulsed light technology should be easily achieved.

T1-05 Fucose and Fucosidase: The Seemingly Contradictive Pair Can Both be Used to Fight Against Norovirus

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Introduction: Human noroviruses (NoVs) have specific interactions with human histo-blood group antigens (HBGAs)-like carbohydrates. The fucose contents are known to play a key role in the binding of NoVs to HBGAs.

Purpose: This study explored the possibilities of using fucose and fucosidase to fight against human NoVs.

Methods: For fucose, the anti-NoV potentials of fucoidan and 2'-Fucosyllactose (2'-FL) were investigated with the use of a zebrafish larvae *in vivo* platform. For fucosidase, we studied the influence of fucosidase-producing bifidobacteria on the HBGA antigenicity of oyster digestive tissue and the associated NoV binding.

Results: For fucose, although both fucoidan and 2'-FL were able to block NoV GII.4 virus-like particle (VLPs) from binding to type A saliva as expected, only fucoidan but not 2'-FL was able to inhibit the replication of NoV GII.P16-GII.4 in zebrafish larvae. For fucosidase, on the contrary to the expected, the binding of NoV GII.4 to *Bifidobacterium bifidum* strain JCM1254 treated oyster digestive tissue was enhanced in both *in vitro* and *in vivo* test. Correspondingly, the type A antigenicity of the oyster digestive tissue extracts was also enhanced after the treatment with *B. bifidum* JCM1254. Further study from our group has isolated bacteria strains decreasing type A antigenicity of oyster extract and, thus, could potentially be used to mitigate the NoV contamination from shellfish.

Significance: With the use of the well-recognized interactions between NoV and fucose-containing carbohydrates, this study explored novel strategies to fight against human NoVs from different perspectives and application scenarios. Specifically, we expect the fucoidan to be developed as an anti-viral candidate for vulnerable populations to prevent NoV infection. Fucosidase-producing bacteria, on the other hand, are potentially able to be used to improve the depuration of NoV from shellfish products.

T1-06 Adaptation of Traditional Foodborne Virus Methodologies for Wastewater-based Epidemiology during the COVID-19 Pandemic

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❖ ***Developing Scientist Entrant***

Introduction: Viruses such as pepper mild mottle virus (PMMoV) have been used as fecal contamination indicators in agricultural waters. Though traditionally used in food safety research, PMMoV is now being employed for wastewater surveillance efforts during the COVID-19 pandemic. The physicochemical and microbiological variability of wastewater creates challenges in adapting methodology for use with enveloped viruses (SARS-CoV-2).

Purpose: The purpose of this study was to adapt methods commonly employed for foodborne viruses for recovery of SARS-CoV-2 from wastewater and to evaluate PMMoV as an indicator and normalization tool.

Methods: Composited wastewater was collected from six locations in New Castle County, Delaware. Samples were incubated, filtered, and concentrated via centrifugal ultrafiltration in triplicate. Viral concentrates were extracted, and detection performed via real-time quantitative PCR (qPCR) for PMMoV and the N1 and N2 SARS-CoV-2 targets. Data analyses were performed for calculated genomic copies and cycle threshold values (CT) using JMP15 statistical software.

Results: PMMoV was detected in all sample replicates ($n = 72$) with an average concentration of 5.25 log (copies/mL). SARS-CoV-2 N1 and N2 were detected in all samples, with an observed 8.3% and 19.4% positivity in unprocessed, and 34.7% and 41.7% positivity in processed replicates. SARS-CoV-2 N1 and N2 averaged 1.79 and 1.77 log (copies/mL) after processing. CT values significantly ($P < 0.05$) decreased by 0.77 cycles for N1, 0.63 cycles for N2, and 3.16 cycles for PMMoV. The variation in detection and recovery efficacy between viral targets suggests distinctive processing methods are needed to generate representative data.

Significance: These data provide evidence that with appropriate modifications, foodborne virus methods can be employed for SARS-CoV-2 surveillance and wastewater-based epidemiology. The data collected through the surveillance of wastewater has been used supplementally to inform public health decisions during the COVID-19 pandemic. The infrastructure that has been created, and research performed, could be utilized for tracking and preventing foodborne illnesses in the future.

T1-07 Assessment of the Infectivity of Coronavirus on Table Grapes during Storage and Following Sulfur Dioxide Fumigation

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Introduction: SARS-CoV-2 is not believed to be transmitted via food. However, the stability of coronaviruses on the surfaces of produce during post-harvest storage conditions is unknown.

Purpose: The overall goal of this project is to determine the efficacy of current SO_2 fumigation processes used on California table grapes to inactivate SARS-CoV-2.

Methods: Human coronavirus 229E was used as a surrogate to study SARS-CoV-2 survival. Grapes were transported to the laboratory immediately after harvest, un-fumigated. Ten µl of 229E suspension at a concentration of 10^5 PFU/mL was applied to the grape surface. Fumigation treatments of 100 ppm, 250 ppm, and 500 ppm were applied to inoculated grapes. Untreated and treated grapes were sampled on day 0 (immediately after inoculation and treatment), and days 1, 2, 3, 5, 7 and 14 after storage at 0.5℃ and 95% RH. To elute virus, grapes were transferred to a Whirlpak bag containing 5 mL of PBS and agitated for 1 minute. The level of infectious virus was determined using plaque assay and viral RNA presence was determined by RT-PCR.

Results: The recovery rate when applying 10 µL of 229E (3.67 log PFU/g) was 2.19 ± 0.18 log PFU/g. Next, we determined the level of infectious 229E recovered from untreated grapes during storage. On day 0, infectious virus was recovered with an average titer of 2.13 ± 0.18 log PFU/g. The level of infectious virus recovered from grapes declined over 4 days of storage to undetectable levels. No infectious virus was detected in samples receiving the lowest SO_2 treatment concentration (100 ppm). However, viral RNA was detected in both treated and untreated samples for 14 days.

Significance: Infectious virus is not detectable in grapes after three days of storage without SO_2 treatment and mild SO_2 treatment inactivates coronavirus on grapes.

T1-08 Study on Persistence and Survival SARS-CoV-2 in Various Foods

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Introduction: Recent reports of the detection of SARS-CoV-2 in foods such as salmon, shrimp, chicken, and ice cream are of serious concern during the COVID-19 pandemic. Studies have shown that the virus can survive for several days on various contact surfaces. Understanding the ability of SARS-CoV-2 to survive on different types of foods to assess the public health risk of contaminated foods is of critical importance.
Purpose: To study the survival of SARS-CoV-2 on different types of foods over time.
Methods: A broad range of food types including chicken, seafood, and produce were cut into 1.5 x 1.5 cm pieces and placed in 12-well tissue culture plates. Foods were inoculated with 20 µL of ~5 log plaque-forming units (PFU) of SARS-CoV-2 in droplets on food surfaces, followed by incubation at 4°C. Viruses were recovered immediately (0 h) to assess the maximum recoverable virus from each food and at predetermined time intervals (1 h, 24 h) by rinsing with 1 mL DMEM media. Samples were stored at -80°C until viral plaque assays were performed on VERO-E6 cells to enumerate the virus.
Results: At all three-time points, recovery of SARS-CoV-2 from chicken skin, salmon, shrimp, and spinach was similar, ranging from 3.4 to 4.3 log PFU/mL infectious virus ($P > 0.05$). However, initial (0 h) virus recovery from apple skin and mushroom was significantly lower, as compared to poultry and seafood ($P \leq 0.05$). Recovery of virus from apple skin decreased over time and was significantly less ($P \leq 0.05$) at 24 h compared to 0 h. The mushroom significantly reduced virus titer after 1 h (to 0.8 logs PFU/mL), and by 24 h infectious virus was undetectable.
Significance: The survival and high recovery of SARS-CoV-2 in certain food signify the importance of safe storage, handling practices, and the use of PPE while handling foods in the supply chains and grocery stores.

T1-09 Study on Survival of Herpes Simplex Virus (HSV-1) on Foods, a Method Development for SARS-CoV-2 Study

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Introduction: The emerging reports about the occurrence of SARS-CoV-2 in a multitude of food is alarming midst the COVID-19 pandemic. To better understand our approaches for SARS-CoV-2 study (a BSL-3 requirement) on foods, method development, and process validation were performed using another enveloped virus, HSV-1.
Purpose: To develop, optimize and validate methods for SARS-CoV-2 survival studies on foods, using HSV-1 at refrigerated conditions over time.
Methods: Food samples (chicken skin, chicken thigh, salmon, apple skin, and mushroom) were chopped in *ca.*1.5 x 1.5 cm size and placed in 12-well tissue culture plates and whirlpak® bags. Ten microliters of ~ 6 logs concentration of HSV-1 were spot inoculated on food surfaces and incubated at 4°C. Virus was recovered from food using a DMEM rinse and hand massage from wells and bags respectively at 0 h, 1 h, and 24 h followed by storage at -80°C. Virus plaque assay was performed on VERO-76 cell line to enumerate the virus.
Results: The viral recovery from the meat sources (chicken skin, thighs, and salmon) was not much different between rinsing and massaging techniques (6.4, 5.7, and 5.1 logs *vs* 6.2, 6.1, and 6.1 logs PFU, respectively, after 24 h). Upon rinsing after 24 h, the recovery from apple skin was 2.8 logs, whereas it was non-detectable in mushrooms. On messaging, the recovery from the apple skin and mushroom was 1.1 log each after 24 h. The result indicated that the initial recovery from apple skin and mushroom were higher (5.8 and 6 logs, respectively) in the rinsing method as compared to the massaging method (2.6 and 3.6 logs, respectively). This could be due to antiviral properties of the apple juice and mushroom extracted during massaging.
Significance: This study shows that the rinsing method is fairly effective when compared to massaging in recovering virus from the food surfaces. Therefore, a time-consuming and less safe massaging method could be avoided for the SARS-CoV-2 study.

T1-10 *Salmonella* Serotypes from FSIS Samples Linked to Outbreaks

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Introduction: The United States Department of Agriculture, Food Safety and Inspection Service (FSIS) samples meat and poultry products for *Salmonella* to verify process control and assist in the detection and investigation of human salmonellosis outbreaks that may be associated with FSIS-regulated products.
Purpose: To determine *Salmonella* serotypes distributions from FSIS-regulated chicken, turkey, beef, and pork samples and identify serotypes that are more likely linked to outbreaks.
Methods: This study compiled *Salmonella* isolates from FSIS samples collected and analyzed during 2009-2018 and identified top-10 serotype distributions by commodity; compared those top serotypes with the serotypes common in human illness outbreaks with confirmed linkage, defined by CDC, to FSIS-regulated products by commodity type.
Results: During 2009-2018, there were 30,781 *Salmonella* isolates recovered from FSIS samples; 17,909 (58.2%) from chicken, 2,416 (7.8%) from turkey, 8,157 (26.5%) from beef, and 2,299 (7.5%) from pork. During 2004-2020, 42 *Salmonella* outbreak investigations coordinated by FSIS and CDC were linked to FSIS-regulated products; 20 (47.6%) were chicken, 6 (14.3%) were turkey, 9 (21.4%) were beef, and 7 (16.7%) were pork. Each commodity exhibited distinct serotype distribution and serotypes linked to illness outbreaks. Enteritidis was the most common serotype for chicken-associated outbreaks and was the 2nd serotype from chicken samples; *Salmonella* Reading was the one of top serotypes from turkey samples and caused the largest turkey-associated outbreak in 2017-2019. Newport and Typhimurium were in top-10 serotypes from beef samples and the most common serotypes in beef-associated outbreaks; I 4,[5],12:i;- was the most common serotype from pork samples and pork-associated outbreaks. Typhimurium was in the top-10 serotypes from all commodities and was associated with outbreaks for each commodity.
Significance: Identifying *Salmonella* serotypes from FSIS samples that are commonly associated with illness outbreaks is key for foodborne illness surveillance and outbreak detection. The meat and poultry industry could use the information to develop novel strategies to reduce the prevalence of those serotypes and to prevent potential foodborne outbreaks.

T1-11 Transfer of Norovirus Surrogate Bacteriophage MS2 from Glass, Stainless Steel and Polypropylene Surfaces to Raspberry

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Introduction: Norovirus (NoV) outbreaks have been widely associated with frozen berries. During processing, NoV can be transferred from surfaces to fruit. Little is known about transfer percentage and further survival of NoV in fruit during frozen storage.
Purpose: To assess the transfer of NoV-surrogate bacteriophage MS2 from glass (GS), stainless steel (SS) and low-density polypropylene (PPS) surface to raspberry and its survival over frozen storage.
Methods: MS2 (9.9 log PFU/cm^2) was inoculated on surfaces (2×2×0.2 cm) previously autoclaved (121°C, 15 min). After drying (30 min), 5 g of fruit were placed on each surface. Transfer was evaluated after 10, 30 and 60 min and MS2 survival after 24 h, 15 and 30 days of frozen storage. MS2 was propagated using *Escherichia coli* C3000 as a host on tryptic soy agar (TSA) plates using the double agar overlay method. TSA plates were incubated overnight at 37°C and viral titer were enumerated. Experiments were performed in triplicate in three genuine replicates. Data were analyzed using ANOVA, followed by the Tukey or Student *t*-test ($P < 0.05$).

Results: The highest transfer of MS2 to raspberry was observed after 60 min-contact with GS (94%). Transfer of MS2 from SS increased with increase of contact-time reaching 90%. Around 85% of MS2 was transferred to raspberry after 10 min of contact with PPS; no changes occurred after 30 or 60 min of contact. A decrease of MS2 up to 2 log PFU/g was observed in raspberries after 30 days of storage.

Significance: Results show that NoV-surrogate MS2 can be easily transferred from surfaces commonly used in food industry to raspberry, suggesting the same behavior for NoV. The transferred viral titer may vary with the surface and contact-time. Findings warn the low reduction in raspberry viral titer transferred to raspberry during frozen storage.

T1-12 Comparative Analyses and Virulence Potential of Incompatibility Group FIB Plasmid Containing *Salmonella* Schwarzengrund Strains Isolated from Food and Clinical Sources

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Introduction: Incompatibility group (Inc) FIB plasmids can contain genes that contribute to antimicrobial resistance and increased virulence.

Purpose: The aim of this study was to determine the genetic relatedness and virulence potential of IncFIB containing *S*. Schwarzengrund isolates from food and clinical sources.

Methods: A total of 55 food and clinical *S*. Schwarzengrund isolates, among which 17 contained IncFIB plasmids were characterized. Food isolates were primarily collected from chicken while clinical isolates were from stool, urine, and blood. Whole genome sequencing (WGS) was performed on 25 clinical isolates using Illumina MiSeq and WGS data for the rest of the isolates were obtained from NCBI. Phylogenetic analyses were performed using single nucleotide polymorphism (SNP) and cgMLST. The virulome was analyzed by the NCTR-developed virulence database. Conjugation was carried out to determine the transferability of plasmids. Invasion and persistence assays were performed using human intestinal epithelial cells (Caco-2) at 1 h and 48 h post infections, respectively.

Results: SNP-based phylogenetic analyses showed that IncFIB-containing food and clinical *S*. Schwarzengrund isolates clustered within the same clade, which was separated from the isolates that lacked IncFIB plasmids. IncFIB plasmids from 9 food and 3 clinical isolates were successfully transferred in to *E. coli* J53 by conjugation. Food and clinical isolates had nearly similar virulome profiles. All food and clinical isolates examined were able to invade the Caco-2 cells. The invasion rate was higher than the persistence irrespective to plasmid contents and sources of the isolates.

Significance: Overall, IncFIB plasmids are self-conjugative and IncFIB plasmid-carrying *S*. Schwarzengrund core genomes of food and clinical isolates are genetically related, yet somewhat distinct from non-plasmid containing isolates. The study is important for better understanding the role *Salmonella* pathogenesis, hence significant for determination of microbiological hazards associated with food.

T2-01 Farm-to-Consumer Quantitative Risk Assessment Model for Listeria monocytogenes on Fresh-Cut Cantaloupe

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❖ ***Developing Scientist Entrant***

Introduction: Cantaloupe contamination with foodborne pathogen *Listeria monocytogenes* (LM) may occur at various points along the supply chain.

Purpose: The purpose of this study was to develop a quantitative microbial risk assessment (QMRA) model for LM on cantaloupe along the fresh-cut supply chain in order to identify risk reduction strategies.

Methods: A QMRA model was built for LM on fresh-cut cantaloupe, from farm-to-consumer. The preliminary model starts at point-of-harvest and includes the effect of conditions during transportation from the field to packinghouse and processing facility, storage at the processing facility pre-processing, fresh-cut preparation (LM reduction by chlorine washing, LM transfer from cantaloupe rind to flesh during cutting); as well as conditions during distribution, retail, transportation to home, and home storage. Model parameters were defined based on published literature or expert opinion. The model was simulated for 100,000 iterations using @Risk 8.1 software, providing an estimate of LM concentration in a single serving (134 g) of fresh-cut cantaloupe. Exponential dose-response model was applied to estimate the annual number of associated listeriosis cases in the United States.

Results: The model estimated a median of 1.31 log CFU of LM (5^{th}, 95^{th}, 99^{th} percentiles: 0.11, 5.21, 8.16) per contaminated serving of fresh-cut cantaloupe at consumption. The median (5^{th}, 95^{th}, 99^{th} percentiles) predicted number of illnesses annually attributed to fresh-cut cantaloupe was 0 (0, 1, 25), which agrees well with unreported sporadic cases and rare reported large outbreaks. Sensitivity analysis identified (i) initial concentration of LM on whole cantaloupes at point-of-harvest, (ii) distribution temperature, (iii) retail temperature, and (iv) home storage temperature had the greatest impacts on LM concentration per serving.

Significance: The preliminary QMRA model suggests reduction of LM risk to consumers requires development of a multipronged approach aimed at preventing or reducing LM contamination at multiple stages of the production chain.

T2-02 Farm-to-Fork Quantitative Microbial Risk Assessment Model for *Escherichia coli* O157:H7 on Fresh-Cut Lettuce

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Introduction: In the United States (US), a number of foodborne illnesses have been linked to lettuce contaminated with *Escherichia coli* O157:H7 (EC).

Purpose: The purpose of this study was to evaluate the impact of various contamination sources, interventions and parameters along the farm-to-fork continuum on the safety of fresh-cut lettuce.

Methods: A quantitative microbial risk assessment (QMRA) model was developed for EC contamination along the fresh-cut lettuce supply chain. The model included impacts of animal feces (from field application of manure, wildlife intrusion, and runoff from adjacent cattle farms) and irrigation on EC levels present on lettuce in the field at pre-harvest. From the harvest to consumer, the effects included were cross-contamination (due to harvesting blades and in the processing facility), washing in processing facility, conditions during retail storage, transport from retail to home, and storage by the consumer. The model was parametrized based on published literature and expert opinion. It was simulated for 50,000 iterations using @Risk 8.1 Software and EC concentration in a single serving of fresh-cut lettuce (100 grams) was estimated.

Results: The model estimated a median of 3 CFU EC present per contaminated serving of fresh-cut lettuce (5^{th}-95^{th} percentile: 1 – 162 CFU). However, the probability of a serving being contaminated was 1.4%. Estimated annual number of illnesses caused by contaminated lettuce (mean = 8,000) is comparable with the number of cases attributable to leafy vegetables in the US. Sensitivity analyses indicated retail storage temperature, home storage temperature and time, and chlorine washing in post-harvest had a strong effect on the estimated concentration of EC per serving, though parameters describing the pre-harvest and harvest stages were also influential.

Significance: The preliminary QMRA model suggests that effective reduction in EC contamination on fresh-cut lettuce can be achieved by simultaneous implementation of intervention strategies at multiple stages of the supply chain.

T2-03 Heat Transfer Analysis of Dry Roasting Peanuts to Achieve Food Safety Goals

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❖ ***Developing Scientist Entrant***

Introduction: Microbial inactivation in dry roasted low-moisture products is influenced by product temperature at the surface of these products because bacteria are located at the surface. There are no published models which show how surface temperature changes during a dynamic process such as dry roasting.

Purpose: This study quantifies the log reduction of *Enterococcus faecium* in peanuts under various roasting conditions.

Methods: A 10-cm peanuts layer was heated in a pilot-scale convection oven at 149°C for 20 minutes, then cooled. Peanuts with a surface embedded thermocouple were placed at the top, middle, and bottom of this layer. Airflow was initially downward and reversed after 10 minutes. Inactivation was modeled as a function of time and product temperature using an existing model for *E. faecium* inactivation in peanuts. A heat transfer model is used to elucidate the impact of different layer depths, air temperatures, and process times on microbial inactivation in peanuts.

Results: Modeled log reductions differed due to temperature differences in the layer, up to 52.4°C difference at the beginning of the process, becoming more homogenous with time. A 5-log reduction was achieved in top, middle, and bottom after 12.9, 16.6, and 17.7 min, respectively. When the hottest (top) layer achieved 5-log reductions, middle and bottom layers had only achieved 2.76- and 1.95-log reductions, respectively. Modeled inactivation ranged from 8.23- to 9.45-log reductions after cooling.

Significance: This study clarified the impact of layer depth on microbial inactivation in peanuts. This study also underscored the importance of understanding thermal variability in a nut roasting process to ensure nuts consistently meet required log reductions.

T2-04 Secondary Model for the Survival of *Salmonella* in Model Low Water Activity Matrix Based on Intrinsic and Extrinsic Factors

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❖ ***Developing Scientist Entrant***

Introduction: Dry foods are a potential vehicle for transmission of foodborne pathogens. A model incorporating extrinsic (environmental), intrinsic factors and inoculating conditions has not yet been created.

Purpose: This research evaluates and models survival of *Salmonella* in dry foods as influenced by storage temperature, product water activity, growing, and inoculating conditions.

Methods: Cocktails of 3 *Salmonella* strains were prepared using an overnight broth or lawn grown culture, and inoculated into milk powder using either a wet or dry method. Inoculated powder was placed in high (70% RH) or low (30% RH) humidity environment. Survival was measured over 112 days. Regression models were fit to each dataset, secondary models and summary statistics were created using R. A stepwise procedure was used to eliminate variables not shown to be significant.

Results: Survival was generally better at lower temperatures regardless of other conditions. Rates of decline ranged from -0.004 to -0.014 log CFU/day at 7°C across all conditions. As temperature increased, so did rates of decline, with a_w appearing to have a greater influence. Rates of decline ranged from -0.006 to -0.013 log CFU/day at 21°C and 0.3 a_w but increased to -0.013 to -0.042 log CFU/day at 0.7 a_w. Rates of decline were slower for all inoculum conditions at 37°C and 0.3 a_w compared to 21°C and 0.7 a_w, ranging from -0.013 to -0.030 log CFU/day. Organisms at 37°C and 0.7 a_w had fastest rates of decline of -0.103 to -0.122 log CFU/day. R^2 values for primary models ranged from 0.32-0.98 indicating potentially high variability. Secondary modeling of rates of decline after stepwise selection had an R^2 value of 0.88, indicating good fit. Temperature, water activity, and their interaction were statistically significant ($P < 0.05$) terms.

Significance: Survival of *Salmonella* is influenced by storage temperature, product water activity and interaction between these factors. Inoculating conditions are clearly important but did not rise to statistical significance ($P > 0.05$).

T2-05 Using Machine Learning to Predict Antimicrobial Resistance Non-Typhoidal *Salmonella enterica* from Poultry Products

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Introduction: Nontyphoidal *Salmonella* is the world's leading cause of foodborne bacterial morbidity and mortality. The role of animal food sources in the emergence and spread of both antimicrobial resistance (AMR) bacteria and their determinants of resistance to humans is currently poorly understood and controversial.

Purpose: The objective of this study was to use the machine learning (ML) algorithms to predict *Salmonella* isolates conferring antimicrobial resistance using *Salmonella* Whole Genome Sequences isolates collected from poultry products and human samples.

Methods: Ensemble machine learning models were used in this analysis. A total of 570 *Salmonella* isolates isolated from chicken breast ($n = 271$; susceptible = 95, resistant = 176), ground turkey ($n = 199$; susceptible = 43, resistant = 156), and human ($n = 100$; susceptible = 23, resistant = 77), and combined dataset (susceptible ($n = 317$) and AMR ($n = 253$)) from chicken breast, ground turkey, and human were selected for study and used to train our model.

Results: The support vector machine (SVM) (radial kernel) model was the best performing model for chicken breast, ground turkey, and human isolates with an average accuracy of 0.94 and random forest (RF) was the best performing model for the combined dataset with an average accuracy of 0.87. ML algorithm identified important antimicrobial genes associated with resistance to sulphonamide, tetracycline, streptomycin and spectinomycin, and beta-lactams.

Significance: Our results indicate a possible role of animal food sources in the emergence and spread of both AMR bacteria and their determinants of resistance to humans. Therefore, analyzing WGS using a machine learning approach has the potential to be a powerful tool for AMR surveillance programs.

T2-06 An Exploratory Quantitative Risk Assessment Model for *Salmonella enterica* by Chicken Consumption at Home in the Central Region of Mexico: Inclusion of the Pathogen's Genotypic and Phenotypic Print

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❖ ***Developing Scientist Entrant***

Introduction: Chicken is one of the products most associated with *Salmonella* in Mexico.

Purpose: To propose a QMRA for salmonellosis linked to the chicken consumption among the population of the central region of Mexico, including genotypic and phenotypic characteristics of *Salmonella* isolates.

Methods: Raw chicken samples (n = 304) were collected in the central region of Mexico to detect and quantify *Salmonella* in markets/street-markets/poulterer-shops (RP1) and supermarket/mini-super (RP2). Considering the presence of virulence genes and multidrug-resistance (MDR), isolates were classified into six groups: low-virulent/non-MDR, low-virulent/MDR, moderate-virulent/non-MDR, moderate-virulent/MDR, high-virulent/non-MDR, and high-virulent/MDR; the prevalence of each group was estimated. The exposure assessment at home was evaluated among 16 scenarios considering retail, transportation, storage, cooking, and/or cross-contamination. The predictive growth and inactivation models were obtained from literature, and consumer practices were surveyed. Published dose-response models for low, moderate, and high virulence were used. The probability of illness (P_{ill}) according to the virulence level, and the years of lost due to the disability (YLD) caused by *Salmonella* groups were estimated using @RISK.

Results: The overall prevalence of *Salmonella* in RP1 and RP2 was 21.3% and 25.5%, ranging from 0 to 16.6% among the six *Salmonella* groups. The pathogen concentration in RP1 (-0.497 ± 0.532 log MPN/g) was higher than RP2 (-0.806 ± 0.569 log MPN/g). The average P_{ill} among the scenarios ranged from $2.4x10^{-9}$ to $3.7x10^{-6}$, $7.4x10^{-8}$ to $1.2x10^{-4}$, $6.5x10^{-4}$ to $7.8x10^{-2}$ for low, moderate, and high virulent groups. The high-virulent/MDR group caused the majority of salmonellosis estimated cases (66.6%), despite their low prevalence at RP1 (0.5%) and at RP2 (5.0%). The YLD for MDR *Salmonella* was double than non-MDR.

Significance: The refinement of QMRA using genotypic and phenotypic *Salmonella* characteristics highlights the importance to focus on those groups that include high-virulent and MDR strains, which are not the most frequent but represent a high risk.

T2-07 Using Bayesian Statistics to Model the Growth of Shiga Toxin-producing *Escherichia coli* (STEC) in Raw Meat during Dynamic Chilling and Freezing Conditions

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Introduction: Existing models that predict bacterial growth in Quantitative Microbial Risk Assessment (QMRA) for food are deterministic and owing to inherent biological (stain, growth history) and experimental (temperature, pH) differences, do not account for the effect of uncertainty in their predictions.

Purpose: The development of a Bayesian model to describe the growth of Shiga toxin-producing *Escherichia coli* (STEC) in raw beef under dynamic post-slaughter chilling and freezing conditions.

Methods: One of four STEC strains (virulent *E. coli* O157:H7, O26, and O103) were separately inoculated onto the surface of portions of recent post-slaughter (within 1 h) beef, which were subsequently cooled (37 to 5°C) under conditions that simulated the temperature change associated with one of the three commercial processes: hot, warm, and cold boning. Over time, the number of bacteria present on the beef was assessed using standard diluting, plating, and incubation methods. The number of bacteria over time was fitted to primary and secondary models to obtain growth parameters, which were used as priors for the Hierarchical Bayesian Model. Computations and MCMC analysis were performed using the '*rjags*' package of R software. The model was validated using the remaining bacterial counts that were not used for model development.

Results: The number of *E. coli* O157:H7, O26, and O103 in beef increased from an initial inoculum of ~4 log CFU/g to ~10 log CFU/g within 10 h for hot, warm, and cold bone temperature profile, which emphasizes the significance of estimating the accurate numbers of STEC during processing. Also, there is a significant difference in growth rates between different serotypes. Given the complex and dynamic nature of the data, Bayesian inference is required for the accurate prediction of STEC growth during post-slaughter beef chilling and freezing conditions.

Significance: This work outlines the use of Bayesian inference in a practical food-science problem and illustrates its applicability to food microbiology and safety.

T2-08 Comparison between Lasso and Classification and Regression Tree for Predicting of *E. coli* Prevalence in Pasture Poultry Farms

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❖ ***Developing Scientist Entrant***

Introduction: Though most strains of *E. coli* are normal components of intestinal flora, certain pathogenic *E. coli* strains are the cause of diseases and outbreaks. Poultry is identified as a reservoir for pathogenic *E. coli*. It is important to identify farm practices factors associated with *E. coli* in pastured poultry environment.

Purpose: The objective of this study was to develop models that can predict *E. coli* counts and select farm practices factors contributing to *E. coli* counts in pastured poultry farms.

Methods: Fecal, soil, whole carcass rinse after processing, final product after chilling and storage, and ceca samples (a total of 2,295 samples) were collected for *E. coli* counts from 11 pastured poultry farms. Classification and regression tree (CART) and lasso method were developed for each sample type. The farm management practices and processing factors such as source of eggs, type of feed used, appearance of other animals on farm, chilling method, and storage time and temperature were used. Models were developed to predict the counts of *E. coli* and select the most important features used in predicting *E. coli*. Model performance were compared using prediction error.

Results: For fecal samples, whether cattle were present on farm, flock size, and type of animal source were the top three important variables affecting *E. coli* counts by lasso method. The CART method selected flock size, type of animal source, brood cleaning frequency, pasture time and age of flock at time of sampling as the most important rules in predicting *E. coli* counts. The prediction error of lasso method was 1.32 where CART method was 0.84. CART method performed better than lasso method in predicting *E. coli* counts.

Significance: The predictive models will provide practical and effective tool to predict *E. coli* counts and identify farm practices factors that affect *E. coli* counts.

T2-09 Development of a Predictive Modeling Approach to Evaluate Food Safety Compliance

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Introduction: Global retail chains and restaurants have challenges to evaluate and enhance the compliance with food safety proper practices in stores/restaurants located in different regions.
Purpose: This study investigated the application of predictive modeling in the evaluation of compliance and recommended the most successful approach.
Methods: The used data (421 observations) were collected from 100 stores of one retail chain, located in different states in the US, through three years (2017 – 2019). The data included results of microbiology swabs for aerobic plate count collected from the stores (food contact and non-food contact surfaces), results of third-party food safety inspections and the results of the routine health inspection conducted by the local authorities. Each local authority has its technique to score the overall inspection per store. The results of the microbiological swabbing and the third-party food safety inspections were used as predictors, while the routine health inspection result was used as criteria for compliance. The criteria of compliance were tested in two configurations: (1) Standardized numerical variable: The overall store score of the health inspection was standardized for different regions, and (2) Categorical variable: The standardized numerical variable was converted to a categorical variable with four levels (Good, Adequate, Needs Improvement, Poor). Various machine learning models (regression/classification), the most successful model was selected using the quality score of the model and the cross-validation result.
Results: The most successful model was developed using the categorized criteria of the routine health inspection. The successful algorism was machine learning classification (Multiclass Decision Jungle). The model predicted the level of compliance by classifying the stores into four categories: Good, Adequate, Need Improvement and Poor. The model scored an average of 83% accuracy among each category.
Significance: The predictive model approach is likely to unify the compliance evaluation methods in different regions and enhance the capability to improve.

T2-10 Comparative Exposure to Antibiotic-Resistant *Salmonella enterica* in Beef from Different Countries

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❖ ***Developing Scientist Entrant***

Introduction: Antimicrobial resistant (AMR) foodborne pathogens are a unique challenge to public health, as decisions and policies made far afield from consumers can have drastic impacts on their health. Regional differences in antibiotic use and regulations may affect the exposure of consumers to foodborne pathogens, such as *Salmonella enterica* with AMR.
Purpose: The purpose of this study is to perform a quantitative exposure assessment comparing European Union (EU) consumer exposure to AMR between beef raised and produced in the EU, United Kingdom (UK) and United States (US).
Methods: Prevalence of AMR *S. enterica* in beef in the EU, UK, and US was estimated from longitudinal national surveillance data and production volumes. Using beef consumption databases and trade data, beef servings per year by country of origin were estimated for EU member states. Prevalence and consumption estimates were paired to estimate EU country-level exposure to AMR *S. enterica* per meal containing beef. Parameter uncertainty was modeled using Bayesian inference and integrated using Monte Carlo simulation to compare and probabilistically rank exposure to AMR *S. enterica* per serving between countries longitudinally, aggregated at different levels.
Results: This study will compare estimates of EU nation-specific exposure to antibiotic resistant *S. enterica* through beef produced in EU, UK and US. Sensitivity and scenario analysis will be conducted to assess the impact that the source of beef has on EU consumer exposure to AMR *S. enterica*. Year-to-year and aggregated estimates will help understand the impact of policy changes in consumer exposure risk to AMR *S. enterica*.
Significance: The study aims to quantitatively compare differences in EU consumer exposure to AMR *S. enterica* in beef from different sources. The longitudinal comparison between and within countries will provide valuable information for foodborne AMR prevention policy.

T2-11 Characterizing the Risk of SARS-CoV-2 Infection Among Essential Food Workers: A Quantitative Microbial Risk Assessment Approach

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❖ ***Developing Scientist Entrant***

Introduction: Essential food workers in the United States are at an increased risk of SARS-CoV-2 infection due to potential occupational exposures during produce harvesting and packing, paired with shared lodging and transportation to and from on-farm facilities.
Purpose: The purpose of this study was to quantify the daily risk of SARS-CoV-2 infection for essential food workers from exposures during a 12 h work shift and shared lodging and examine the impact of CDC COVID-19 guidelines and existing FSMA requirements as risk reduction strategies.
Methods: A quantitative microbial risk assessment (QMRA) model was created in R using a two-dimensional Monte Carlo package and 10,000 simulations. Four distinct modules were parameterized to simulate the daily routine of an essential food worker: shared lodging, shared transportation, outdoor harvesting field, and an indoor packing facility. Aerosol, close-contact droplet, and fomite-mediated SARS-CoV-2 transmission pathways were examined based on the size distribution of droplets containing infectious SARS-CoV-2 released from an infected index case while coughing, and the distance between an infected and susceptible worker (1, 2, 3, or >3 m).
Results: Without any mitigation strategies, the risk associated with each module included: 1 h shared transportation (55.1%), 10 h residential lodging (90.2%), 12 h indoor packing facility shift (12.9%), and a 12 h outdoor harvesting shift (14.9%). Relative to no intervention, mask use reduced infection risk by 82.7% (cloth), 88.8% (surgical), or 99.8% (N95 respirator). Furthermore, surface disinfection reduced fomite-mediated transmission when applied daily (4.1%), bi-hourly (89.4%), and hourly (99.9%), relative to no intervention.
Significance: Consistent with the numerous documented SARS-CoV-2 outbreaks within the food supply chain, this work estimates considerable risk to essential workers who have extended occupational exposures and with limited risk mitigation procedures in place. Based on this work, risk mitigation procedures should be focused on the specific risk derived from exposure at each work and living location.

T2-12 SARS-CoV-2 Transmission Risks and Risk Mitigation Strategies Among Essential Workers in an Indoor Food Processing Facility

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❖ ***Developing Scientist Entrant***

Introduction: The SARS-CoV-2 global pandemic poses significant infection risk to food workers who are essential to maintaining the supply chain and whose jobs often preclude infection control measures (physical distancing).
Purpose: To estimate the SARS-CoV-2 infection risk and evaluate risk reduction strategies for controlling SARS-CoV-2 transmission among essential workers in food processing facilities.

Methods: A stochastic quantitative microbial risk assessment model was developed in R using the mc2d package with 10,000 simulations. The model structure consisted of close contact droplet (1, 2, 3 m), aerosol (>3 m), and fomite-mediated (1 to >3 m) exposures. Sensitivity analysis and model calibration according to super-spreader conditions were conducted on parameters derived from the literature and by expert elicitation. SARS-CoV-2 cumulative infection risks were estimated for a susceptible worker following an 8-h shift with an infected worker. Relative risk reductions were calculated for interventions (physical distancing, mask use, handwashing, surface disinfection).

Results: Under the baseline model, the SARS-CoV-2 infection risk was largest for exposures at 1 m (97.5%, 95% CI: 76.0–100%); followed by 2 m (15.3%, 95% CI: 6.8–31.6%); 3 m (8.7%, 95% CI: 3.7–18.3%); and greater than 3 m (4.6%, 95% CI: 1.4–13.4%). Infection risk reductions resulted from increasing physical distancing from: 1 to 2 m (84.3% reduction), 2 to 3 m (43.2%) and 1 to 3 m (91.1%). Mask use by the infected and susceptible workers reduced infection risk by 52.1% (cloth mask risk: 46.7%, 95% CI: 19.5–87.1%) and 64.3% (surgical mask risk: 34.8%, 95% CI: 12.1–75.6%), relative to no masks. Interventions targeting fomite-mediated exposures resulted in minimal risk reductions: 1% reduction for handwashing (2-log removal) hourly (96.4% risk, 95% CI: 67.6–100%) and 0.97% reduction for surface disinfection (3-log removal) four times/8-h shift (96.6% risk, 95% CI: 69.3–100%).

Significance: While individual interventions should prioritize physical distancing and mask use as effective SARS-CoV-2 infection mitigation strategies, combined interventions will likely enhance the risk reduction impact.

T3-01 *Ex Vivo* Evaluation of the Effectiveness of *Lactobacillus* Metabolites with Berry Phenolic Extracts Against *Campylobacter*

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❖ ***Developing Scientist Entrant***

Introduction: Previously it was reported that cell free cultural supernatant (CFCS) of probiotic *Lactobacillus casei* with *mcra* (myosin-cross-reactive-antigen) over-expressed (LC^{+mcra}) and/or berry phenolic extracts (BPEs) can reduce growth of *Campylobacter* in *in vitro* condition efficiently but their effect on normal bacterial-flora in complex gut ecosystem is not known yet.

Purpose: The purpose of this study was to evaluate combined effect of cell-free-cultural-supernatant of *Lactobacillus casei* with *mcra*-over-expressed (CFCS-LC^{+mcra}) and BPEs against *Campylobacter* in a simulated *ex vivo* poultry gut condition as well as their effect on normal microflora.

Methods: Freshly obtained cecal content from chicken (inoculated with kanamycin resistance *Campylobacter jejuni*, CJ-KM) were incubated at standard condition with CFCS-LC^{+mcra} and/or BPEs. Effect on growth of CJ-KM and natural *Campylobacter* was observed by cultural methods. The16S metagenomic analysis was used to determine the effect on microbiome of control/treated samples. Significance in control/treatments difference was determined by ANOVA.

Results: Combined effect of CFCS-LC^{+mcra} (10%) and BPEs (0.1 mg GAE/ml) on CJ-KM or *Campylobacter* growth reduction was more efficient than their individual effect at both 24 h and 48 h time points. After 24 h of incubation, either CFCS-LC^{+mcra} or BPEs or their combination reduced growth of CJ-KM and *Campylobacter* by 1.65 log CFU/mL and 2.8 log CFU/mL, by 1.5 log CFU/mL and 2.67 log CFU/mL ($P < 0.05$), and by 1.85 log CFU/mL and 3.3 log CFU/mL, respectively ($P < 0.05$). The levels of Firmicutes decreased more in control group (from 88.5% at 0 h to 55.17%) than treatments ($P < 0.05$) and for Proteobacteria, treatments reduced the level of increment compared to control (cecal content without CFCS-LC^{+mcra} or BPEs) at 48 h time point. There was also notable change in percentage of abundance various genus at both 24 h and 48 h time points ($P < 0.05$).

Significance: This *ex vivo* observation recommended that combination of CFCS-LC^{+mcra} and BPEs can effectively reduce colonization of *Campylobacter* without detrimental effect on gut microbiome.

T3-02 Adaptation of *Listeria* to Increasing Sanitizer Concentrations

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Introduction: In food processing environments, *Listeria* can be exposed to sublethal concentrations of sanitizers, such as Benzalkonium chloride (BC), which can promote physiological changes conferring increased tolerance. Genes encoding efflux pumps such as *bcrABC* and *qacH* are associated with increased *Listeria* tolerance to BC.

Purpose: Assess the potential of *Listeria* isolates to achieve increased tolerance through adaptation to increasing concentrations of BC, maintain increased tolerance without selective pressure, and to survive exposure of use level concentrations after adaptation.

Methods: A total of 28 *Listeria monocytogenes* isolates and 40 *Listeria* spp. isolates (including *seeligeri* (*n* = 14), *innocua* (*n* = 12), *welshimeri (n* = 9*)*, *marthii (n* = 3*)*, and *ivanovii (n* = 2*)*) were adapted to increasing sanitizer concentrations by subculturing every 24 h or 48 h if growth was detected. If no growth was detected after 48 h, isolates were plated on brain heart infusion (BHI) agar. Single colonies from adapted isolates were substreaked seven times on BHI agar. Isolate minimum inhibitory concentrations (MIC) to BC were determined with broth microdilution for (i) parent isolates, and adapted isolates (ii) before and (iii) after substreaking seven times. Parent and adapted isolates were also assessed for their survival after exposure to BC at use level concentrations.

Results: Initial MIC of parent (i) isolates ranged from 2-6 mg/L BC. Isolates carrying tolerance genes (*bcrABC* or *qacH*) showed significantly higher MIC than isolates lacking these genes ($P < 0.001$). Through adaptation, isolates grew at concentrations up to 20 mg/L BC, but acquired tolerance was reduced after removal of selective pressure to at most 6 mg/L and 8 mg/L for isolates lacking and carrying tolerance genes, respectively. Tolerance genes were no longer significantly associated with increased tolerance to BC ($P = 0.139$). Adapted (ii and iii) *L. monocytogenes* isolates showed significantly higher MIC compared to *Listeria* spp. ($P < 0.001$).

Significance: Our results suggest that tolerance genes play a limited role in the adaptation of *Listeria* to BC.

T3-03 B-Phenylethylamine as a Natural Food Additive Shows Antimicrobial Activity Against *Listeria monocytogenes* on Ready-to-Eat Foods

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Introduction: *Listeria monocytogenes* (*Lm*) is an important foodborne pathogen and a major cause of death associated with bacterial foodborne infections. Its control on ready-to-eat (RTE) foods remains a challenge.

Purpose: Evaluate β-phenylethylamine (PEA) as a potential organic antimicrobial for *Lm* control.

Methods: Using sixty-two clinical and food-related isolates we determined the minimum inhibitory concentration (MIC) of PEA against *Lm* in a meat simulation media, and Brain heart infusion (BHI) broth, and agar. Challenge tests using bologna-type sausage (lyoner) and smoked salmon as food model systems supplemented with PEA (0 to 20 mg/g) were done at appropriate storage (4°C) and abusive temperature (10°C) to validate the *in vitro* findings. Transcriptomics was employed to identify PEA mechanism of action. Statistical analysis of data was done using ANOVA and post-hoc tests.

Results: PEA had a significant ($P < 0.05$) growth-inhibitory (growth rate 0.28 in PEA [1.56 mg/mL] vs 0.31 OD_{600}/hour in BHI) and bactericidal effect against *Lm* both in *in vitro* experiments and on lyoner and cold smoked salmon. In broth and agar, the MICs were 8 mg/mL (1/62), 10 mg/mL (57/62), and 12.5 mg/mL (4/62 strains). PEA treatment caused 0 to 2.1 log CFU and 0 to 0.35 log CFU reduction in bacterial counts at concentrations between 7.5 mg/g to 20 mg/g on lyoner and smoked salmon, respectively, in comparison to the non-PEA treated controls. Furthermore, PEA also significantly ($P < 0.05$) inhibited *Lm* biofilm formation at sub-MIC levels (3.13 and 6.25 mg/mL). PEA exposure was associated with expression induction of the cold shock protein family genes (*csp*: >2-fold change).

Significance: Based on good manufacturing practices as a prerequisite, PEA application to RTE products might be an additional hurdle to limit *Lm* growth in foods whereas its biofilm inhibitory effects suggest a potential PEA role as a surface disinfectant in food processing environments.

T3-04 Network Assisted Variant Analysis Reveals Novel Genetic Elements Linked to Nisin Tolerance and Sensitivity in *Listeria monocytogenes*

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* poses numerous response mechanisms that enhance its resistance to different stresses, including nisin, a widely used bacteriocin. Nisin resistance mechanisms of *L. monocytogenes* are not yet fully unknown thus necessitating novel approaches for their identification.

Purpose: Apply growth phenotype and genome-based analyses to identify natural mutations underlying altered nisin stress responses in *L. monocytogenes*.

Methods: *L. monocytogenes* field strains (*n* = 356) isolated from food associated environment (*n* = 279) and human listeriosis cases (*n* = 77) were examined. Nisin growth responses of the strains were determined through spectrophotometry for 24 hours at 37°C. Absorbance data was modeled to area under the curve (AUC) using spline fitting method and normalized to percentage change in AUC (ΔPAUC) to account for inherent growth variability without stress. Strains were classified as high nisin resistance (HNR; ΔPAUC<10%) and high nisin sensitive (HNS; ΔPAUC>90%). Comparative sequence analysis of 30 known *L. monocytogenes* nisin response genes and 254 genes within a nisin response network created through text mining using StringApp was carried out for the HNR and HNS strains.

Results: Seventeen and twenty-three strains were categorized as HNR and HNS, respectively. Analyzing known nisin response genes revealed mutations in *PBPB3* and *rsbU* genes in two HNR strains. Sixteen NHS strains had mutations in *sigB*, *vir* and *dlt* operon genes. Network assisted variant analysis revealed further mutations in six genes in six other HNR and HNS strains. Genes identified are involved in cell envelope modification (*ddl*, *mtrA* and *gtcA*), transport (*lmo0194* and *lmo1966*) and house-keeping (*prs2*) functions. Further studies, involving mutagenesis and complementation, to fully characterize the contribution of these six candidate genes to nisin tolerance are currently underway.

Significance: These data show that a combination of growth parameter-based phenome and sequence analysis can be used to identify nisin response genes in *L. monocytogenes* that can be targeted to improve food safety.

T3-05 Effect of *Ohelo* berry (*Vaccinium calycinum*) Juice on Physicochemical Properties, Biofilm Formation, and Virulence Gene Expression of *Listeria monocytogenes*

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Introduction: *Ohelo* berry (*Vaccinium calycinum*) is a Hawaiian wild relative of cranberry (*Vaccinium macrocarpon*). Previous research indicates that *ohelo* berry is a rich source of polyphenolic compounds and possesses antimicrobial properties.

Purpose: This study aimed to understand the mode of action of antimicrobial compounds in *ohelo* berry against the pathogen *Listeria monocytogenes*.

Methods: Growth potential, physicochemical properties (auto-aggregation, hydrophobicity, and motility), biofilm formation capability, and virulence gene expression patterns of *L. monocytogenes* were evaluated in the presence of *ohelo* berry juice. Additionally, transmission electron microscopy (TEM) was used to assess the damages of *ohelo* berry juice to *L. monocytogenes* cell structures.

Results: The minimum inhibitory concentration (MIC) of *ohelo* berry juice against *L. monocytogenes* was 12.5%. Sublethal concentrations of *ohelo* berry juice (3.125% and 6.25%) significantly increased the auto-aggregation and decreased the hydrophobicity, swimming motility, swarming motility, and biofilm formation capability of *L. monocytogenes.* The relative expression of genes for invasion (*iap*), listeriolysin production (*hly*), biofilm formation (*sigB*), motility (*flaA*), and survival-related phosphatidylinositol phospholipase C (*plcA*) was significantly downregulated in *L. monocytogenes* with 6.25% of *ohelo* berry juice. TEM disclosed that *ohelo* berry juice caused irregular shapes, discontinuous and collapsed membranes on *L. monocytogenes* cells.

Significance: These findings highlight the potential of *ohelo* berry as a natural preservative. Furthermore, *ohelo* berry extracts could potentially be used as an oral supplement to prevent *L. monocytogenes* infection.

T3-06 Disinfectant Efficacy Against *Staphylococcus aureus* and *Pseudomonas aeruginosa* Dry Surface Biofilms is Product, Time Point and Strain Dependent

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❖ ***Developing Scientist Entrant***

Introduction: Dry surface biofilms (DSB) can be prevalent on environmental surfaces. They are harder to kill than planktonic bacteria and wet surface biofilms (WSB). However, sanitizers are tested against planktonic bacteria of *S. aureus* and *P. aeruginosa* prior to registration and products that have biofilm claims are tested against WSB.

Purpose: This study tested the bactericidal efficacy of seven EPA-registered products against *S. aureus* and *P. aeruginosa* DSB.

Methods: WSB of *S. aureus* ATCC-6538 and *P. aeruginosa* ATCC-15442 were established on borosilicate glass coupons following EPA MLB SOP MB-19. Coupon-holding rods were harvested and dehydrated for 24-72 h using incubators set at 25°C or 21°C for *S. aureus* and *P. aeruginosa,* respectively, to generate DSB. After 24 h and 72 h of dehydration, three coupons with DSB were harvested, treated with disinfectant liquid, neutralized at the label-defined contact time and viable bacteria were recovered on media following EPA MLB SOP MB-20.

Results: There were differences in *S. aureus* DSB inactivation among disinfectant active ingredient classes ($P = 0.0006$). Specifically, quaternary ammonium plus alcohol (QA), sodium dichloro-s-triazinetrione (CL), sodium hypochlorite (SH), and hydrogen peroxide (HP) products were more bactericidal than the quaternary ammonium products (QT; $P < 0.05$). For *P. aeruginosa*, HP and CL products were more bactericidal against *P. aeruginosa* DSB than SH, QT and QA products ($P < 0.05$). There were no significant differences in the bactericidal efficacies of disinfectants against 24 h and 72 h DSB of *S. aureus*; statistically lower log reductions against 72 h DSB of *P. aeruginosa* were recorded compared to their 24 h DSB.

Significance: Disinfectant efficacy against DSB is dependent on product type, bacterial strain and DSB "age." DSB may better represent biofilm challenges in food systems.

T3-07 Efficacy and Quality Attributes of Antimicrobial Agents Applied Via a Commercial Electrostatic Spray Cabinet to Inactivate *Salmonella* on Chicken Thigh Meat

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Introduction: Poultry meat, including chicken, is an important protein source among consumers, but it is highly susceptible to *Salmonella* contamination. Therefore, a conscious control needs to be applied in the poultry industries.

Purpose: This study aimed to determine the efficiency and quality attributes of two antimicrobial agents to reduce *Salmonella* on raw chicken meat

when applied individually and in combination using an electrostatic spray cabinet.

Methods: Five log CFU/g of non-pathogenic, rifampicin resistant *Salmonella* Typhimurium were aseptically inoculated on skin/bone less raw chicken thigh meat and passed through a commercial electrostatic spray cabinet while being sprayed with 5% lauric arginate (LAE), and 100, 1000, 1500, 1750 ppm of peracetic acid (PAA). Three experiments were carried out to analyze microbiological aspects as follows: (1) optimal concentration and exposure time of PAA (2) ideal exposure time of LAE, and (3) effect of a combination of treatments with LAE and PAA. Each sample was stored at 4ºC for 0, 1, 2, and 3 days and subjected to microbiological analysis. Organoleptic sensory evaluation, color measurement and water holding capacity (WHC) were performed to understand the meat quality attributes.

Results: Spraying of 5% LAE for 45 s, significantly reduced *Salmonella* by 5 logs ($P < 0.05$). The 1500 ppm of PAA reduced *Salmonella* significantly within 45 s (1.157 logs). Spraying of 1500 ppm PAA followed by LAE within 15 s reduced *Salmonella* significantly more than vice versa ($P < 0.05$). The treatments did not cause significant ($P > 0.05$) differences in color, water holding capacity or texture, but did result in a significantly ($P < 0.05$) strong aroma and flavor.

Significance: Both LAE and PAA efficiently reduced *Salmonella* when applied in an electrostatic spray cabinet on raw chicken thigh meat. The results suggest that the sequential order of application of antimicrobial agents and dwell time in the spray cabinet is important to improve the safety and quality of raw chicken thigh meat.

T3-08 In-Plant Validation of Novel On-Site Ozone Generation Technology (Bio-Safe) Compared to Lactic Acid on Beef Carcasses and Trim Using Natural Microbiota and *Salmonella* and *E. coli* O157 Surrogate Enumeration

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Introduction: The recent enhancement of ozone aqueous solution on-site generation has been evaluated as an alternative for improving beef safety.

Purpose: To determine the antimicrobial efficacy of an aqueous ozone (Bio-Safe) treatment and lactic acid on natural microbiota and *E. coli* O157:H7 and *Salmonella* surrogates on beef carcasses and trim in a commercial beef processing plant environment.

Methods: For every repetition, 40 carcass and 40 trim swabs (500 cm^2) were collected, 20 before and 20 after each intervention. Samples were taken using EZ-Reach™ swabs pre-hydrated with 25 mL buffered peptone water, diluted, and plated into aerobic plate count (APC), coliform, and *E. coli* Petrifilm™ for enumeration. In addition, a 5-strain cocktail (BAA-1427, 1428, 1429, 1430, and 1431) of surrogates was inoculated onto trim targeting 5 log CFU/cm^2 attachment. For every trim surrogate repetition, 15 chuck and 15 foreshank pieces were sampled after 30 min attachment and after ozone intervention. Samples were diluted, and counts were determined using the TEMPO® system for *E. coli* enumeration. In each trial, 6 repetitions were conducted, and comparisons were done using a two-way ANOVA ($\alpha = 0.05$).

Results: Ozone and lactic acid interventions significantly reduced ($P < 0.003$) APC, coliform, and *E. coli* in carcasses and trim samples. Moreover, lactic acid further reduced APC and coliforms in trim samples compared to ozone intervention ($P < 0.009$). In the surrogate trials, ozone significantly reduced ($P < 0.001$) surrogate concentration on average by 1.17 log CFU/cm^2. Historical data from the plant revealed a reduction ($P < 0.001$) of presumptive O157:H7 in trim from 1.06% (102/9609) to 0.26% (25/9439) after a year of ozone intervention implementation.

Significance: The novel technology for ozone generation and application as an antimicrobial can become an alternative option that may also act synergistically with existing interventions in the beef industry, minimizing the risk of pathogens such as *Salmonella* and *E. coli* O157:H7, thus improving the safety of beef.

T3-09 Association between Antibiotic Resistance and Sanitizer Resistance of *Escherichia coli* Isolated from Agricultural Water in Hawaii

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Introduction: Pathogenic *Escherichia coli* is notoriously known as a common cause of foodborne infections. The food industry utilizes sanitizers to minimize the risk of foodborne illness outbreaks. Implementing sanitizers to wash fresh produce has been found effective in reducing pathogens. However, there are concerns about bacteria acquiring resistance against sanitizers. Bacteria may utilize the same resistance mechanisms to resist antibiotics.

Purpose: This study aimed to explore the prevalence of antibiotic resistance and sanitizer resistance in *E. coli* isolated from agricultural water in Hawaii and evaluate the association between them.

Methods: Antibiotic and sanitizer susceptibility profiles of 182 *E. coli* isolates were constructed using the Kirby-Bauber disk diffusion method. The association between antibiotic resistance and sanitizer resistance of the *E. coli* strains was evaluated. Furthermore, six selected strains were plasmid cured using sodium dodecyl sulfate. It was investigated whether plasmids are involved in the resistance and cross-resistance of the strains against the antimicrobials.

Results: Of 15 tested antibiotics, *E. coli* isolates showed a high prevalence of resistance to cefotaxime (61.5%), ampicillin (42.3%), and erythromycin (41.74%). Ninety-nine isolates (54.3%) showed resistance against two or more antibiotics. One isolate showed resistance against 11 antibiotics. Most of the tested strains demonstrated higher resistance against peroxyacetic acid than bleach. There was a moderate correlation between tetracycline resistance and peroxyacetic acid resistance, with a correlation coefficient of 0.4. After plasmid curing, 6 cross-resistant *E. coli* isolates showed increased sensitivity to some antibiotics and sanitizers. The ampicillin resistance gene *ampC* was only detected in the parent strain and lost during plasmid curing.

Significance: This study lays the groundwork for understanding the relationship between antibiotic resistance and sanitizer resistance in foodborne pathogenic bacteria, which could pose a serious threat to public health.

T3-10 Selection of a Potential Synbiotic Against *Cronobacter sakazakii*

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❖ ***Developing Scientist Entrant***

Introduction: *Cronobacter sakazakii* (*Cs*) is an opportunistic foodborne pathogen that can be fatal to infants and is commonly associated with powdered infant formula (PIF) due to contamination in manufacturing processes, hospitals, or homes.

Purpose: This project aimed to select a synbiotic, a combination of probiotic strains with a prebiotic product, to inhibit the growth of *Cs* in an *in vitro* dynamic infant gut model (Simulator of the Human Intestinal Microbial Ecosystem [SHIME]).

Methods: A total of 16 probiotic bacteria were tested for their inhibitory properties against four *Cs* strains by the drop-plate method. An aliquot of 2 µL of the probiotic culture was pipetted onto BHI agar containing a lawn of *Cs* with *Lactobacillus plantarum* ATCC 14365 as the positive control and *Enterobacter tabaci* 3E7 as the negative control. Following overnight incubation, the clear zones of inhibition (mm) were measured ($n = 9$). The selected probiotic strains that inhibited *Cs* were grown in MRS broth containing 1% dextrose or 1% commercial PIF prebiotic (w/v) to compare their ability to metabolize the prebiotic product ($n = 3$). The probiotic strains that produce inhibition zones and grow better in 1% prebiotic will be used in the synbiotic mixture.

Results: Four out of the 16 probiotic bacteria, based on growth inhibition of *Cs* and prebiotic metabolism, were chosen to be part of the synbiotic

mixture. *Lactobacillus* and *Pediococcus* species were able to inhibit the growth (>15 mm inhibition zones) of all *Cs* strains tested (n = 9). *L. plantarum* ATCC 14365, *Lactobacillus rhamnosus*, and *Pediococcus pentosaceus* strains grew >0.5 log CFU/mL more in MRS containing 1% prebiotic compared to 1% dextrose (n = 3; P < 0.05).

Significance: This research will help to develop a synbiotic that may reduce the prevalence of *Cs* in PIF and the gastrointestinal tract.

T3-11 Antifungal Activity and Bioprotective Potential of Lactic Acid Bacteria Isolated from *Kunu-Zaki*, a Nigerian Indigenously Fermented Beverage

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Introduction: Safety and stability concerns resulting from contamination of indigenously fermented cereal foods by fungi, have continued to deter wide acceptance and commercial production in Africa. Current measures to protect cereal foods do not conform with consumer demand for clean-labelled foods. Bioprotective cultures represent promising alternatives.

Purpose: This study aims to screen and characterize antifungal activity of LAB isolates from *Kunu-zaki* against plant food spoilage fungi and determine their bioprotective potential in millet-sorghum beverage.

Methods: Antifungal activity against 8 mold species were screened among 220 LAB strains from *kunu-zaki* by agar overlay method on millet-sorghum flour hydrolysate (MSFH) agar. Cell-free supernatants (CFS) of strains displaying strong and broad inhibition were further screened by MSFH agar diffusion. The nature of antifungal compounds in CFS was determined after neutralization (pH 7) and proteinase (1 mg/mL) treatment. Antifungal effect of selected LAB was validated in *kunu-zaki* challenged with *Aspergillus flavus* and *Penicillium citrinum.*

Results: LAB strains, belonging to *Lactobacillus fermentum* and *Weissella confusa* were the most active against 7 test mold species, with average inhibition score range of 2.25 – 2.88. Antifungal activity against all target molds was retained in CFSs of selected LAB, with the highest inhibition (P < 0.05) against *A. flavus* and *P. citrinum* demonstrated by *Lb. fermentum* 5KJEU5 (9.06%) and *W. confusa* YKDIA1 (24.55%), respectively. Inhibition by CFS was mainly due to occurrence of organic acid. Challenge test showed that the *Lb. fermentum* 5KJEU5 delayed growth of *A. flavus* and *P. citrinum* on *kunu-zaki* for 6 days at ambient temperature (25°C).

Significance: LAB from *kunu-zaki* displayed strong antifungal activity. The cultures or metabolites could be used to prevent fungal growth and mycotoxin accumulation in cereal foods.

T3-12 Antimicrobial Food-Grade Coatings on Hydrophobic Plastics for Reducing Cross-Contamination of Fresh Produce

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❖ ***Developing Scientist Entrant***

Introduction: Antimicrobial coatings using food-grade compositions were developed to reduce the risk of cross-contamination from hydrophobic plastic surfaces.

Purpose: The objectives were to develop antimicrobial food-grade coatings on plastics, evaluate their antimicrobial activity in the presence of water and organic matter, and determine their efficacy in reducing cross-contamination.

Methods: Wax-based coating solutions were prepared by mixing 5 or 10% Bio-Mos (yeast-derived *N*-halamine precursor that binds chlorine), ethanol, glycerol, Tween 80, and beeswax at 70°C. Polypropylene coupons were dip-coated three times for 10 s, and *N*-halamine compositions were generated by reacting Bio-Mos/beeswax coatings with commercial bleach for 1 h. The level of surface-bound chlorine and its chemical stability was enhanced by encapsulating ε-poly-L-lysine into Bio-Mos. Uncoated and uncharged coupons were used as controls. The antimicrobial activity was evaluated against *Escherichia coli* O157:H7 (10^6 CFU/mL, 20 µL) for 2-10 min. The stability was tested after water immersion (1-8 h) or in the presence of organic matter (500-20,000 ppm chemical oxygen demand). Cross-contamination was simulated using baby spinach. Viable bacteria were enumerated by plate counting, and significant differences (P < 0.05) of the results were determined using ANOVA (n = 3/treatment).

Results: Submillimeter-scale uniform coatings were achieved on plastic coupons, resulting in the surface-bound chlorine concentration of 262.50-700.00 nmol/cm^2, depending on the Bio-Mos composition. The chlorine-charged 5% Bio-Mos/beeswax coatings resulted in >4-log reduction of inoculated bacteria within 2 min. The coatings with 10% Bio-Mos/beeswax modified with ε-poly-L-lysine maintained their antimicrobial activity after water immersion for 8 h or in the presence of high organic load (20,000 ppm). These coatings significantly reduced cross-contamination (>2-log reduction) of baby spinach from the plastic surfaces.

Significance: The antimicrobial food-grade coatings developed in this study would enable stable and rapid deposition on existing food contact surfaces, reducing cross-contamination of fresh produce.

T4-01 An Evaluation of Inter-Laboratory Analytical Results for Mycotoxins in Cereal Grains

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Introduction: Cereal grains and other organic materials contaminated by molds that produce mycotoxins can result in illness and disease when consumed so it is important to accurately quantitate mycotoxin levels in foods.

Purpose: This work evaluates quantitative results for deoxynivalenol in cereal grains collected by seven analytical laboratories over a three-year period.

Methods: Ground corn and wheat samples with known amounts of deoxynivalenol were provided to each laboratory. Analyses were conducted in triplicate for each sample on three days for the first evaluation and in triplicate on one day for subsequent testing. Samples were analyzed by GC/ECD or UHPLC/MS depending on the laboratory. A statistical analysis of results was conducted using Mini-Tab and Excel.

Results: In the latest round of testing conducted in 2020, the inter-laboratory mean for a 0.50 ppm DON wheat check sample was 0.59 ± 0.16 (Coefficient of Variation (CV) = 28%) ppm DON and ranged from 0.44 to 0.92 ppm. The mean for a 2.1 ppm DON wheat check sample was 1.97 ± 0.56 ppm (CV = 28%) and ranged from 1.5 to 3.8 ppm DON. The mean result for the 4.5 ppm DON wheat check sample was 4.08 ± 0.97 (CV = 24%) and ranged from 2.4 to 5.6 ppm. The mean result for the 4.7 ppm DON corn check sample was 4.46 ± 1.77 (CV = 40%) and ranged from 1.9 to 7.1 ppm. Relatively large variability was due mainly to greater variability in results from 2 of the labs. One lab had *Z*-scores exceeding 1.9 for 2 of the check samples and another lab showed long-term drift in results.

Significance: The comparison of quantitative deoxynivalenol results from each laboratory provided information on proficiency of the laboratories and consistency of the check samples. Significant differences in results, greater variability and long-term drift were detected in results from a couple of the analytical laboratories which reinforces the need for laboratories to participate in periodic proficiency evaluations and improve their analytical methods when necessary.

T4-02 Effect of Changes in Fermentation Conditions on the Selection of Appropriate Calibrants for the Quantitation of Gluten in Fermented-Hydrolyzed Foods

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Introduction: Accurate quantitation of gluten in fermented-hydrolyzed foods is challenging due to the lack of appropriate calibrants and variable proteolysis. A multiplex-competitive ELISA was recently developed that recognized the protein/peptide profile differences among the different types of fermented foods such as wheat beers, barley beers, sourdough breads or soy-based sauces.
Purpose: Using gluten-incurred (spiked before fermentation) yogurt as a model food, this study evaluated the similarities/differences in protein/peptide profiles arising due to differences in fermentation conditions as recognized by the multiplex-competitive ELISA. This is essential for the selection of appropriate calibrants for accurate quantitation of gluten in fermented-hydrolyzed foods.
Methods: Yogurts prepared by incurring 0, 20, 100, and 500 µg/g gluten and by varying certain fermentation conditions (fermentation time, incurred gluten concentrations and starter culture type and concentrations) were analyzed by the multiplex-competitive ELISA. Western blot was performed in order to evaluate similarities/differences in gluten protein-antibody binding patterns. Cluster analysis of the apparent gluten concentration values was performed by hierarchical clustering to evaluate similarities/differences in protein/peptide profiles due to differences in the fermentation conditions.
Results: Analysis indicated epitope specific responses with glutenin epitopes being less susceptible to longer fermentation time and higher starter culture concentration compared to gliadins. Incomplete proteolysis observed after 24 hours of fermentation became more efficient after 48 hours as the high gluten concentration values initially observed reduced close to the initial incurred levels for nearly all the antibodies ($P < 0.05$). Western blot confirmed the ELISA results. Cluster analysis indicated similar gluten protein/peptide profiles due to changes in incurred gluten concentrations, starter culture type and concentrations, but distinctive profiles due to changes in fermentation time.
Significance: For yogurt or products with similar fermentation chemistry, fermentation time could make a difference in protein/peptide profiles, especially if the initial proteolysis is incomplete. It will likely require appropriate calibrants with similar protein/peptide profile as both intermediate and complete proteolyzed products for accurate quantitation of gluten.

T4-03 Maximum Levels for Carcinogenic Pyrrolizidine Alkaloids in Kitchen Herbs Introduced

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Introduction: In the past two years a number of product recalls of oregano due to high content of pyrrolizidine alkaloids (PAs) were published via the European RASFF (Rapid Alert System for Food and Feed). Further herbs are in the focus as well. PAs are naturally occurring substances and potentially carcinogenic. The EU adopted maximum levels of PAs in different foods including kitchen herbs in June 2020 which will come into effect on July 1, 2022. Usually, PAs are not produced by the crop plant itself. They are added to the product through co-harvest of PA-containing weeds that grow in between the crops.
Purpose: The purpose of this study was to provide an overview on the situation of PA-contamination of common kitchen herbs.
Methods: Analysis of PAs is achieved using a simple extraction with 0.05M sulfuric acid and subsequent analysis by means of HPLC-MS/MS. For analysis of concentrations below approximately 20 µg/kg, SPE using strong cation exchange cartridges was applied. Quantification is done by spiking the extract (or sample aliquot before SPE, respectively) with 28 commercially available and certified PA-standards (3-point calibration).
Results: A total of 184 oregano, 45 basil, 21 lovage, 8 marjoram and 8 tarragon samples were analyzed in this study. In only ~9% of the oregano samples no PAs were detected, while 43% showed concentrations above 1000 µg/kg, which is the limit for PAs in oregano currently under discussion. The other herbs also contained PAs but usually to a lesser extent. However, also in marjoram, lovage and tarragon some samples contained more than 1,000 µg/kg PAs. In basil PA-positives do not occur often (16% PA-positive) and did not exceed 1,000 µg/kg.
Significance: These data suggest that PAs in herbs are a serious problem as they occur often and also at concentrations significantly exceeding the maximum levels adopted by the EU.

T4-04 Nanoliposome Loaded with Chitosan-Epigallocatechin Gallate Conjugate: Preparation, Characterization and Its Application for Shelf-Life Extension of Refrigerated Asian Sea Bass (*Lates calcarifer*) Slices

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❖ ***Developing Scientist Entrant***

Introduction: Chitosan (CS) is water-insoluble, thus restricting its applications in various food systems. Therefore, CS-epigallocatechin gallate (EGCG) conjugate (CEC) was prepared to increase water solubility and bioactivities. However, EGCG present in CEC is converted into non-epicatechin isomer (gallocatechin gallate) induced by several intrinsic and extrinsic factors, leading to poorer bioactivities in various food systems.
Purpose: Nanoliposomes (NLs) loaded with CEC (CEC-NLs) were prepared to enhance the storage stability of CEC and the application of CEC-NLs for shelf-life extension of Asian sea bass slices (ASBS) 4ºC.
Methods: NLs using lecithin and cholesterol at various concentrations were prepared for loading CEC at different levels. Encapsulation efficiency (EE), particle size (PS), zeta potential (ZP) and polydispersity index (PDI), storage stability, antimicrobial and antioxidant activities of resulting NLs were examined. NLs were applied on ASBS for shelf-life extension, in which both microbiological (10 g sample) and chemical ($n = 3$) along with sensory properties ($n = 50$) were monitored during storage at 4ºC for 18 days.
Results: CEC-NLs prepared using lipid phase (60 µmol/mL) and CEC (0.5% w/v; EGCG (8% w/w of CS) in CS solution (1% w/v)) (LP-60-0.5) had highest EE (76.96%) and possessed PS, ZP, and PDI of 685.10 nm, -49.46 mV, and 0.276, respectively ($P < 0.05$). LP-60-0.5 retained their antioxidant and antimicrobial activities at a higher extent than free CEC during storage for 28 days at 4ºC ($P < 0.05$). SEM images of bacteria confirmed rapid leakage of intracellular components caused by CEC-NLs. Treatment of LP-60-0.5 (0.05 g/100 g) on ASBS (ASBS-CENL-0.05) resulted in lower microbial loads ($<10^6$ CFU/g) and lower oxidation of polyunsaturated fatty acids than other samples ($P < 0.05$) up to 15 days, mainly caused by antimicrobial activities and free radical scavenging of CEC-NLs.
Significance: CEC-NLs effectively sustained the bioactivities more effectively than free CEC during storage. Furthermore, the shelf life of ASBS with sensory acceptability could be prolonged for at least 12 days at 4°C.

T4-05 Advanced Bioinformatics for Highly Resolved Profiling and Quantification of Spoilage Microbiota and Prediction of Functions Influencing Food Spoilage

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Introduction: Undesirable microbial activity in food can cause food spoilage that renders food unacceptable for human consumption. As high as 25% of all foods produced globally is lost due to microbial spoilage, thereby, making it by far the most common cause for food losses. 16S rRNA sequencing is a method of choice to profile resident microbiota of food, monitor their growth during processing, transportation and storage, and even discover spoilage

causing organism. However, utility of this method is severely hindered by lack of advanced bioinformatics platform that can enable data to discovery workflow.

Purpose: Develop a cloud-based, easy-to-use bioinformatics platform for rapid and accurate profiling, quantification and discovery of spoilage associated microbiota.

Methods: We developed a comprehensive application for 16S microbiome taxonomic profiling (MTP) as part of EzBioCloud platform using our extensive, curated and taxonomically validated database and optimized in-house pipeline.

Results: It lets user easily and accurately characterize 16S data at the species level and enables user to correct and normalize for 16S gene copy number and red depth to get more accurate abundance estimates. It provides an up-to-date prediction of KEGG-based orthologs, modules, and pathways using >10,000 species data. It offers a complete set of comparative cohort analysis to compare taxonomic composition, α-/β-diversity statistics, β-group significance using PERMANOVA, as well as cohort comparison through LEfSe, Kruskal-Wallis H test and Taxon XOR analysis with adequate statistics to facilitate discovery of taxonomic and functional biomarker with just a few clicks. EzBioCloud MTP provides access to various public datasets (>11,000 samples) for users to compare with their own 16S data.

Significance: EzBioCloud 16S MTP offers a one-stop, easy-to-use, data-to-discovery solution for food scientists to discover and quantify spoilage microbiota and identifying potential functions and metabolic processes that influence food spoilage.

T4-06 A Meta-Analysis Assessment of the Bacterial and Fungal Contaminants in Caprine and Ovine Cheese

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Introduction: Artisanal cheese made from caprine or ovine milk is produced in small holdings from untreated milk posing a severe health risk to humans due to microbial contamination.

Purpose: This meta-analysis aims to estimate the overall incidence of microbial contaminants in caprine and ovine cheese.

Methods: A systematic review identified 30 studies assessing microbial contaminants of caprine or ovine milk cheese by plate counting or PCR, where 5,698 cheese were tested. Four studies focus on fungal contaminants (n = 130), whereas 26 studies (n = 5568) elucidate bacterial contaminants. A random-effects (RE) model was applied to the extracted incidence of contamination using R studio and the metaphor package, which assumes that the studies varied from each other and represent a random sample of all observed incidence. A multi-level mixed-effects (ME) model estimated the impact of milk-type, cheese-hardness and treatment on contamination. An Egger's test and funnel plots were used to assess data quality.

Results: The RE model estimated the proportion of contaminated cheese, highlighting several food pathogens and spoilers *such as Penicillium* spp. (25%), *Staphylococcus* spp. (25%), *Escherichia* spp. (15%), *Listeria* spp. (11%) and *Salmonella* spp. (3%) as significant contaminants (P < 0.05) amongst others. *Penicillium* spp. and *Staphylococcus* spp., found in the environment can easily contaminate cheese, whereas *Escherichia* spp., *Listeria* spp. and *Salmonella* spp., associated with pathogenicity, had a lower incidence. The ME model fitting was carried out on these microorganisms only due to data availability, which indicated that contamination was highest in hard cheese made from untreated milk. Most studies are high precision with a low incidence and standard error, indicative of some publication bias favoring reporting pathogens. Overall, more experimental data are required assessing contaminants of caprine and ovine milk.

Significance: Raw milk artisanal cheese is nutritious but prone to contamination. Therefore, educating cheesemakers is critical for its safe manufacturing and consumption.

T4-07 Longitudinal Dynamics and Antimicrobial Resistance Profiles of *Salmonella* in Beef Cattle and the Feedlot Environment

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Introduction: A lack of longitudinal environmental sampling in beef cattle feedlots limits industry knowledge of the transmission dynamics of *Salmonella* between cattle and the feedlot environment.

Purpose: This study determined the prevalence and genotypic antimicrobial resistance (AMR) profiles of *Salmonella* serotypes in feedlot environment and cattle samples and their associations with feedlot pens, geographic location, seasonality, and nutritional treatments.

Methods: Six sample types (cattle feces [n = 824], composite environmental manure pack [n = 210], composite environmental dry [n = 206], water [n = 146], feed [n = 149], and cattle lymph nodes [n = 227]) were collected at the West Texas A&M University Research Feedlot in Canyon, TX, from June-December 2019. The cattle for this study were part of an ongoing nutritional trial that included four dietary treatments (wet distillers grain, sweet bran, combination and control) at the pen level. Thirty pens (n = 360 cattle) were selected, evenly distributed across dietary treatment and geographic location (front, back and side rows) in the feedlot, for this study. *Salmonella* were selectively cultured from samples and confirmed via MALDI-TOF. Whole genome sequencing was conducted on environmental, fecal and lymph node isolates.

Results: Prevalence of *Salmonella* was highest amongst composite environmental manure pack samples (77.6%, n = 163/210) followed by composite environmental dry (72.3%, n = 149/206) and fecal samples (72.2%, n = 595/824). *Salmonella* was identified in 42.3% (n = 96/227) of cattle lymph nodes. *Salmonella* presence varied significantly by month (P<0.0001), feedlot pens (P=0.005) and pen movement (P<0.0001) but not geographic location (P=0.236) or nutritional treatment (P=0.201). Seven *Salmonella* serotypes were identified: Montevideo (n = 110), Kentucky (n = 114), Anatum (n = 81), Lubbock (n = 44), Cerro (n = 28), Virginia (n = 4) and Derby (n = 1). Interestingly, none of the sequenced isolates harbored AMR genes.

Significance: Determining the relationship between *Salmonella* in the feedlot environment and cattle will facilitate the development of pre-harvest environmental treatments, such as phage therapy, to reduce prevalence or shift to pan-susceptible serotypes of *Salmonella* in slaughter-ready beef cattle.

T4-08 Intracellular Autolytic *Salmonella* Vaccine in Preventing Salmonellosis

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Introduction: *Salmonella enterica* is one of the major pathogenic bacteria causing human foodborne disease. Approximately 1.2 million people are annually infected with *Salmonella* in the US, causing 23,000 hospitalizations and 450 deaths. The development of efficient vaccine is important for the prevention of *Salmonella* infection, to reduce *Salmonella* food contamination, thereby relieves the huge burden for medical cost.

Purpose: The purpose of this study is to develop am intracellular autolytic *Salmonella enterica* serovar Typhimurium strain, and to evaluate its effectiveness on poultry gut *Salmonella* colonization.

Methods: *Salmonella* Typhimurium bacteriophage genes were integrated into non-pathogenic *Salmonella* Typhimurium strain for autolysis under intracellular conditions. Adherence activity of the vaccine strain (STLT2 INC-ATLY) was evaluated on chicken macrophage cell line (HD11). One-day-old chicken were provided with STLT2 INC-ATLY for vaccination, followed by pathogenic *Salmonella* challenge. Serum cytokine expressions in chicken were examined by quantitative PCR and enzyme-linked immuno-sorbant assay. Intestinal microbial genomic DNA was subjected to 2×300 bp paired-end 16S rRNA sequencing on Illumina MiSeq system for microbiome analysis.

Results: The intracellular viability of STLT2 INC-ATLY was reduced by ~94% (P < 0.05) in HD11 cells. Vaccination of STLT2 INC-ATLY in chicken successfully prevented (P < 0.05) the follow-up induced *Salmonella* Typhimurium colonization in cecum, jejunum, and ileum; meanwhile, the total *Salmonella* intestinal colonization was significantly reduced (P < 0.05) by ~1.5 log. The expressions of IL-1β, IL-6, IL-8, IL-10, IL-12, IL-18 LITAF, iNOS, TNF-α, TLR3, and TLR7 were

significantly enhanced by differential folds ($P < 0.05$). STLT2 INC-ATLY induced modulation of intestinal microbial composition through decreasing the abundance of Proteobacteria especially *Salmonella*.

Significance: STLT2-INC-ATL, the intracellular autolysis *Salmonella* strain, can be a promising candidate for *Salmonella* vaccination in chicken, to reduce or prevent the risk of *Salmonella* contamination and salmonellosis in human.

T4-09 Effect of Turkey-Derived *Lactobacillus* Probiotics and *Trans*-Cinnamaldehyde Against Multidrug-Resistant *Salmonella* Heidelberg in Turkey Poults

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❖ ***Developing Scientist Entrant***

Introduction: Reducing the cecal colonization of *Salmonella* in turkeys is a critical step to mitigating the risk of pathogen contamination during the subsequent stages of production. Probiotics and essential oil compounds could be effective pre-harvest interventions to reduce multidrug-resistant (MDR) *Salmonella* in turkeys.

Purpose: The objective of this study was to determine the efficacy of two turkey-derived *Lactobacillus* strains (LB; *L. salivarius* UMNPBX2 and *L. ingluviei* UMNPBX19), and *trans*-cinnamaldehyde (TC) against MDR S. Heidelberg in 14-day-old turkey poults.

Methods: Forty, day-old turkey poults were randomly allocated to one of five groups ($n = 8$). Three are treatment groups that were given either LB (~10 log CFU/mL), TC (0.08% vol/vol), or both through drinking water from day 0. Negative (NC; no *S*. Heidelberg inoculated, no treatment) and positive (PC; *S*. Heidelberg inoculated, no treatment) controls were also included. On day 7, poults in the treatment and PC groups were challenged with ~4.5 log CFU MDR *S*. Heidelberg by crop gavage. Cecal *S*. Heidelberg enumeration was performed 7 days post-inoculation on XLD agar. The experiment was replicated twice, and ANOVA was performed to determine significance at $P < 0.05$.

Results: Individual TC and LB supplementation reduced cecal *S*. Heidelberg populations by 1.2 and 1.7 log CFU/g compared to the PC group, respectively ($P < 0.05$). A greater reduction of *S*. Heidelberg populations (2.7 log CFU/g) was obtained when both TC and LB were applied together ($P < 0.05$).

Significance: Based on the reduction in cecal *S*. Heidelberg, supplementation of TC and LB through drinking water could be suitable pre-harvest interventions against *S*. Heidelberg in turkeys. Furthermore, utilizing both in combination has the potential to improve their efficacy as observed with the increased reduction in cecal *S*. Heidelberg populations.

T4-10 Effects of Common Litter Treatments on *Campylobacter jejuni* Cross-Contamination in Broilers

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Introduction: After a flock of chickens is harvested, their litter is reused for the next flock, possibly spreading *Campylobacter jejuni* to subsequent flocks.

Purpose: The objective was to determine if applying common litter treatments after harvesting a *C. jejuni*-contaminated flock can prevent cross-contamination of subsequent flocks.

Methods: To simulate natural contamination of litter, a flock of 1,250 broilers was placed into 25 indoor floor pens with fresh pine shavings for bedding material. On day 14 of the trial, 5 birds from each pen were challenged with a cocktail containing three ciprofloxacin-resistant marker strains of *C. jejuni* and three wild-type strains of *C. jejuni*; the total dosage was approximately 7 log $CFUmL^{-1}$. The spread of *C. jejuni* throughout the flock was monitored by sampling ceca from 5 birds/pen and collecting boot swabs from each pen on days 14, 21, 28, 35, and 42. After day 42, this flock was euthanized and 5 litter treatments were applied to 5 pens each. These treatments consisted of a negative control, a positive control, windrow composting, treatment with sodium bisulfate, and windrow composting in combination with sodium bisulfate treatment. After applying treatments, a second flock of 1,250 broilers was placed and the sampling scheme from the first flock was repeated. All samples were analyzed for *C. jejuni* by spread plate enumeration and enrichment followed by detection using the 3M Molecular Detection System.

Results: The results for flock one showed that by day 21, 80% of samples were positive for *C. jejuni*. In addition, typical colonies were recovered on Campy Cefex supplemented with ciprofloxacin at 1 mg/L. Both of these facts indicate that the marker strains successfully colonized the birds. Results for flock two are currently pending.

Significance: If common treatments for used litter cannot prevent cross-contamination of subsequent flocks with *C. jejuni*, it would indicate the need to develop more effective litter treatment methods.

T4-11 Growth of *Escherichia coli* O157:H7 on Romaine Lettuce Leaves Under Different Conditions of Relative Humidity is Associated with Leaf Properties and Composition of Resident Bacterial Communities

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Introduction: The success of a human pathogen such as *Escherichia coli* O157:H7 to grow and survive as a plant-associated microbe is modulated by its interaction with the plant host, with the resident microbial communities and its adaptability to the environmental conditions.

Purpose: The purpose of this study was to evaluate the growth of *E. coli* O157:H7 on romaine lettuce plants cultivated under different relative humidity (RH) conditions and determine what factors can be associated with high or low growth of the pathogen.

Methods: Plants were grown and evaluated under three levels of RH: 83%, 62%, and 43%. Drop-inoculations of *E. coli* O157:H7 were performed on 24 plants, 48 leaves, and 576 different areas across leaves under each RH, using low (10^3 CFU) and high (10^6 CFU) inoculum doses. Plate counts were recorded after 16 and 112 hours of inoculation. Leaf properties such as leaf wettability, stomatal density, and cuticular waxes were quantified. Illumina sequencing was performed on leaf areas which exhibited high and low growth of *E. coli* O157:H7, to characterize significant differences between the resident bacterial communities in these contrasting cases.

Results: RH was the main factor modulating the fate of *E. coli* O157:H7 and the composition of resident bacterial communities on romaine lettuce. Not only RH at the time of inoculation, but also RH used for lettuce cultivation determined the growth of *E. coli* O157:H7 on these plants. Higher growth or slower decline of *E. coli* O157:H7 was observed with low inoculum dose and humid condition ($P < 0.05$). Also, higher growth was found in the upper halves of leaves ($P < 0.05$) which was associated with decreased leaf wettability in this area. *Microbacterium* and an unclassified genus from the *Rhizobiaceae* family were found as biomarkers of bacterial communities where *E. coli* O157:H7 reached higher and lower population counts, respectively.

Significance: Meaningful data about RH conditions and potential biomarkers are provided for safe indoor lettuce growing systems.

T4-12 Survival and Transfer of Escherichia coli in Soils and on Radishes

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Introduction: Biological soil amendments of animal origin (BSAAO) have been utilized to improve crop yield and soil quality. However, BSAAO may introduce pathogens and increase their transfer to root crops and potential for foodborne illness.

Purpose: Evaluate the survival of *E. coli* in soils containing BSAAO and transfer to radishes.

Methods: In summer 2019 and 2020, twenty soil plots (3 m^2) were amended with composted poultry litter (CPL), or inorganic fertilizer (UN) in quadruplicate. Each plot was spray-inoculated with *E. coli* TVS355 (1 L, 6 log CFU/mL). Plots were planted with radishes (Early Scarlet). *E. coli* were recovered from composite soil samples (n = 80) collected every 20 days (up to day 40-days post inoculation (dpi)) and radishes (n = 60), harvested by day 40. Climatic factors such as rainfall, soil and air temperatures were recorded. Data were analyzed using one-way ANOVA (P < 0.05).

Results: A significant (P < 0.05) decrease in *E. coli* populations (~4.7 log CFU/g) was observed between 0 and 40 dpi in all plots. In 2019, *E. coli* populations were reduced significantly to undetectable levels in CPL- and UN-amended soils by 40 dpi. However, in 2020, CPL-plots supported significantly higher levels of *E. coli* (4.4 log CFU/g) compared to UN-plots (2.4 log CFU/g). *E. coli* levels decreased to 2.9 log CFU/g in CPL- and to undetectable levels in UN-plots (<0.4 log CFU/g) by 40 dpi. Significantly greater transfer of *E. coli* to radishes (4 log CFU/radish) was observed in CPL-plots compared to UN-plots (1.3 log CFU/radish) in 2020. Greater *E. coli* transfer to radishes was observed in 2020 (4 log CFU/radish) than in 2019 (2.0 log CFU/radish) in CPL-plots. More rainfall was recorded in 2020 (12.2 in) compared to 2019 (4.8 in).

Significance: *E. coli* survived in soils and transferred to radishes at higher levels in 2020 and CPL-plots compared to UN-plots, indicating that the combination of CPL and precipitation extended bacterial survival in soils.

T5-01 Farms Preparedness for FSMA PSR Inspections Based on Farm Size

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Introduction: On-Farm Readiness Reviews (OFRR) have been conducted since 2018 to assess farm preparedness nationally for Food Safety Modernization Act Produce Safety Rule (FSMA PSR) inspections.

Purpose: Our objective is to track readiness for FSMA PSR inspections, identify areas that should be prioritized for improvement including practices necessary for FSMA PSR compliance, and to inform technical assistance providers of educational outreach needs.

Methods: The anonymous survey has been completed at the conclusion of OFRRs conducted on U.S. farms. This survey tracks the FSMA PSR covered farm practices, priority improvement areas, FSMA PSR inspection readiness, and priority needs for compliance, as inputted by assessors. The results of this survey are shared with individual states, regional centers, the Food and Drug Administration, and the National Association of State Departments of Agriculture.

Results: Since April 2018, 1,427 OFRRs representing activity in 43 states and one U.S. territory have been completed with anonymous online survey responses. Farms selling over $500,000 of produce in an average year had 48% (n = 328) meet the FSMA PSR requirements, farms selling between $250,000 and $500,000 had 39% ($n$ = 90) meet the FSMA PSR requirements, and farm selling between $25,000 and $250,000 had 27% ($n$ = 115) meet the FSMA PSR requirements. Time and technical assistance were the top two priority needs for farms in all size ranges who did not meet the FSMA PSR standards during their OFRR. Time was the highest priority with 37% (n = 761) of the farms needing to implement changes to meet the FSMA PSR requirements. Technical assistance was the second priority need for farms with 24% (n = 492) requiring it to meet the FSMA PSR requirements.

Significance: This survey assesses the preparedness of farms for FSMA PSR inspections, and provides a framework for generating national, regional, and state assistance to help growers meet FSMA PSR implementation needs.

T5-02 Harvest Practices Aid in Pathogen Transfer as a Result of Animal Intrusion

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Introduction: Foodborne outbreaks have been linked to events in which animal intrusion was suggested as the source. This is evident in the 2006 *E. coli* O157:H7 outbreak linked to feral pigs and contaminated spinach.

Purpose: The purpose of this study was to evaluate microbial contamination and transfer from romaine lettuce to harvesting tools and equipment as a result of a simulated animal intrusion event under commercial scale growing and harvesting conditions in Yuma, AZ.

Methods: Fecal slurries were prepared from three different animal species: horse, goat, and pig and inoculated with *E. coli* TVS353 at 6.0 x 10E7 per 0.5 mL of slurry. In each trial, 25 mL of slurry was directly applied to the surface of romaine heads, field furrows, and/or beds. At 7 days post inoculation, a standard "S", and "Z" pattern were collected for pre-harvest raw product sampling n = 60. Additionally, 1-acre field plots were evaluated during harvest. Swab samples were collected from workers gloves, knifes, table, conveyer belt, and elevator for a total of 5 stops per acre of harvested product. Following harvest, 1,500g of grab samples were collected from four bins of harvested romaine. Raw product and swab samples were enriched and plated onto ChromAgar ECC + 80 µg/mL Rifampicin to determine +/- for *E. coli* TVS353.

Results: The pre-harvest results indicated 0/4 of *E. coli* on both "S", and "Z" patterns demonstrating that pre-harvest sampling was unable to detect animal intrusion contamination. Alternatively, positive swabs were detected at stops 2 through 5 for all zones except the conveyer belt. Samples from post-harvest bins demonstrated 9/12 positive indicating the ability of field contamination to transfer from localized areas to harvesting tools and equipment.

Significance: Results indicated that harvest practices aid of the spread and "amplification" of bacterial contamination. Best management practices may include improved or increased frequency of equipment sanitation, changing gloves periodically.

T5-03 Sunlight Affects Both Viability and Die-Off of *Salmonella* and *Escherichia coli*

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Introduction: Sunlight is crucial for crop production, and has been known to induce different levels of inactivation among bacterial species.

Purpose: The decay in bacterial population of *E. coli* O157: H7, *Salmonella* Newport and a pond isolate of *E. coli* over time due to sunlight exposure was compared.

Methods: *E. coli* O157: H7, *S.* Newport and a pond water-*E. coli* isolate were suspended in sterile deionized water (8 log CFU/mL) and exposed to sunlight for a duration of six hours on three different days (D1, D2, D3). Bacterial population was enumerated every 30 min. UV, temperature and light fluence were also measured. Live: Dead staining was also performed to determine cell viability. The mean population of bacteria from 3 repeats each day were compared for significant differences. Controls experiments were performed in the dark.

Results: The average temperatures on D1, D2 and D3 were 20.08, 28.32 and 26.57°C, respectively (P < 0.05). The mean light intensity was D1- 69.67, D2- 68.97 and D3- 44.85 klux with D3 being significantly lower (P < 0.05). The mean UV intensity on D1, D2 and D3 were 5.65, 4.59 and 2.82 mW/cm^2, respectively (P < 0.05). Population decrease of *E. coli* was different on all 3 days (P < 0.05). A significant drop in *E. coli* O157: H7 population occurred on day 2 (5.28 log CFU/mL) and on day 3 (6.5 log CFU/mL), at 60 min of sunlight exposure. Population reduction of *S.* Newport was different on all three days (P < 0.05). *S.* Newport population on day 3 dropped within 30 min (6.06 log CFU/ml) of sunlight exposure (P < 0.05). Live: Dead staining indicated that sunlight-exposed bacterial cells transitioned to a non-culturable state after 6 h of exposure.

Significance: The findings could help growers evaluate the risk of foodborne pathogens presence in agricultural waters.

T5-04 Fate of *Salmonella* and *Listeria monocytogenes* on the Surface of Whole Mangoes during Storage

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Introduction: Previously linked to outbreaks of salmonellosis, highlight the importance of understanding the fate of pathogens on mango surfaces during storage.

Purpose: The growth kinetics of *Salmonella* (SAL) and *Listeria monocytogenes* (LM) on the surface of whole mangoes was explored.

Methods: Experiments were conducted in triplicate with duplicate samples (n = 6) to determine the growth curves. Three varieties of mangoes (Tommy Atkins, Ataulfo, and Kent) were spot inoculated with a rifampicin resistant multi-strain cocktail of SAL, or LM (100 µL) at 6 log CFU/mango, allowed to dry, stored at 12, 20 or 30°C and sampled up to 21 days. SAL and LM were enumerated following a rub-shake-rub on selective (XLT4 and modified oxford agar) and non-selective (tryptic soy agar and brain heart infusion agar) supplemented with rifampicin. Populations were expressed as log CFU/mango. To describe pathogen growth as a function of time and temperature, primary and secondary modeling was performed.

Results: SAL is more likely than LM, to grow on the surface of whole mangoes. SAL increased linearly with temperature on Kent; growth rates were ~0.004, 0.01, and 0.06 log CFU/mango/h at 12, 20, and 30℃, respectively. At 20 and 30℃, growth rates were significantly higher than 12℃ (P < 0.05). However, no clear pattern was observed on Tommy Atkins and Ataulfo. On Tommy Atkins, at 12 and 20℃, no difference in *Salmonella*'s growth rates (0.006 log CFU/mango/h) was observed, while on Ataulfo, the growth rate decreased at 30℃. Populations of *L. monocytogenes* declined regardless of temperature.

Significance: No meaningful reductions of *Salmonella* were observed on the surface of whole mangoes. These results imply that postharvest microbial reduction rates should not be relied on as a corrective measure to meet mangoes' produce safety rule requirements.

T5-05 Effect of Sanitizers and Organic Load on Removal of Silver Nanoparticles from Contaminated Lettuce

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Introduction: Accelerated agricultural use of silver nanoparticles (AgNPs) may increase its residue levels on fresh produce, thus raising possible food safety and public health concerns.

Purpose: This study aimed to evaluate the impact of sanitizers and simulated leafy green processing water on removal of AgNPs from romaine lettuce using a simple bench-top model system.

Methods: Romaine lettuce pieces were first contaminated with AgNPs and then washed in a bench-top carboy system (4-L). Peroxyacetic acid (PAA; 80 mg/L; Tsunami 100) and chlorine (100 mg/L and pH 6.5 – 7.0; XY-12, Ecolab) were used with/without 2.5% (w/v) organic load (OL) as washing treatments and deionized water served as the control. Wash water samples were collected at 30-s intervals during 5 min of washing and analyzed for Ag using inductively coupled plasma mass spectrometry.

Results: Washing contaminated lettuce with PAA solution, chlorine solution, and water decreased the Ag residue levels by 7%, 4%, and 5%, respectively. The presence of OL decreased Ag residue levels by 3%, 3% and 0.006% when used chlorine, OL alone and PAA respectively, after 5 min of washing. Ag concentrations in the wash water increased during 5 min of lettuce washing, except for the PAA treatment with OL.

Significance: Commercial postharvest washing may be inadequate for removing AgNPs from Romaine lettuce. This highlights the necessity to develop an efficient washing method for AgNP removal from contaminated produce.

T5-06 Efficacy of Chlorine and Peroxyacetic Acid Against *Salmonella* and Shiga-Toxigenic *Escherichia coli* in Simulated Postharvest Water Systems with Varying Levels of Chemical Oxygen Demand

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Introduction: Postharvest water systems which recirculate water must be properly managed with antimicrobials to mitigate risk of foodborne pathogen cross-contamination in the presence of increasing organic load.

Purpose: The goal of this study was to determine the efficacy of commonly used antimicrobials [sodium hypochlorite (NaOCl) and peroxyacetic acid (PAA)] against Shiga-toxigenic *Escherichia coli* (STEC) and *Salmonella enterica* with water quality representative of that observed in apple packing flumes.

Methods: Organic load was varied by mixing varying amounts of soil, applesauce, and humic acid with deionized water and coarse filtered to simulate wash water with organic load of 30, 500 and 2,500 ppm of chemical oxygen demand (COD). Target concentrations of NaOCl (10 to 100 ppm) or PAA (25 to 80 ppm) were added to water. Water was portioned into 500 mL samples and inoculated with 7 log CFU/mL 5-strain cocktail of *Salmonella* or STEC. Five mL aliquots were taken at 15, 30, and 60 s, neutralized with 0.1 N sodium thiosulfate, serially diluted into Buffered Peptone Water (BPW) and enumerated onto Tryptic Soy Agar and selective media to determine inactivation.

Results: Keeping concentration and time constant, increasing organic load resulted in reduced inactivation of *Salmonella* and *E. coli* for both PAA and NaOCl. At a COD of 2,500 ppm, 50 ppm of PAA or NaOCl was required for at least a 3-log inactivation within 60 s for STEC and *Salmonella.* Exposure to 80 ppm PAA for 60 s, caused both microorganisms to be near or below detection limit (1 log CFU/mL) regardless of organic load. Overall, the efficacy of microbial inactivation was highly dependent on COD level, sanitizer concentration and exposure time for STEC and *Salmonella*.

Significance: This research highlights the need to set antimicrobial limits based upon expected COD to manage risk of cross-contamination within these postharvest washing systems.

T5-07 Isolation and Characterization of AmpC and Extended Spectrum β-Lactamase-producing *Enterobacterales* from Fresh Vegetables

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Introduction: AmpC β-lactamases and extended spectrum beta-lactamases (ESBL) inactivate commonly used β-lactam antibiotics, including penicillin and cephalosporin. Infections caused by ESBL-producing *Enterobacterales* are considered serious threats. Vegetables may serve as a reservoir for antibiotic resistant bacteria and resistance genes.

Purpose: We determined the prevalence of AmpC and ESBL-producing *Enterobacterales* in retail vegetables in the United States.

Methods: Eighty-eight conventional and organic vegetable samples were purchased from local grocery stores in Arkansas. Pre-enrichment was performed in buffered peptone water (BPW) for 3 hours prior to selective enrichment in *Enterobacteriaceae* enrichment (EE) broth. CHROMagar ESBL was used to isolate AmpC and ESBL-producing *Enterobacterales.* Blue and pink colonies grown on CHROMagar ESBL agar were selected for AmpC and ESBL production assays using double-disk combination tests following CLSI procedures. AmpC and ESBL-positive isolates were also tested for the susceptibility to 11 antibiotics using standard disk diffusion methods. The confirmed AmpC and ESBL-producing isolates were sequenced using Illumina Nextseq 500. Antibiotic resistance genes were identified using RGI (resistance gene identifier) against CARD database.

Results: Eight vegetable samples (9.09%) were positive for ESBL-producing *Enterobacterales* based on double-disk combination test using cefotaxime

with or without clavulanic acid. These included three *Enterobacter hormaechei* isolates and five *Serratia fonticola* isolates. In addition, two *E. hormaechei* isolates from two vegetable samples (2.27%) showed AmpC activity. The MIC of cefotaxime against these isolates ranged from 4 to > 32 µg/ml. *E. hormaechei* strains, S11-1, S17-1, and S45-4 possess an ESBL gene, bla_{SHV66}; whereas, five *Serratia fonticola* isolates contain genes encoding a minor ESBL, FONA-5. Genes encoding AmpC β-lactamases were found in *E. hormaechei* strains S43-1 and 74-2, which were consistent with AmpC production phenotypes. *E. hormaechei* isolates, S11-1, S17-1, and S45-4, were non-susceptible to multiple antibiotics, including ampicillin, amoxicillin/clavulanic acid, cefiderocol, chloramphenicol, gentamycin, nalidixic acid, trimethoprim/sulfamethoxazole, and tetracycline.

Significance: The study highlights the need for antimicrobial resistance surveillance in fresh produce products.

T5-08 Tomato Fruit Surface Metabolome Changes as Fruit Ripen Affect *Salmonella* Newport Association with Fruit

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Introduction: Tomato fruit may be harvested at the mature green stage or fully ripened. Ripe fruit has been implicated in several foodborne illness outbreaks of *Salmonella enterica.* Red fruit has been reported to be more favorable for *Salmonella* association.

Purpose: To determine how fruit surface phytocompounds shift during ripening and assess any relationship with *Salmonella*-fruit association.

Methods: Field-grown mature green and red tomato fruit cultivar 'Nyagous' were harvested. Some fruit were placed in sterile water for 3 h and the resultant solutions containing fruit exudates were filter-sterilized. *S.* Newport was inoculated at 3 log CFU/sample onto fruit or in fruit exudates and incubated at 37°C for 24 h. *S.* Newport was enumerated by serial plating. Pooled fruit exudate solutions were chemically profiled by gas-chromatography time-of-flight mass spectrometry (GS-TOF-MS). Data were analyzed for differences between mature green and ripe red fruit using JMP Pro 14.

Results: Fruit surfaces and exudate solutions of both mature green and ripe red fruit were able to support growth of *S*. Newport. Higher population counts of *S*. Newport were recovered from the surface of ripe red fruit than green mature fruit ($P < 0.05$). The exudates collected from ripe red fruit were more supportive of *S*. Newport growth than those from green tomatoes ($P < 0.05$), with a difference of 0.9 log CFU/fruit. GC-TOF-MS analysis revealed 145 identified compounds which included amino acids, sugars, sugar alcohols, fatty acids and phenolics. Peak heights for various amino acids and sugars were higher in ripe red fruit exudates than mature green fruit exudates. Sugar alcohols decreased in fruit surfaces as fruit ripened, as did several fatty acids and phenolics.

Significance: Various compounds found on the surface of fruit may support or inhibit association of *Salmonella* with mature tomato fruit and may explain differences in *Salmonella* association between mature green and ripe red fruit.

T5-09 Differences in Colonization and Internalization of *Salmonella enterica* serovars in Cucumber Fruit

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Introduction: Recent outbreaks of *Salmonella* have been associated with consumption of slicer cucumbers. Numerous studies have used cocktails consisting of multiple *S. enterica* serovars to identify serovar-specific responses in a variety of produce commodities. However, this approach may introduce *Salmonella* competition during enrichment.

Purpose: This study investigated the ability of five *S. enterica* serovars to individually colonize and internalize cucumber fruit following pre-harvest blossom inoculation.

Methods: Cucumber plants (*Cucumis sativus* var. *sativus*) from two slicing cultivars, Thunder and Marketmore76, were grown from commercial seed. Plants were maintained in the NCSU BSL-3P phytotron greenhouse. *Salmonella* contamination was introduced individually (serovar Javiana, Montevideo, Newport, Poona, or Typhimurium) via blossoms at ca. 2.4 log CFU/blossom. Cucumbers 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 to confirm single serovar inoculation and recovery. The Pearson Chi-Square Fisher's Exact test was used to determine significant differences in sample positivity (i.e., fruit colonization) obtained for inoculated fruit for both cultivars over each serovar treatment.

Results: Prevalence of surface contamination was equivalent when comparing cultivars [Thunder vs. Marketmore76 ($X^2 = 3.306$, $P = 0.0690$)] and serovars [Javiana: 22/32, 68.8%, Poona: 26/38, 68.4%; Montevideo: 15/26, 57.7%; Typhimurium: 20/35, 57.1%; Newport: 20/38, 52.6% ($X^2 = 3.143$, $P = 0.5342$)]. Percent positivity for internal samples was comparable among serovars [Montevideo: 5/26, 19.2%; Javiana: 5/32, 15.6%; Poona: 5/38, 13.2%; Typhimurium: 3/35, 8.6%; Newport: 3/38, 7.9%, ($X^2 = 2.621$, $P = 0.6231$)]. Interestingly, the probability for serovar Poona to internalize cucumber fruit was greater for Thunder (5/19, 26.3%) than Marketmore76 (0/19, 0.0%) [$X^2 = 5.758$, $P = 0.0164$].

Significance: This study demonstrated that produce-associated *Salmonella enterica* serovars can colonize cucumber fruit with equivalent frequencies when inoculated through the blossoms during pre-harvest. Enrichment and selection procedures may bias serovar fitness when using a cocktail of bacteria.

T5-10 Plant Growth-promoting Rhizobacteria *Pseudomonas* strains as Possible Agents to Enhance Food Safety by Limiting *Salmonella enterica* Association with Kale

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❖ ***Developing Scientist Entrant***

Introduction: Plant growth-promoting rhizobacteria (PGPR) promote plant health and can protect plants from biotic and abiotic stresses. Abiotic stresses such as drought alter plant physiology and can influence plant microbe-interactions. The impact that PGPR have on *Salmonella*-plant associations under adequate watering or drought is not understood.

Purpose: Evaluate the effects of PGPR and drought on the kale-*Salmonella* association.

Methods: Kale cultivar 'Improved Dwarf' plants were grown in a greenhouse (23°C, 16h L:8h D). Two separate root PGPR inoculations with *Pseudomonas* strains S2 or S4 (10^8 CFU/mL) or 0.1% peptone water (negative control) were carried out at the stem base of kale plants 2-days and 9-days post-germination. All plants were watered regularly for 5 days before being subjected to drought for 6 days or maintained under regular watering (control). About 10^6 *Salmonella* Newport cells were inoculated onto the adaxial surface of the third true leaf of plants and kept at room temperature. Inoculated leaves were clipped 24 hours post-inoculation for *Salmonella* quantification. Data were analyzed using JMP Pro 14.

Results: The retrieval of *S.* Newport was 2.75 log CFU/plant from control (regularly watered) and 2.18 log CFU/plant from drought-subjected kale plants that were not colonized by PGPR ($P < 0.05$). In plants whose roots were inoculated with *Pseudomonas* S2 and S4, exposure to drought did not affect *Salmonella* populations compared to regularly-watered plants. However, under regular watering conditions, the recovery of *S*. Newport was significantly lower from kale plants inoculated with *Pseudomonas* S4 (0.63 log CFU/plant) than from plants without the PGPR inoculation (2.13 log CFU/plant; $P < 0.05$). In drought-subjected plants, no further decline was detected in *Salmonella* from plants whose roots were colonized with PGPR and plants whose roots were uninoculated.

Significance: PGPR may be a promising strategy to enhance food safety by helping to limit *Salmonella* association with kale leaves during plant growth.

T5-11 Prevalence of *Listeria* Species on Food Contact Surfaces in Washington State Apple Packinghouses

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❖ ***Developing Scientist Entrant***

Introduction: The 2014 caramel apple listeriosis outbreak was traced back to cross-contamination between food contact surfaces (FCS) of equipment used for packing and fresh apples. For Washington State, the leading apple producer in the U.S, managing the risk of apple contamination with *Listeria monocytogenes* within the packing environment is crucial.

Purpose: To determine the prevalence of *Listeria* spp. on common FCS in Washington State apple packinghouses over two packing seasons, and to identify those FCS types with the greatest likelihood to harbor *Listeria* spp.

Methods: A range of 27-50 FCS were sampled in five commercial apple packinghouses to detect *Listeria* spp. at two timings for each sampling event, (i) post-sanitation and (ii) in-process (three hours of packinghouse operation). Each packinghouse was visited quarterly over two year-long packing seasons from August 2018 to July 2020. The isolation, detection, and confirmation of *Listeria* spp. were conducted following a modified FDA bacteriological analytical manual (BAM) method with PCR confirmation.

Results: Among all tested samples (n = 2,988), 4.6% (n = 136) samples were positive for *Listeria* spp. The FCS that showed the greatest prevalence of *Listeria* spp. were polishing brushes, dividers and brushes under fans/blowers, and dryer rollers. In 17.2% of the positive samples, *Listeria* spp. were detected at both timings of sampling ($P \leq 0.05$). Wax coating was the unit operation from which *Listeria* spp. were most frequently isolated (17.3%; 19/110). The prevalence of *Listeria* spp. on FCS increased throughout apple storage time.

Significance: This research is the first to assess the prevalence of *Listeria* spp. on FCS in apple packinghouses, and to identify those FCS most likely to harbor *Listeria* spp. Such findings are essential for the apple packing industry striving to exhaustively mitigate the risk of contamination with *L. monocytogenes* to prevent future listeriosis outbreaks and recalls.

T5-12 Characterization of the Relationship between Postharvest Fungal Rot and *Listeria Innocua* Die-Off Rates on Gala Apples during Long-term Storage

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❖ ***Developing Scientist Entrant***

Introduction: *Botrytis cinerea* (BC) and *Penicillium expansum* (PE) can colonize and rot apple tissue as storage time increases, which may impact the growth of saprophytic foodborne pathogens like *Listeria monocytogenes*. Decayed apples are exposed to other co-mingled fruit and packing equipment and may serve as a source of contamination.

Purpose: To determine population changes of *Listeria innocua* (LI) as a surrogate for *L. monocytogenes* on apples co-inoculated with BC or PE during 11 months of controlled atmosphere cold storage.

Methods: Gala apples (n = 540) were drenched in pyrimethanil and inoculated with LI in two-6.25 cm^2 spots on the equator, one of which was wounded. Apples were assigned to one of three treatments: LI-only or co-inoculation with BC or PE. Apples were treated with 1-methylcyclopropene and stored under controlled atmosphere conditions (1°C, 1% CO_2/2% O_2). LI population was determined at 1 week, and 1, 3, 6, 9, and 11 months. The inoculation site was excised, and cells eluted in 24 mL 0.1% peptone with 0.2% Tween 80, serially diluted, and plated on Modified Oxford Medium and incubated 48 h at 35°C. After three months, LI consistently fell below the limit of detection (1.35 log CFU/g) and samples were enriched following a modified BAM method with PCR confirmation.

Results: Populations of LI decreased by up to 5.57 log CFU/cm^2, with significant differences in populations based on wound status and treatment. LI survival was greater across timepoints on wounded surfaces ($P < 0.0001$). LI survival was greater in LI-only and BC treatments for all but one time point on wounded surfaces ($P < 0.05$). Prevalence of LI varied from 6-11 months in storage on wounded [80% (72/90)-62% (52/84)] compared to unwounded [74% (67/90)-5% (4/84)] samples.

Significance: LI survival depended on wound status and fungal species, suggesting risk may depend on type of postharvest rot. Postharvest interventions should emphasize removal of infected or punctured fruit as soon as possible before or during packing.

T6-01 Remote Inspection and Audit: First Pilot Project in the World That Uses Augmented Reality to Conduct Remote Inspections on Food Safety with the Official Italian Authority in Italy

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Introduction: We have officially launched a pilot test to test augmented reality to guide official inspections in Italian-based food industries from remote locations.

Purpose: Our aim was to study the effectiveness and usability of these tools in the real food industry environment.

Methods: We chose a smart glass available on the market, usually used for remote assistance for maintenance needs. This tool has never been used internationally in the food industries for live streaming inspections. Using the official checklist developed by the official Italian veterinary services of Parma (AUSL Parma) we tested this new tool in the ham factory. From the factory headquarters we started guiding the Veterinary Officers through the factory from the outside area following the company flow chart. The tool gives the possibility to virtually share checklists, photos and videos with the inspectors thanks to a wifi connection.

Results: The instrument adopted was able to cancel the noise of the system giving a real possibility to ask questions related to the official check list and receive answers from the floor; Veterinarians were able to take pictures via direct streaming, share company registers, labels and monitors with food safety measures (temperature) with the "Driver" office. In the meantime, the "Driver team" was able to record the streaming video and all the photos and activities carried out on the track from the main office. The detected limits are related only to the wifi connection, which could be easily enhanced in several internal industrial areas.

Significance: These new tools are the future of the next generation of inspections and audits that can also enhance and increase the capabilities of officials in shorter time frames.

T6-02 Crop-Livestock Integration in Vegetable Production; Survival of Generic *E. coli* and Non-O157 STEC in Organic Fields Grazed by Sheep

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Introduction: Livestock grazing of cover-cropped fields in produce operations may enhance soil health benefits. However, microbial food safety is not clearly understood while this practice has been expanding. It may introduce foodborne pathogens into the soil, potentially transferring to produce.

Purpose: The objective of this study is to evaluate generic *E. coli* (gEc) and Non-O157 Shiga toxin-producing *E. coli* (STEC) prevalence and persistence in sheep grazing winter cover-cropped fields.

Methods: Two-year (2019-2020) randomized complete block design study was performed on USDA-NOP certified organic research field, with 4 replicates of three treatment types (winter cover-crop, grazed with sheep (WG) or tilled (WT), fallow (WF)). Grazing was conducted once in 2019, and twice in 2020 before planting vegetables. Feces was tested for foodborne pathogens before each grazing. Prevalence of gEc and STEC in soil samples were evaluated pre- and post-grazing (4 samplings after the last grazing day (D-0)) each year. Descriptive statistics and ANOVA were used to determine pathogen prevalence and compare gEc Most Probable Number (MPN) in soil among treatments each sampling.

Results: In 2019, gEc prevalence in WG (71.7% (43/60)) was higher than in WT (26.7% (16/60)) or WF (40% (24/60)). A significant difference in gEc MPN (log MPN/g) among treatments was observed until D-82 ($P < 0.001$). In 2020, gEc prevalence was also higher in WG (62.5% (90/144)) than in WT (35.4% (51/144)) or WF (34.7% (50/144)), with a significant difference observed until D-46 ($P = 0.05$). STEC was detected once in WF in 2019 (0.5% (1/188)) and 2020 (0.2% (1/432)), and its prevalence in feces pre-grazing was 30% (6/20) in 2019 and 23% (11/48) in 2020.

Significance: Results will aid the sustainability of food systems through valuable science-based knowledge and tools to enhance the food safety of produce in integrated crop-livestock systems.

T6-03 How Does Cross-Contaminated Escherichia coli O157:H7 on Fresh-Cut Lettuce Behave?

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❖ ***Developing Scientist Entrant***

Introduction: Since cross-contamination of bacterial pathogen to food surfaces would be one of the main sources of foodborne illness, many studies on cross-contamination of bacterial pathogens have been conducted. However, there are few studies on growth kinetics of cross-contaminated pathogenic bacteria on foods due to complicated experimental setup. Therefore, the growth kinetics of bacterial pathogen on food surface after cross-contamination would play an important role in evaluation of bacterial behavior for quantitative microbial risk assessment.

Purpose: The objective of this study was to investigate growth kinetics of *Escherichia coli* O157:H7 on fresh-cut lettuce after cross-contamination from stainless steel surfaces.

Methods: Pre-grown *E. coli* O157:H7 were inoculated on a stainless steel piece (1 cm × 1 cm) surfaces (ca. 10^7 CFU/cm^2) and dried in safety cabinet for 60 min, and then the contaminated stainless steel pieces were stored at 5°C and 50-60% relative humidity for up to 7 days. Fresh-cut lettuces (3 cm × 3 cm) were attached to the contaminated stainless steel pieces to realize cross-contamination. The number of *E. coli* O157:H7 on the cut lettuce was determined during storage at 25°C for 30 h. The observed growth kinetics was described by Baranyi-Roberts model using Bayesian inference.

Results: The growth kinetics of cross-contaminated *E. coli* O157:H7 on the cut lettuce showed almost same the growth behavior with directly inoculated *E. coli* O157:H7 on cut lettuce. No lag times and the similar maximum specific growth rate (0.4 -0.5 (1/h)) were observed in the growth kinetics regardless with or without cross-contamination. The *E. coli* O157:H7 on the lettuce regardless the inoculation methods reached by stationary phase after 21-30 h incubation.

Significance: The growth kinetics of *E. coli* O157:H7 on lettuce would not be influenced by prior exposure to desiccation stress on stainless steel surface and the contamination process.

T6-04 Pre-Exposure to Protective Bacterial Cultures in Food Attenuates Listeria monocytogenes Virulence

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Introduction: Commercially available protective bacterial cultures (PCs) have been shown to exhibit bacteriostatic or bactericidal effects against foodborne pathogens such as *L. monocytogenes*. However, pathogen survival and growth in foods have been demonstrated and the potential effect of PCs on *L. monocytogenes* virulence in the absence of growth inhibition is not known.

Purpose: To determine the effect of pre-exposure to PCs on *L. monocytogenes* virulence *in vitro* using milk as a model food.

Methods: *L. monocytogenes* Scott A was cultured alone (control) or cocultured with PCs of *Lactococcus lactis* (PC1) or *Lactobacillus plantarum* (PC2, PC3) in UHT milk incubated at 37°C for 16 h. *L. monocytogenes* were separated from coculture by immunomagnetic separation to isolate the effect of pre-exposure to PCs. Captured cells were immediately subjected to simulated gastrointestinal survival assays. Changes in adhesion, invasion, and translocation capabilities were also determined using Caco-2 cells. All experiments were repeated three times and significance was considered when $P < 0.05$.

Results: *L. monocytogenes* cells separated from cocultures with PC2 or PC3 were more sensitive to simulated gastrointestinal conditions with counts ~ 1.3 log CFU/mL lower than control (~ 4 log) at the end of simulation ($P < 0.001$). Pre-exposure to PCs did not affect adhesion capabilities of *L. monocytogenes*. However, pre-exposure to PC1 and PC3 significantly reduced invasion by 1.5 and 2.3 log CFU/mL, respectively, compared to control (3.6 log CFU/mL) ($P < 0.001$). Pre-exposure to PC1, PC2, or PC3 also reduced translocation by 1.8, 2.1, and 1.5 log CFU/mL, respectively, compared to control (3.8 log CFU/mL) ($P < 0.001$).

Significance: These findings demonstrate the potential ability of PCs to enhance the safety of food products by attenuating *L. monocytogenes* virulence, thereby limiting its ability to cause infection upon ingestion.

T6-05 Persistence of the Enveloped phi6 Bacteriophage on the Surface Farmers' Markets Fomites

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Introduction: With an increase in farmers markets across the U.S., consumers' demand for fresh produce has been growing. Cross-contamination issues could be a concern within farmers markets from contaminated surfaces (fomites) to hands, produce, or other surfaces.

Purpose: The goal of this study was to investigate the survival and persistence of phi6 bacteriophage on farmers market fomites and simulate cross-contamination events within the market.

Methods: The persistence of phi6 was assessed over 30 days. Plastic, cardboard, molded pulp fiber, wicker, and tablecloth coupons were inoculated with 10^8 PFU mL^{-1} phi6, and plaque-forming units were quantified over 30 days (23 ± 2°C). The virus transfer rate from fomites and hands to produce was

also assessed at high (10^7 PFU mL^{-1}) and low (10^3 PFU mL^{-1}) concentrations at 23 ± 2°C.

Results: The bacteriophage persisted for 13 days on plastic, 4 days on cardboard and molded pulp fiber, 16 days on wicker, and up to 2 days on the tablecloth. For the transfer rate, the mean transfer rate from fomites to produce ranged from 21% to 30%, and hands to produce ranged from 21% to 29%. While with a low virus level (10^3 PFU mL^{-1}), the transfer rate from fomites to produce ranged from 10% to 30%, and no virus was detected from hands to produce. The results indicated that phi6, a surrogate for SARS-CoV-2, could survive and be transmitted through fomites to hands and produce.

Significance: The results highlight the high risk of cross-contamination from fomites. Single-use containers such as cardboard, molded pulp fiber, wicker, and fomites that can be washed, rinsed, and sanitized are recommended for use at farmers markets.

T6-06 Development and Characterization of Electrospray Starch/Yellow Mustard Mucilage Coatings for the Preservation of Cherry Tomatoes

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❖ ***Developing Scientist Entrant***

Introduction: There is an urgent need to develop biodegradable material from natural polymers for active packaging. The usage of synthetic polymers is high in generating active packaging material, and the advantage of natural polymers is still undiscovered.

Purpose: The objective of the current study is to use a natural polysaccharide, water soluble yellow mustard mucilage (WSM), with starch, to fabricate an antimicrobial coating material to extend the shelf life of agriculture products using cherry tomatoes as a model.

Methods: Nanoemulsions containing thymol and carvacrol (1:1) and shell materials (WSM and starch) at various ratios were homogenized by a high-pressure microfluidizer. The emulsions were electrosprayed to fabricate the essential oil-loaded WSM/starch nanoparticles. The emulsion's viscosity, surface tension, and conductivity were optimized to generate materials with ideal structural, functional, and physicochemical properties. The morphology, encapsulation efficiency, molecular interaction, release kinetics, and antimicrobial activity of the particles were studied. The particles were subjected to coating on package containers for tomatoes storage. The shelf life, color, texture, sensory attributes were further analyzed.

Results: The SEM confirmed the uniform non-porous microcapsules with the particle diameter ranged from 120.8 to 150 nm. The infrared spectrum showed a band at 1708 cm^{-1} due to the WSM-starch interaction. Encapsulation efficiency (EE) of 84.5% ($P \leq 0.05$) was obtained as the interactions lead to surface hydrated layers. Based on EE, oil loaded nanoparticles (30% w/w) were chosen for further studies. The methyl group in WSM holds the oil and controlled their diffusion up to 120 h. For the application of tomatoes, nanoparticles possessed high antibacterial activity against *E. coli, Salmonella dublin, S. aureus,* and *Pseudomonas fluorescens* for 14 days ($P \leq 0.05$), without affecting the color, texture, and sensory properties.

Significance: The fabricated particles exhibited superior properties in microbial prohibition and shelf life extension.

T6-07 The Physicochemical Properties of Powders are Associated with the Ease of Removal from Surfaces Using Scraping and Brushing

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❖ ***Developing Scientist Entrant***

Introduction: Dry cleaning activities are used to remove food soils and allergenic residues from equipment surfaces. However, their relative efficacy in different environments is poorly understood.

Purpose: The purpose of this study was to investigate the efficacy of scraping and brushing in removing milk powder and wheat flour equilibrated at different relative humidity (RH) levels from stainless steel coupon surfaces.

Methods: Wheat flour or milk powder was deposited evenly on 10.16 ×10.16 cm^2 stainless steel coupons (thickness: 1.69 ± 0.09 mm) and equilibrated at 11%, 76%, and 88% RH before brushing/scraping. The brushing/scraping was performed using a custom platform for consistent application of the cleaning treatment. The weight (mass) of residual powder after each pass of scraper/brush was measured using an analytical balance. Coupons were considered clean when the mass difference of remaining soil and coupon was within 0.0005 g for two consecutive passes. Coupon surfaces were tested using ATP swabs, protein swabs, and specific allergen LFDs to detect remaining residues. All experiments were conducted in triplicate.

Results: Scraping was significantly less effective than brushing in the removal of powders under all conditions. RH did not significantly affect the removal of wheat flour, although residual protein was detected following brushing at 88% RH. The number of brush passes required to remove milk powder at 88% RH was significantly greater than for samples at lower RH levels. Moreover, surface protein residues were detected following brushing of milk powders at 76% and 88% RH. Greater cohesion among milk powder particular and adhesion to coupon surfaces were detected for milk powder at 76% and 88% RH.

Significance: These results will assist in the development of targeted dry cleaning processes. Additionally, the outcomes highlight the potential for allergenic residue retention after currently available dry sanitation. The need for condition-dependent utility of different sanitation verification activities is evident.

T6-08 Prevalence and Mapping of *Listeria* spp. and *Listeria monocytogenes* in Small and Very Small Food Manufacturing Facilities in Nebraska

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Introduction: Under the FSMA-PCHF rule, environmental monitoring of *Listeria monocytogenes* is required especially for ready-to-eat foods if post lethality contamination is a risk. Meeting this requirement presents more obstacles for small and very small business operators due to limited resources and lack of in-house expertise.

Purpose: To determine the prevalence of environmental *Listeria* spp/*Listeria monocytogenes* in small and very small food manufacturing facilities in Nebraska.

Methods: Three food processing facilities (A, B, and C) in Nebraska were involved and included two RTE and one non-RTE frozen food company. In each facility, 25 to 30 sites representing zones 1 to 4 were mapped and swabbed with cellulose sponge sticks and swab samplers pre-hydrated in D/E neutralizing broth. Each facility was visited three times and samples collected at least three hours into production. *Listeria* spp. was detected using the 3M™ Petrifilm Environmental *Listeria* Plates by extracting 2 mL of broth from respective samples, performing a 1:2 dilution in 4 mL of BPW, and incubating for an hour at 25°C to cell resuscitation before plating. Detection in the 3M™ Molecular Detection System involved sample enrichment in 100 mL of Demi-Fraser broth for 28 h at 35°C. *Listeria* spp colonies in Petrifilm were streaked on MOX agar to observe for typical colonies.

Results: Overall, *Listeria* spp were detected in 14 of 266 (5.3%) samples. *Listeria* spp were prevalent in all facilities with B having the highest prevalence (5.9%; 5/85) followed by C (5.6%; 5/89) and finally A (4.9%; 4/92). Facility B was also the only facility that had *Listeria* spp. positive samples from food contact surfaces. Non-food contact surfaces like drains and floors had the most contamination. This data supports the importance of management and sanitation of non-food contact surfaces like drains and floors.

Significance: Our study provides data that will enable small companies to comply with FSMA-PCHF rules through the establishment of an environmental monitoring program.

T6-09 Evaluation of the Ability of Two *Enterococcus* spp. Strains to Inhibit *Listeria monocytogenes* in Monoculture and in the Context of a Complex Microbiome

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Introduction: *Listeria monocytogenes* (Lm) is a concern in produce packinghouses because of its ability to persist through formation of biofilms with the environmental microbiome. Previous studies demonstrated lactic acid bacteria (LAB) to be effective biocontrols for Lm in both lab and post-harvest meat processing facilities.

Purpose: We aimed to evaluate the ability of LAB strains ATCC PTA-4761 and PTA-4759 to inhibit Lm in a monoculture, and in attached microbiota from apple packinghouses.

Methods: Twenty-two phylogenetically distinct Lm isolates (10^8 and 10^7 CFU/mL) grown as microbial lawns were spot-inoculated with 1 µL of each strain and incubated for 3 days at 15, 20, 25, or 30°C. Microbiome samples from packinghouses were inoculated into polypropylene conical tubes with Lm at 10^5 CFU/mL and either ATCC PTA-4761 or PTA-4759 at 10^7 CFU/mL, and then incubated at 15°C for 3, 5, or 15 days to form a quasi-biofilm. Attached cells were removed for aerobic plate count, Lm enumeration by BAM MPN, and DNA extraction. 16s rRNA V4 amplicon sequencing of extracted DNA was conducted using Illumina Miseq. Sequences were analyzed using Mothur v.1.44.2. Lm reductions were evaluated using ANOVA and the effect of putative biocontrol strains and growth period on packinghouse microbiota composition was evaluated by performing PERMANOVA and differential abundance analysis.

Results: ATCC strains PTA-4761 and PTA-4759 were identified as *Enterococcus faecium* and *Enterococcus lactis*. Both strains showed greater inhibition against 10^7 CFU/mL Lm compared to 10^8 CFU/mL Lm lawns ($P < 0.0001$). Lower temperatures increased PTA-4759 inhibition against Lm at both concentrations ($P < 0.001$). Lm reductions in the 15-day quasi-biofilms were greater than those of the positive control without added LAB ($P < 0.01$). PERMANOVA analysis found that the microbiota of samples grown for 3, 5, and 15 days were different.

Significance: These findings may inform further studies to evaluate biological strategies for controlling Lm in packinghouse environments.

T6-10 Inactivation of *Listeria monocytogenes* on Cantaloupe by Eugenol Nanoemulsion in Combination with Commercial Sanitizers

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* (LM) is a major foodborne pathogen that has been implicated in outbreaks related to consumption of contaminated cantaloupes. The complex surface topography of cantaloupes enhances bacterial attachment thereby reducing antimicrobial efficacy of sanitizers. Therefore, there is a need to develop novel sanitizers with improved antimicrobial efficacy against LM.

Purpose: This study investigated the efficacy of eugenol nanoemulsion (EGNE) wash either alone or in combination with commercial sanitizers in inactivating LM on cantaloupe surface at 25℃.

Methods: EGNE (Eugenol-1.25%, Gum-Arabic-0.5%, Lecithin-0.5%, and Ethanol 16.67% co-surfactant) was prepared by high-speed homogenization and sonication. LM (5 strain mixture, ~8.5 log CFU/plug) was inoculated on circular, cantaloupe rind plugs (2 cm^2 area) followed by attachment at 25℃ for 120 minutes. The cantaloupe rind plugs were washed with EGNE (0.3, 0.6, 1.25%) alone or in combination with chlorine (200 ppm) or hydrogen peroxide (2%) for 1, 5, 10 or 15 minutes at 25℃. Following the treatment, the plugs were transferred to DE broth, and homogenized for 1 minute. The surviving LM were enumerated on Oxford agar. All experiments had duplicate samples and repeated twice. Data were analyzed using one-way ANOVA.

Results: The EGNE had a particle size of ~85 nm, Poly Dispersity Index of 0.26 and a high negative surface charge (-30 mV). EGNE treatments (0.6, 1.25%) reduced LM population on cantaloupe by ~1.5 log CFU/mL as early as 5 minutes of treatment ($P < 0.05$). The EGNE (1.25%; 15 min time) treatment in combination with hydrogen peroxide or chlorine reduced LM population by 1 log or ~2.5 log CFU/ml, respectively.

Significance: Eugenol nanoemulsion could be potentially used as a natural, safe and effective disinfectant in combination with commercial sanitizers for inactivation of *Listeria monocytogenes* on cantaloupes. Follow up experiments testing efficacy of EGNE with enhanced hydrophilicity and combinations with peracetic acid are currently underway.

T6-11 Cleaning and Sanitizing in Produce Facilities: Training Gaps, Opportunities and Industry Preferences

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Introduction: Cleaning and sanitizing (C&S) requirements under the Produce Safety Rule (PSR) have been identified as one of the more challenging pieces for compliance, with inadequate practices and program requirements frequently reported during farm inspections.

Purpose: To better understand the produce industry's needs related to C&S, we worked with produce associations and FDA to develop and administer a national survey.

Methods: A 44-question anonymous survey was administered via Qualtrics over a 3-week period in June 2020. Survey was shared with produce growers through social media, Oregon State University food safety extension listservs, Oregon Department of Agriculture Produce Safety Program, United Fresh, National Farmers Union and Produce Marketing Association.

Results: The survey received 300 clicks with 162 responses, with respondents representing 185 produce operations across 18 different states. The preferred format for C&S education was an in-person workshop (55/126); followed by a self-paced online course (27/126); hybrid course with pre-requisite background sessions online and in-person interactive sessions (25/126); and a live delivery through an online platform (19/126). The most common materials reported for harvesting containers were plastic (40/72), wood (16/72), cardboard (8/72), and canvas (6/72), while stainless steel (34/91) and plastic (30/91) were most commonly reported for post-harvest equipment. Most commonly used sanitizers included bleach (40/114), quaternary ammonium compounds (28/114), and peracetic acid (26/114). A large proportion indicated the need for resources related to C&S (45/69), also translated to Spanish and other languages. Topics included: principles and practical implementation of C&S, identification of hazards and prioritizing C&S activities, establishing a C&S program, and verification of C&S effectiveness. High turnover and seasonal workforce (37/122) and no time to clean and sanitize (19/122) were indicated as major barriers for improving C&S in a facility.

Significance: The results of the survey helped guide the development of a virtual cleaning and sanitizing workshop, piloted in January-March 2021.

T6-12 Characterization of Lytic *Escherichia coli* O157:H7-Specific Phage Focusing on Its Novelty

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❖ ***Developing Scientist Entrant***

Introduction: *Escherichia coli* O157:H7 is one of major foodborne pathogens associated with fresh produce. Lytic phages as a novel biocontrol agent have been recently employed for the prevention and control of *E*. coli O157:H7. Thus, searching and investigation of novel phages against *E*. coli O157:H7 would be helpful for developing a novel biocontrol methodology.

Purpose: The purpose of this study was to isolate and characterize a novel and lytic *E. coli* O157:H7-specific phage.

Methods: *E. coli* O157:H7-specific (EC) phage was isolated from wastewater of slaughterhouse and purified by using CsCl gradient ultracentrifugation. The specificity and efficiency of plating (EOP) of EC phage was investigated against 10 inclusive and 40 exclusive foodborne pathogens using a plaque assay. The morphology of EC phage was observed using TEM. DNA of EC phage was extracted using an extraction kit and performed a whole genome sequencing. The open reading frames (ORFs) of EC phage was predicted and annotated using BLAST, and then compared with ResFinder 2.1, virulence factor database, and allergen database.

Results: EC phage was isolated and purified with a concentration of 1.23×10^{10} PFU/mL. EC phage infected *E. coli* O157:H7, *Shigella sonnei*, *Salmonella* Enteritidis, *S*. Mission, as well as *S*. Senftenberg with high EOP values of ≥ 0.52. EC phage belonged into *Myoviridae* family with contractile tails of 129.95 ± 11.49 nm and icosahedral heads of 96.35 ± 6.84 nm. EC phage was consisted of 167,440 bp genome with GC content of 40.5% and 265 ORFs. Comparative genomic analysis revealed that EC phage had very low similarity to other phages in NCBI databases and there was no homologous genome. Furthermore, genes encoding lysogenic property, virulence, antibiotic resistance, and potential allergens were absent in EC genome.

Significance: This study confirmed the specificity and novelty of EC phage as a promising biocontrol agent against *E. coli* O157:H7.

T7-01 Effect of Poultry Litter Moisture Content on Litter to Dust Transfer of Salmonella

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❖ ***Developing Scientist Entrant***

Introduction: Recognizing the impact of litter moisture on dust-associated microorganisms will help to understand airborne transmission at the production level.

Purpose: This study was aimed at evaluating the role of litter moisture content on litter to dust transfer of bacteria.

Methods: An *in vitro* study was performed in which five batches (110 g each) of litter were placed separately in 2 L flasks. Four were then inoculated with *Salmonella* Typhimurium. In three of the inoculated flasks an increasing amount of sterilized water (10 mL, 15 mL, and 20 mL) was added. After 24 h at room temperature, 20 g of litter from each flask was removed to analyze litter moisture and microbial levels. The remaining litter of each flask was used to create dust through blowing air (143-155 m/s) directly onto litter. Generated dust was collected by impingement into buffered peptone water. Litter and dust samples were analyzed for aerobic plate counts (APC), *Salmonella*, and naturally occurring *Escherichia coli* and coliform counts and prevalence. Following five replications, microbial data by moisture level and the relationship between dust *Salmonella* and litter moisture were analyzed with ANOVA and simple logistic regression, respectively.

Results: Moisture content (%) by batch were 13.0, 18.2, 23.0, 28.2, and 33.3. APC, *Salmonella*, *E. coli* and coliforms counts did not differ ($P > 0.05$) with moisture and were on average 10.30, 5.92, 5.97, and 5.99 log CFU/g, respectively. Dust sample counts significantly decreased with increasing moisture levels ($P < 0.0001$). *Salmonella*, *E. coli*, and coliforms were below the detection level (1.10 log CFU/L) in dust obtained from litter having moisture ranges 27.2–34.8. *Salmonella* in dust decreased with increasing litter moisture ($P <0.0001$).

Significance: Increasing levels of moisture tended towards lower dust contamination with bacteria. However, the drawbacks of high litter moisture content on bird health need to be considered.

T7-02 Effect of Litter Treatments on Persistence of *Salmonella* Enteritidis in Reuse Poultry Litter

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Introduction: Nontyphoidal *Salmonella* contributes to a significant number of cases of foodborne illness in the United States. Reuse of litter in broiler houses can lead to the accumulation of *Salmonella* in litter.

Purpose: The purpose of this study was to evaluate persistence of *Salmonella* Enteritidis in poultry litter after challenging one flock with *Salmonella* Enteritidis, treating litter with *Bacillus* spp. Probiotic Blend (PB), Sodium Bisulfate (SB), Sodium Formate Salts (SF), Windrow Composting (WC), or untreated control (UC), then raising two flocks on the same litter.

Methods: One thousand broiler chicks were spread over 40 pens (25/pen) with 8 pens per treatment and were challenged with 10^7 CFU of a nalidixic acid-resistant strain of *Salmonella* Enteritidis at 6 days of age. Following flock termination(s), WC was performed by removing litter from all pens and creating a pile for 6 days between flock placements. Other treatments were applied one day prior to chick placement. SB was applied at 45.35 kg/92.90 m^2, PB at 4,535 kg/92.90 m^2 and SF at 151.4 L/92.90 m^2. *Salmonella* persistence was measured before and after both second and third flock termination using boot covers that were then enriched in Tetrathionate broth and enrichment streaked on selective media. Data were assessed based positive or negative results and analyzed by chi-square test.

Results: *Salmonella* persistence decreased significantly between flocks within each treatment group ($P < 0.01$). Following the first flock, persistence was >96% for all treatments. Persistence for the second and third flock was 15/32 and 2/32 for UC, 18/32 and 5/32 for PB, 22/32 and 5/32 for SB, 16/32 and 3/32 for SF, and 14/24 and 1/24 for WC, respectively. There were no significant differences between treatments following each flock or overall.

Significance: These data demonstrate that *Salmonella* persists in litter after multiple reuses although persistence decreases from flock to flock regardless of litter amendments.

T7-03 Characteristics of Multi-Jurisdictional Poultry Associated Whole Genome Sequencing Clusters in Canada

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Introduction: The implementation of prospective whole genome sequencing (WGS) in May 2017 allowed for the more accurate identification of salmonellosis clusters in Canada, including those associated with poultry.

Purpose: To describe the frequency and characteristics of multi-jurisdictional, poultry-associated salmonellosis clusters in Canada since the implementation of WGS, and their impacts on outbreak assessment and response activities at the national level.

Methods: *Salmonella* clusters are identified by the National Microbiology Laboratory using whole genome multi-locus sequence typing (wgMLST). In addition to human isolates, clusters may include non-human isolates identified through routine surveillance programs or during food safety investigations. Multi-jurisdictional clusters are reported to federal epidemiologists at the Public Health Agency of Canada for assessment, prioritization and potential investigation. Clusters may be classified as poultry-related based on available epidemiologic or laboratory evidence (i.e., poultry food isolate within 10 wgMLST). Trends and characteristics of multi-jurisdictional poultry-related clusters were examined.

Results: The routine integration of non-human data in WGS analyses has allowed for improved detection of poultry-associated clusters at the national level. Since the implementation of WGS until end of March 2021, 25% (n = 154) of multi-jurisdictional *Salmonella* clusters in Canada were classified as poultry-related. These poultry-related clusters include approximately 6,000 human isolates, representing an estimated burden of illness of 156,026 cases. Clusters range in size from 2 to 642 human isolates (median = 13). The top *Salmonella* serovars associated with multi-jurisdictional poultry clusters in Canada are Enteritidis (62%), followed by Heidelberg (11%) and Typhimurium (8%).

Significance: Despite previous successes surrounding the investigation and control of *Salmonella* in frozen raw breaded chicken products in Canada, the burden of illness associated with fresh chicken remains high, as there are no current regulations surrounding the level of *Salmonella* in fresh poultry

products. The exploration of control measures used in other countries may prove beneficial for addressing this issue in Canada.

T7-04 Effect of Antimicrobial Interventions on *Salmonella* Percent Positive in Raw Poultry Slaughter Establishments

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Introduction: Poultry slaughter establishments commonly use antimicrobial interventions to control *Salmonella* in raw poultry carcasses.

Purpose: FSIS analyzed *Salmonella* percent positive in raw poultry carcasses to correlate these data with the reported chemical interventions used at the point in the process prior to FSIS sample collection and in the context of policy changes.

Methods: FSIS evaluated the presence of *Salmonella* in poultry carcass samples (35,590) collected between 2016-2019 as part of FSIS HACCP verification sampling programs.

Results: Data show establishments increased the use and changed the type of chemical interventions on poultry carcasses when FSIS implemented the use of a neutralizing buffered peptone water (nBPW) in July 2016 as sample transport media. As of 2019, 79% (6675/8431) of raw poultry samples originated from establishments that use chemical interventions to control *Salmonella*, compared to 67% (3868/5755) of samples in 2016. In response to the nBPW implementation and FSIS posting of establishment categories, establishments changed the type of antimicrobial intervention used. We noted increased peroxyacetic acid (PAA) application in 2019 (87%; 7602/8762) compared to 2016 (64%; 5190/8074) and decreased use of cetylpyridinium chloride (CPC; 25% in 2016 and 9% by 2019). In the three years following implementation of nBPW, percent positive *Salmonella* samples increased from 1.8% to 4.3% (310/7292) at establishments using PAA and from 1.5% (32/2081) to 8.8% (63/716) at establishments using CPC. Overall, *Salmonella* percent positive for poultry carcasses did not change significantly from 2016 to 2019.

Significance: These data support the hypothesis that nBPW is effective in preventing carryover effect for specific types of chemical antimicrobial interventions. These data also suggest that FSIS-collected sampling results provide an important tool to evaluate the effectiveness of interventions and assess continued intervention use. These results can help improve food safety measures by guiding establishments to consider chemical interventions to control pathogens.

T7-05 Application of Yellow Mustard Mucilage in Microencapsulation of Essential Oil and Polyphenols for Targeted Delivery to Gastrointestinal Tract of Poultry

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Introduction: The synergistic antimicrobial effect of essential oils thymol and carvacrol with polyphenols have been proposed to improve digestive health and growth performance of poultry. The microencapsulation acts as a reservoir and a controlled release system to protect bioactive compounds from volatile and degradation during processing and storage. Water-soluble yellow mustard mucilage (WSM) is a novel polysaccharide material which is proven with thermal and acid resistant properties.

Purpose: The current study is to investigate the potential of using the natural polymer, WSM, as a microencapsulation coating material for protection and delivery of bioactive components into lower section of poultry gastrointestinal tract where the microorganisms are preferentially located.

Methods: Emulsions with the core to coat ratios of 4:9 and 1:5 (v/v) were prepared by mixing core materials carvacrol, thymol and polyphenol at the ratios of 0.5/0.5/1.0 (w/w/w) with coating materials WSM, maltodextrin and gum Arabic as at WSM/MD/GA ratios of 0/3/6 (w/w/w) as control, 1/2/6 and 2/2/5 as treatments. The emulsions were spray dried to obtain the microparticles. The fabricated microencapsulates were assessed in terms of particle morphology, size distribution, encapsulation efficiency, and releasing profile at *in-vitro* stimulated gastric and intestinal digestion system.

Results: Spray dried EOs and polyphenols microcapsules coated with WYM/MD/GA at ratio of 2/2/5 with core to coat ratio of 1:5 has exhibited highest encapsulate efficiency and best stability after exposed to the *in vitro* gastric digestion system. The addition of WSM has significantly reduced the total released core compounds at pH 2 and slowed down the releasing rate at pH 7. The microcapsules coated with WSM showed a smaller particle size, a larger surface area per unit volume and a relatively uniform size distribution.

Significance: WSM has exhibited unique properties in protecting bioactive compounds under acidic environments, which has a great potential as a coating material for encapsulation.

T7-06 A Value Chain Approach to Identify Hazards and Risks to Child Health Associated with Enteropathogens Carried by Chickens in Maputo, Mozambique

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❖ ***Developing Scientist Entrant***

Introduction: The morbidity and mortality associated with exposure to animal feces is unquantified yet likely contributes considerably to the global burden of diarrheal disease in children. Given the encouragement of small-scale poultry farming as a development strategy in low and middle-income countries, it is important to understand risks of childhood exposure to chicken feces from the contamination of food and the environment.

Purpose: The purpose of this study was to identify and map hazards along the chicken production process while simultaneously testing for *Campylobacter jejuni* and *Salmonella* spp.

Methods: From July – September 2019, 118 pooled feces and 75 carcass rinse samples were collected from broilers, layers, and indigenous chickens at depots (stores selling day-old chicks), small-scale farms, informal markets, grocery stores, and households. qPCR analyses were performed for *Salmonella* spp., *Campylobacter* spp., and *Campylobacter jejuni*.

Results: Prevalence of *Campylobacter* spp. was 81.8% (*N* = 110) in feces and 88.7% (*N* = 62) in carcass rinses, while prevalence of *Salmonella* spp. was 11.0% (*N* = 118) in feces and 21.6% (*N* = 74) in carcass rinses. Of samples positive for *Campylobacter* spp., 86.7% (*N* = 60) of carcass rinses and 88.8% (*N* = 89) of pooled fecal samples were also positive for *C. jejuni*. Cross-contamination of chicken meat during butchering processes at informal markets warrants further investigation. Contaminated indigenous chicken feces at households suggest potential exposure risks associated with chicken husbandry and food preparation practices.

Significance: Mapping value chains for chicken production concurrent with assessing microbial contamination at key nodes highlights food safety risks and opportunities for foodborne disease transmission. Future studies should go beyond traditional water, sanitation, and hygiene exposure routes commonly considered in the field of diarrheal disease research, to explore additional exposure routes and cross-contamination along food production systems.

T7-07 Probability of *Salmonella* Foodborne Outbreaks by Consumption of Chicken Cooked with Different Methods

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❖ ***Developing Scientist Entrant***

Introduction: Poultry consumption is a major cause of *Salmonella* foodborne outbreaks, but there is no microbial risk assessment for *Salmonella* foodborne outbreaks with a scenario from carcasses to cooking in Korea.

Purpose: This study estimated the risk of *Salmonella* outbreaks by consumption of chicken cooked with various methods.

Methods: Prevalence of *Salmonella* in 270 chicken carcasses was investigated by plating the samples on Xylose Lysine Deoxycholate agar. Data for storage temperature and time of poultry were collected, and probabilistic distributions for the data were also determined. Predictive models describing the fates of *Salmonella* in chicken were developed, using the Baranyi model (primary model) and polynomial model (secondary model), and the amount and frequency of consumption were surveyed. Reductions in *Salmonella* cell counts by cooking methods (moist-heating and dry-heating) were examined. A dose response model for *Salmonella* infection was searched. Subsequently, these data were used in a simulation model in @RISK to estimate the risk of *Salmonella* foodborne illness caused by chicken consumption.

Results: Of 270 chicken samples, 5 samples (1.9%) were contaminated with *Salmonella* and thus, the initial contamination level (-3.1 log CFU/g) of *Salmonella* was estimated by RiskBeta (6, 266). Temperatures for transportation and market display were fitted with RiskUniform (2.12, 12.54) distribution and RiskUniform (-2, 10) distribution, respectively. The average chicken consumption amount fitted by Lognormal distribution [RiskLognormal (119.73, 153.71, RiskShift (-4.0294))] was 115.7 g at 13.7% of frequency and at the consumption phage, chicken was cooked by dry-heating (36%) and moist-heating (64%). Beta-Poisson model [$1-(1+Dose/4.4\times10^{5})^{-0.89}$] was selected for the dose response of *Salmonella*. The simulation with these data showed that the probability of *Salmonella* foodborne illness by chicken consumption was 2.99×10^{-10} per person per day in Korea.

Significance: This result indicates that the risk of *Salmonella* foodborne illness in chicken seems low in Korea.

T7-08 Thermal Inactivation of *Salmonella* in Chicken Wings Cooked in an Air Fryer and a Convection Oven

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Introduction: Chicken wings are one of the most popular poultry products for domestic and food service consumption. However, poultry products are often linked to foodborne illness caused by *Salmonella* spp. Novel domestic appliances such as air fryers and convection ovens need to be validated for their capacity to achieve pathogen reductions and ensure food safety for consumers.

Purpose: To determine the thermal inactivation of *Salmonella* in chicken wings cooked in an air fryer (AF) and a convection oven (CO).

Methods: Chicken wings (average weight 46.5 ± 4.3 g) were inoculated with a five-strain cocktail of poultry-borne *Salmonella* spp. (10^{8} CFU/per wing). Wings were cooked in an air fryer (set temperature 176.6°C) or a convection oven (set temperature 179.4°C), for 2, 5, 10, 15, 20, 22, or 25 min. Temperature profiles of wings and appliance environment were recorded with thermocouples. *Salmonella* counts (SC) were determined on XLD plates for rinsates (100 mL/sample). Rinsates were incubated for 24 h at 37°C to recover bacteria below the limit of detection. Experiments were performed in triplicate (three wings/per treatment/per replicate), with inoculated untreated wings as controls.

Results: The recommended internal temperature of 73.8°C was achieved after 8.2 ± 1.5 minutes (AF) and 7.7 ± 1.2 min (CO), with reductions of 6.61 ± 0.05 log CFU/wing in both appliances. Cumulative lethality (F_0) calculations predicted a 7.4-log reduction around this time (8.5 min) and a 12.7-log reduction by 8.75 min. However, sample enrichments tested positive for *Salmonella* for all cooking times below 22 min. At this time, SC reductions were 8.61 ± 0.05 log CFU/wing in both appliances.

Significance: Both household appliances are able to achieve safe cooking temperatures for chicken wings. SC decreased drastically when the internal temperature of wings reached 73.8°C, as predicted; however, additional cooking time was required to ensure complete reductions.

T7-09 Microbial Assessment of Fresh Meats and Blends Sold as Raw Pet Foods by Online Retailers

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❖ ***Developing Scientist Entrant***

Introduction: Raw meat–based diets (RMBDs) for companion animals are gaining popularity due to perceived health benefits to pets, including reduced chances for acquiring infectious and degenerative diseases. However, raw meats are often contaminated with bacteria that may put the safety of pets and humans in the household at risk.

Purpose: The purpose of this study was to survey the level of microbial contamination in different kinds of frozen ground meat blends and livers sold by online retailers.

Methods: Frozen ground meat blends and livers of four species (beef, chicken, pork, and turkey) were purchased from four online retailers at two different times of the year and tested for microbial quality: aerobic plate count (APC), *Enterobacteriaceae* (EB), lactic acid bacteria (LAB), yeast and molds (Y&M), *Salmonella*, *Escherichia coli*, and *Listeria.* APC, EB, and Y&M were enumerated using 3M Petrifilm™, while the other microorganisms were enumerated using selective media, namely, De Man, Rogosa and Sharpe (MRS) agar with leucine for LAB, xylose lysine deoxycholate (XLD) agar for *Salmonella*, Sorbitol-MacConkey (SMAC) agar for *E. coli*, and Oxford Listeria agar for *Listeria*.

Results: Generally, ground blends had higher microbial counts compared to those of liver samples. There was an interaction between species and meat type on the microbial counts across the variety of pet foods tested ($P < 0.05$), except for *Listeria* and *Salmonella*. Even though supplier and season had no effect on the microbial counts, the overall quality was poor as *Salmonella, Listeria* and *E. coli* were detected in 34.2%, 97.4% and 97.4%, respectively, of the 38 product samples tested in the study.

Significance: These data showed meats sold online as raw pet foods have high levels of spoilage microorganisms and are contaminated with *Salmonella, E. coli*, and *Listeria*. Suitable control guidelines should be set for this growing trend in the pet food industry.

T7-10 Comparison of the Inactivation of *Salmonella* spp. and *E. coli* O157:H7 during the Manufacture of Dry Fermented Sausages: Is It Necessary to Perform a Challenge Study for Each Pathogen?

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Introduction: *E. coli* O157:H7 and *Salmonella* spp. contaminated dry fermented sausages (DFS) have been linked to several outbreaks, thus requiring challenge studies for both organisms to validate DFS production processes. Challenge studies are time consuming, expensive and require specialized facilities and expertise to conduct.

Purpose: Compare the inactivation of *Salmonella* spp. to *E. coli* O157:H7 during the manufacture of DFS and assess whether separate challenge studies for each pathogen is necessary.

Methods: Population reductions of 5-strain cocktails of *E. coli* O157:H7 and *Salmonella* spp. were compared at different stages throughout the production of DFS; individual studies compared sausage casing material, fat content, caliber size, and salt. All processes employed a fermentation, maturation and

prolonged drying typically used to produce DFS. Surviving pathogen populations (CFU/mL) were enumerated by serial dilutions and plating on selective media and transformed to log-reductions and log-reduction rates calculated at different stages during DFS production. Data presented are mean of values from three separate batches of sausages produced for each pathogen.

Results: In all studies, log-reduction and log-reduction rates of *Salmonella* spp. were significantly (P <0.05) higher during fermentation than *E. coli* O157:H7. During the 24-48 h fermentation, when the pH of the sausages dropped to ≤5.0, *Salmonella* spp. reduced by 1.5 to 2.0-logs compared to 0.5-log reduction of *E. coli* O157:H7. However, during drying although log-reduction rates of *Salmonella* spp. and *E. coli* O157:H7 were significantly reduced with increasing sausage caliber size, log-reduction rates of *Salmonella* spp. were not significantly different from *E. coli* O157:H7 in all other studies. In all cases a 5-log inactivation of *Salmonella* spp. was achieved within 25-30 days of DFS production compared to 35-40 days for *E. coli* O157:H7.

Significance: DFS production processes capable of achieving a 5-log inactivation of *E. coli* O157:H7, should be able to achieve a similar log-reduction of *Salmonella* spp., suggesting that a separate challenge study for *Salmonella* spp. might not be necessary.

T7-11 Assessing Shiga Toxin-producing *Escherichia coli* in FSIS Regulatory Pork Samples

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Introduction: Shiga toxin-producing *E. coli* (STEC) are associated with severe disease and recalls of contaminated beef, but their presence in pork products has not been well defined.

Purpose: To assess the frequency of STEC in pork and characterize *E. coli* responsible for discrepancies between molecular screening tests and culture isolation.

Methods: Pork samples (n = 1,477) were shipped from USDA-FSIS to USDA-ARS for analysis. Each broth was screened using the Bio-Rad STEC VirX and SerO test kits. All broths positive for Shiga toxin (*stx*) and intimin (*eae*) genes were taken forward for culture isolation by direct plating on to modified Rainbow, ChromSTEC, and washed sheep's blood agar. For samples that were determined to additionally have any of the 7 most common STEC serogroup(s) present (O26, O45, O103, O111, O121, O145 and O157; a.k.a. Top7) immunomagnetic concentration was used with plating the same agars, with all suspect colonies selected and characterized for *stx*, *eae* and serogroup.

Results: Of the 1,477 samples, 327 (22.1%) screened positive for the presence of *stx* and *eae*. Most (91%) of these also screened positive for the presence of one or more Top7 serogroups. Culture isolation yielded *E. coli* possessing at least one of the targeted genes (*stx*, *eae*, serogroup) from 223 samples, while 104 yielded no isolate. The source of the *stx* gene was confirmed in 63 samples while the source of the *eae* gene was confirmed in 151 samples. This included 5 samples containing a potential enterohemorrhagic *E. coli* (*stx*+ *eae*+), 4 being of Top7 serogroups, and 39 samples containing multiple *E. coli* possessing either *stx* or *eae,* and/or *a* serogroup.

Significance: While many pork samples screened positive for STEC on molecular tests, culture identified most to be mixed *E. coli* contaminants rather than potential enterohemorrhagic *E. coli* of less common serogroups.

T7-12 NARMS Expansion Project: Exploring *Salmonella* Isolates from Cattle Lymph Nodes in Fsis

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Introduction: The National Antimicrobial Resistance Monitoring System (NARMS) is a national surveillance system that monitors changes in the antimicrobial susceptibility of enteric bacteria found in clinical, retail, and food animal samples in the US. As part of a One-Health framework, FSIS expanded NARMS sample collections to include mesenteric lymph nodes (MLNs) from cattle in Fiscal Year 2020. MLNs and cecal samples are pre-intervention samples taken prior to applying antimicrobial interventions during slaughter or processing.

Purpose: The objective of this study was to assess antimicrobial resistance of *Salmonella* from cattle MLNs.

Methods: Paired sampling of MLN and cecal contents was conducted for several cattle classes. The FSIS laboratories isolated *Salmonella* following the Microbiology Laboratory Guidebook (MLG) Chapter 4 from MLN and NARMS Interagency Laboratory Manual from cecal samples. Whole genome sequencing (WGS) was performed for all *Salmonella* isolates per MLG Chapter 42. WGS data was analyzed on NCBI Pathogen Detection Browser for phylogenetic relatedness to other *Salmonella*, including FSIS product isolates. Antimicrobial susceptibility was determined on the isolates.

Results: Forty-five *Salmonella* isolates were recovered from 251 MLN samples. Of MLN *Salmonella* sequenced, 97.5% (39/40) were susceptible to antibiotics on NARMS phenotypic testing panels. Twenty MLN *Salmonella* isolates had a paired positive cecal isolate and four were related. MLN isolates (6/40) appeared potentially related to FSIS product isolates.

Significance: The current results suggest *Salmonella* isolates from MLN samples, which have a 17.93% positivity rate, do not possess unique antimicrobial resistance profiles compared to cecal and product *Salmonella*. However, genetic information from *Salmonella* from these samples may provide insight into survival mechanisms, antimicrobial resistance and their potential association with food product and public health. In addition to other data sources, this information can help generate hypotheses about likely sources of foodborne illness and outbreaks by potentially identifying missing links when food product and clinical *Salmonella* isolates cannot be closely connected.

T8-01 Evaluation of Consumers Behavior, Knowledge, and Attitudes Around the Recommendation Not to Wash Raw Poultry

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❖ ***Developing Scientist Entrant***

Introduction: The practice of washing raw poultry continues to be widespread despite consumer education efforts about the cross-contamination risk associated with the behavior.

Purpose: The purpose of this study was to determine the frequency with which consumers continue to wash raw poultry and barriers to consumers adopting the correct behavior.

Methods: A survey was developed and administered online to a sample of 1,822 consumers in the United States via Survey Monkey®. The survey sought to determine whether consumers wash raw poultry, and if they were aware of the USDA recommendation not to wash poultry. Consumers who indicated that they were not aware of the recommendation were presented with USDA's educational message online and then asked how confident they were that they could stop washing poultry. The survey also included questions to identify barriers that prevented consumers who were aware of the recommendation from adopting the proper behavior.

Results: Results found that 73.5% (n = 1,340) of consumers reported washing raw poultry. Of those consumers, 68.8% (n = 920) indicated that they were not aware that the practice was incorrect. When these consumers were presented the educational message, 81.9% (n = 748) indicated that they were somewhat to very confident that they could stop washing raw poultry. Of the consumers who were aware of the message but continued to wash raw poultry, 58.5% (n = 244) reported that they continued to do so because they thought they cleaned surfaces well, and 47.2% (n = 197) thought they were careful not to splash when washing raw poultry. That is, consumers did not believe that their washing raw poultry was a risk for cross-contamination.

Significance: A large percentage of consumers are still not aware of the recommendation not to wash raw poultry and a large subset of that group may easily adopt the practice if made aware of the correct behavior.

T8-02 Everyday Risks Every Time We Eat – Global Poll Findings of Perceived and Experienced Risks from Unsafe Foods

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Introduction: There were no comparable global data on people's perceptions of risk of harm from unsafe food and their experiences of those risks.
Purpose: To improve understanding of how people perceive and experience risk of harm from unsafe food.
Methods: The World Risk Poll was conducted by Gallup using face-to-face or telephone interviews with over 150,000 respondents. The poll used nationally representative samples of more than 95% of the world's population, across 146 countries and 145 languages.
Results: Seventeen per cent of poll respondents – equivalent to one billion people worldwide – experienced serious harm, or know someone who experienced serious harm, caused by the food they ate in the two years prior to polling.

- The greatest levels of harm from food occur in East Africa (29 per cent experienced harm) and the Middle East (27 per cent experienced harm).
- Over half of the world's population, 60 per cent of people worldwide, say they are worried about the food they eat.
- Countries and territories that had experienced the most harm from food were those in the developing world; top three countries: Liberia (52%), Zambia (51%) and Mozambique (45%).
- However, in these regions levels of worry about food are lower than the levels of harm experienced. EG, East Africa (25 per cent worried) and Middle East (22 per cent worried).
- Although the world's population suffers many problems from the food they currently eat, genetically modified (GM) food is seen as a high risk with 48% of people worldwide saying that GM foods will mostly harm people in their country over the next twenty years.

Significance: The poll provides the first globally comparable, publicly available data set on public understanding of risk from unsafe food. It creates insight that aids the development of evidence-based interventions that empower people to take action, that saves lives and helps people feel safer.

T8-03 Development of a Rational Approach to Identify and Characterize Hypervirulent Non-Typhoidal *Salmonella* and Associated Genomic Signatures

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❖ ***Developing Scientist Entrant***

Introduction: Non-typhoidal *Salmonella* (NTS) cause an estimated 1.03 million human foodborne illnesses annually in the US. Virulence differences have been observed across serovars and clades within a serovar.
Purpose: To identify and characterize genomic signatures that are associated with SNP clusters of *Salmonella* Saintpaul that have significantly higher proportions of human clinical isolates (and thus could be "hypervirulent").
Methods: Metadata for all *S.* Saintpaul isolates in the NCBI pathogen detection (PD) database were downloaded (June 23, 2020). Odds ratios were calculated to identify SNP clusters with significantly higher or lower proportions of human clinical isolates (representing putatively hyper- or hypovirulent clusters, respectively); statistical significance was assessed using one-sided Fisher's exact tests with FDR correction. Genomic assemblies for isolates (10 per SNP cluster) selected from five putative hyper- and four hypovirulent SNP clusters were characterized to identify accessory genes and core SNP alleles associated with hypervirulence.
Results: For *S.* Saintpaul, a total of 222 SNP clusters were assessed; 29 and 25 of the clusters had significantly higher or lower proportions of human clinical isolates ($P < 0.05$), respectively, suggesting that isolates in some SNP clusters may be more frequently associated with human infections. Comparative genomic analyses identified 384 genes and 338 core SNPs that were significantly associated with hypervirulent SNP clusters, including four virulence genes (*sodCI*, *gtgE*, *sseI*, and *gtgA*) carried on Gifsy-2 prophage. Among core SNPs present in isolates associated with hypervirulent SNP clusters, a nonsense mutation in *stfC*, encoding the Stf fimbrial usher, was significantly underrepresented among hypervirulent isolates.
Significance: In this study, we developed a novel framework for using metadata and WGS data from the NCBI PD database to identify putatively hyper- and hypovirulent SNP clusters. Combined with experimental characterizations, genomic signatures identified in this study may be used as markers for identification of hyper- and hypovirulent *Salmonella.*

T8-04 Dietary and Socioeconomic Risk Factors for Fumonisin Exposure Among Reproductive Age Women in Guatemala

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Introduction: Exposure to mycotoxins, such as fumonisins, is associated with adverse health outcomes, and prevalent in populations where maize is a dietary staple, such as Guatemala.
Purpose: The objective of this cross-sectional study was to estimate fumonisin B_1 exposure among reproductive-aged women in Guatemala and assess dietary and socio-demographic risk factors for exposure.
Methods: Reproductive-aged women from 18 municipalities in Guatemala were recruited to participate in the study. Data on individual/household demographics and socioeconomic status were collected. A 60-item food frequency questionnaire was administered to estimate the amount and types of food products consumed during the previous week. A spot urine sample was collected and urinary Fumonisin B_1 (uFB_1) concentration was measured. Univariable analyses were conducted to identify predictors of low/high uFB_1. Multivariable logistic regression was used to calculate adjusted odds ratios (ORs) and 95% confidence intervals (CIs).
Results: A total of 775 women had analyzable urine samples. Higher uFB_1 levels were significantly associated with speaking Mayan (OR=2.266, 95% CI:1.393,3.685), less than high school education (OR=1.606, 95% CI:1.122,2.297), percent of total diet consisting of maize-based foods (OR=1.020, 95% CI:1.009,1.031), and tostadas consumption (OR=1.106, 95% CI:1.020,1.199). Lower uFB_1 levels were significantly associated with consumption of highly processed maize-based foods (OR=0.932, 95% CI:0.874,0.993). Tortillas were the most frequently consumed maize-based food and significantly associated with high exposure in the univariable analysis but not in the multivariable analysis. Consumption of maize-based foods, locally produced maize-based foods, and tortillas were also significantly associated with high exposure in the univariable analysis.
Significance: These findings suggest that populations with low socio-economic status/education levels and high consumption of maize-based foods are at risk of higher exposure to fumonisins. Increasing diet diversity through inclusion of non-maize-based foods can lower fumonisin exposure.

T8-05 Food, Agriculture, and Veterinary Defense (FAV-D) Architectural Framework

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Introduction: The Securing Our Agriculture and Food Act directs DHS to provide oversight to and coordinate with federal, state, industry, academia and international partners to defend the country's food, agriculture, and veterinary systems against terrorism and other high-consequence events. This presentation highlights the architectural framework developed to build stronger resilience across the sectors.

Purpose: The goal of this effort was to develop a FAVD architecture that would help to identify existing capabilities and gaps across stakeholders that could inform efforts to ensure the level of readiness and resiliency needed for high-consequence food and agricultural events.

Methods: To develop this framework, we: conducted a scan of existing preparedness, detection, response, mitigation and recovery programs across food systems and One Health; developed readiness frameworks for crop-based agriculture, livestock and poultry, companion animal, equine and wildlife; formed focus groups to review the findings and discuss capabilities needed for an optimized readiness framework to identify critical strengths and gaps; and, used multisector and catastrophic-scenario workshops to provide ground-truth assessment of cross-sector coordination and communication.

Results: The FAVD architectural framework has determined that 20% of the national GDP is reflected by the food/agriculture sector which is represented by 10% of the labor force. The framework has ensured that existing efforts can be optimized and gaps can be effectively addressed to minimize stove piping of information and resources. The framework has also identified strengths, vulnerabilities, and opportunities to strengthen readiness and resiliency of the 5 functional pillars from a One Health perspective that will better integrate aspects of human, animal, and plant health from the local to global level.

Significance: The development of an effective program for defending America's food, agriculture, and veterinary systems requires a thorough understanding of the various sectors, infrastructure components, stakeholders, and existing programs to maximize collaboration among them.

T8-06 Testing and Evaluation Countermeasure Tools for African Swine Fever Preparedness, Response, and Recovery

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Introduction: African Swine Fever (ASF) is a highly transmissible, viral disease that can cause up to 100% mortality in swine. ASF is the highest transboundary animal disease threat to the U.S. swine industry. An ASF outbreak would potentially cost the U.S. swine industry as much as $50 billion over 10 years. Highly effective and rapidly deployable ASF countermeasure solutions are urgently needed.

Purpose: The purpose of these studies was to test and evaluate (i) a field deployable detection device, (ii) a swine carcass composting disposal method, and (iii) chemical disinfectants for ASF virus (ASFV).

Methods: A commercial off-the-shelf, portable pathogen detection device (Solas 8®) was evaluated for diagnostic sensitivity, speed, and sample type. An ASFV-infected swine carcass compost windrow was constructed in BSL-3Ag containment according to established procedures outlined in the U.S. Department of Agriculture Livestock Mortality Composting Protocol. Samples were collected for virus viability over a 35-day period. Commercial chemical disinfectants relevant to the U.S. pork producer industry were tested for the ability to kill ASFV on porous and non-porous surfaces.

Results: The Solas 8® was effective in detecting ASFV within three hours in clinical samples (blood, oral swabs, meat, bone marrow, ear punch, and spleen) and comparable to current lab-based methods. Virus isolation and reverse transcriptase-polymerase chain reaction showed that the ASFV-infected pig carcass composted windrow successfully inactivated ASFV within 10 days coincident with a significant increase in windrow temperature. Virocid® (1:256 dilution) reduced infectious ASFV by >4 log in the presence of organic soil loads on non-porous stainless steel and porous concrete following a 10-minute contact time. This result meets U.S. Environmental Protection Agency standards for demonstration of Virocid® ASFV virucidal efficacy.

Significance: Highly effective and rapidly deployable agricultural defense countermeasure solutions (detection device, disinfectant, and disposal methods) for ASF outbreak preparedness, response, and recovery were identified.

T8-07 Potential Utility of the Intentional Adulteration Assessment Tool (IAAT) – Survey of Food Industry

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Introduction: Department of Homeland Security (DHS) performs chemical hazard and threat characterization for defense of the food supply, including the consequences and risk of the intentional adulteration of foods with chemicals, bacteria, viruses, and toxins. DHS developed the Intentional Adulteration Assessment Tool (IAAT) to enable to food industry to have access to DHS data when evaluating the risk of contaminants in company-specific food processes.

Purpose: The purpose of this study was to conduct representative market research on the potential benefits to the food industry of utilizing IAAT to create food defense plans for compliance with the intentional adulteration (IA) rule of the Food Safety Modernization Act (FSMA).

Methods: DHS collaborated with MilTech and the Michigan Manufacturing Technology Center (MMTC) interviewed SMEs from different food industries for their insight about the IAAT, and developed an online assessment survey that was completed by 120 representative food firms.

Results: The results show that 97% of large companies vs. 83% of small firms are familiar with the IA rules. More than 60% of all companies agree that industry-specific assistance is needed to comply with IA rules. Sixty-two percent of all firms considered the IAAT concept as positive. All of the respondents supported a report that details the risk-based vulnerabilities followed by a report format that supports the IA requirements.

Significance: This study provides a statistically relevant datasets to evaluate and understand the food industry needs for the IAAT and the importance of documentation priorities during the development of food defense plans.

T8-08 Characterization and Prioritization of Ingested Chemical Threats

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Introduction: There are thousands of toxic chemicals that can be potentially be used to intentionally adulterate food, especially if the chemicals are undetectable or easily masked by the food or beverage. Characterizing the toxicity and organoleptic profiles (detection by test and smell) of select chemicals provides critical information to focus mitigation and prevention tactics for intentional adulteration events.

Purpose: Based on prior models, the acceptance of adulterated foods by rats would indicate that humans would also eat the adulterated food. Laboratory testing was performed to characterize this critical knowledge gap.

Methods: A rat model was developed to determine the potential risk of toxic chemicals. The chemicals assessed were aldicarb, carfentanil, nicotine, potassium cyanide, and 4-aminopyridine. Solubility of the agents in the different matrices was determined and acute toxicity was quantified by 24-hour lethality following gavage administration. Organoleptics were also assessed via voluntary oral consumption of 3.0 mL of adulterated bottled water, apple juice, or 2% milk (n = 10 rats per beverage and concentration of adulterant).

Results: The estimated median lethal doses were lowest for aldicarb (0.83 mg/kg) and carfentanil (1.65 mg/kg) and were highest for nicotine (114.1 mg/kg). Aldicarb was readily consumed (M = 2.83 mL, SD = 0.35 mL) at supralethal doses in all beverages, producing rapid death. Consumption of lethal

doses of carfentanil occurred in juice and milk (M = 2.83 mL, SD = 0.24 mL) but not water (M < 1.0 mL). Rats never consumed lethal amounts of potassium cyanide or nicotine. Consumption of 4-aminopyridine was highest in juice (M = 2.65 mL; LD_{10}), followed by milk (M = 1.36 mL; LD_{01}), and then water (M = 0.44 mL; LD_{01}).

Significance: These results suggest that some toxic chemicals may be unwittingly consumed in toxic or even lethal concentrations by humans in a variety of beverages.

T8-09 Developing a New Quantitative Risk Metric Tool to Support Individual Sanitary Measure Equivalence Reviews

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Introduction: The Food Safety and Inspection Service (FSIS) is a regulatory agency that uses quantitative risk assessments (QRAs) to inform food safety decisions. Traditional QRA models can be complex and data intensive, and are not always practical for comparing the safety of food produced under different systems.

Purpose: FSIS identified a risk metric (i.e., based on a non-inferiority statistical test) to provide a streamlined approach in evaluating data on foodborne hazards that allows robust comparison of the safety of products produced under differing systems (e.g., Individual Sanitary Measure reviews).

Methods: FSIS' risk metric is used to determine if the different systems for producing comparable products are as good at controlling a foodborne hazard (i.e., "non-inferior"). Risk tolerances are considered to find an appropriate balance in misclassification errors. Margin of acceptance is defined as the maximum acceptable difference in prevalence between independently tested product (i.e., data gathered from product produced under a different system) and FSIS-tested product. The probability of misclassifying an independently tested product prevalence as non-inferior or inferior, i.e., Type I error and Type II errors, respectively, can be determined. Misclassification determination depends on the selected margin, the FSIS prevalence, and the number of samples collected.

Results: A margin of acceptance has been identified that provides clear criteria to ensure a high level of confidence that less safe product will be correctly classified (Type I error ≤ 5%) and that product that is as safe product will be correctly classified (Type II error ≤ 20%) to inform FSIS decisions.

Significance: FSIS' risk metric provides a consistent, objective and transparent approach to analyze external data and compare the safety of products produced under different food safety systems.

T8-10 A Novel Approach to FSIS Species Identification in Response to the 2018 Farm Bill

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Introduction: The 2018 Agriculture Improvement Act (commonly known as the Farm Bill) prohibited the slaughter or shipment of dogs, cats, or their parts for human consumption. The USDA Food Safety and Inspection Service (FSIS) determines animal species content of cooked and raw meat products to ensure label compliance. After passage of the Farm Bill, FSIS species methodologies were expanded to include the ability to detect canine and feline tissue.

Purpose: To support enforcement, FSIS validated a new technology for species identification capable of detecting the presence of multiple species in FSIS regulated food products using a single test. This technology detects canine and feline tissue per the requirement of the Farm Bill in addition to amenable meat and poultry species.

Methods: Canine and feline tissues and a variety of cooked and raw meat products were tested for species content using a novel DNA-based macroarray technology (n = 80). The technology utilizes a universal primer system targeting a small area (125-165 bp) of vertebrate 16S rRNA. Biotinylated PCR products of this region are generated from nucleic acid extracts of sample DNA. Labeled amplicons are hybridized to species-specific capture probes immobilized on the LCD-Chip surface and visualized.

Results: Validation findings were in accord with expected results. Each of the species examined showed no cross reaction or nonspecific binding during the study. The limit of detection was determined to be 0.2% on a weight basis; current methods have a limit of detection of 1% by weight.

Significance: FSIS validated a method to detect canine and feline tissue, enabling verification that the nation's food supply is accurately labelled. The method also provides enhanced detection for current species, can more easily add new targets, and is more cost-effective than current methodology.

T8-11 Risk Ranking of FSIS Shiga Toxin-producing *Escherichia coli* (STEC) Based on Virulence Genes

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Introduction: The USDA Food Safety and Inspection Service (FSIS) participates in Codex Alimentarius and is exploring whether aligning with the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) report on categorizing Shiga toxin-producing *Escherichia coli* (STEC) is both feasible and appropriate. In the FAO/WHO report, STEC are divided into categories based on virulence factors associated with poor clinical outcomes such as diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome.

Purpose: The objective of this study was to demonstrate feasibility of applying the FAO/WHO guidance criteria to STEC isolates from FSIS testing.

Methods: Whole genome sequencing (WGS) data exist for all STEC isolates derived from FSIS raw beef verification testing programs. WGS data was analyzed for the specific Shiga toxin type (*stx*) and the presence of intimin (*eae*) and transcriptional regulator *aggR* using output from the NCBI Pathogen Detection AMRFinderPlus tool and then assigned to one of the FAO/WHO categories. FSIS isolates were then searched on NCBI Pathogen Detection browser for inclusion in a phylogenetic tree to assess relatedness to clinical isolates.

Results: Based on the presence of both *stx* and *eae*, 100% (n = 725) of the FSIS isolates were placed into FAO/WHO categories 1 through 4, defined by poor clinical outcome. Twenty-seven percent of FSIS isolates were categorized as Level 1 (potential hemolytic uremic syndrome and/or bloody diarrhea). Approximately 99% of the clinical isolates from the NCBI Pathogen Detection also adhered to the four FAO/WHO categories. For STEC isolates belonging to categories 1-3, 83.4% were in a phylogenetic cluster on NCBI, whereas 59.8% of STEC from category 4 were in phylogenetic clusters on NCBI.

Significance: FSIS raw beef STEC isolates and clinical STEC isolates belong to FAO/WHO categories 1-4 indicating a public health impact on outcome. Adopting the FAO/WHO approach would allow FSIS to direct resources to riskier pathogens.

T8-12 Aligning Confirmation Criteria for *E. coli* O157:H7 and the "Top Six" non-O157 Shiga Toxin-producing *E. coli* (STEC)

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Introduction: The Food Safety and Inspection Service (FSIS) has aligned *Escherichia coli* O157:H7 laboratory confirmation criteria with criteria for non-O157 Shiga toxin-producing *Escherichia coli* (STEC) to harmonize the definition of laboratory-confirmed STEC and to clarify the FSIS STEC definition for stakeholders.

Purpose: This updated definition for a positive STEC in FSIS-regulated products addresses stakeholder confusion with the previous "two definition"

criteria for O157 and non-O157 STEC when developing new test methods and ensures worldwide applicability of the FSIS STEC identification method.

Methods: FSIS evaluated communications with stakeholders regarding concerns with the STEC criteria and the constraints placed on their processes. FSIS also analyzed STEC isolate data to assess the two definition criteria and the need for H7 gene identification. FSIS updated the Microbiology Laboratory Guidebook (MLG) STEC identification method (MLG Chapter 5C) to reflect the public health conclusion.

Results: FSIS aligned the two STEC definitions into a single definition. To confirm an *E. coli* isolate as an adulterant STEC, the isolate must contain a *stx* gene, an *eae* gene, and genetically identify as one or more of the "Top Seven" adulterant O-groups. Further, FSIS refined the STEC method to remove the identification of the H7 gene in certain *E. coli* O157:H7 isolates. The MLG 5C rapid screening criteria has not changed since H7 gene identification was part of the confirmation criteria. MLG 5C has been updated to a format that clearly defines laboratory criteria for potential, presumptive, and confirmed positive STEC isolates.

Significance: Aligning the STEC definition to have a single set of criteria 1) clarifies criteria for public health partners while meeting FSIS's public health priorities; 2) streamlines confirmation criteria across all STEC adulterants to facilitate efficient lab processes; and 3) can be implemented with no impact to existing STEC laboratory screening methods in use by stakeholders.

T9-01 Trace Amounts of Antibiotic in Feed Modified Fecal Microbiota of Weaning Pigs Experimentally Infected with a Pathogenic *Escherichia coli*

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❖ ***Developing Scientist Entrant***

Introduction: Trace amounts of antibiotics may delay the growth and development of animals. Our previous studies demonstrated that trace amounts of antibiotic supplements exacerbated growth performance and systemic inflammation of weaned pigs infected with a pathogenic *Escherichia coli* (*E. coli*).

Purpose: The objective of this study was to investigate the influence of trace amounts of antibiotic in feed on fecal microbiota of pigs experimentally infected with *E. coli* F18.

Methods: Thirty-four weaned pigs (6.88 ± 1.03 kg body weight) were individually and randomly allotted to one of three dietary treatments (9-13 pigs/treatment). The three dietary treatments were basal nursery diet (control), and 2 additional diets supplemented with trace amounts (TRA; 0.5 mg/kg) or recommended dose (REC; 50 mg/kg) of antibiotic (carbadox), respectively. The experiment lasted 18 days [7 days before and 11 days after the first inoculation (day 0)]. The *E. coli* F18 inoculum (10^{10} CFU/3 mL) was orally provided to all pigs for 3 consecutive days. Fecal samples were collected on days 5 and 11 post-inoculation (PI), and fecal microbiota was analyzed using 16S rRNA gene sequencing at the V4 hypervariable region. All compositional data were statistically analyzed using R software with QIIME2 plugin.

Results: Bray-Curtis PCoA analysis displayed that pigs in both control and TRA groups were separately clustered from REC on days 5 and 11 PI. Pigs in TRA group had a greater ($P < 0.05$) relative abundance of *Murbaculaceae* and *Lactobacillaceae* than other treatments on days 5 and 11 PI, respectively. REC increased ($P < 0.05$) the relative abundances of *Clostridiaceae*, but reduced ($P < 0.05$) *Lactobacillaceae* compared with other treatments on day 11 PI.

Significance: Trace amounts of antibiotic in feed could modify gut microbiota of weaned pigs challenged with *E. coli* F18. These results fill current knowledge gaps to understand the potential antibiotic residues' risks and mechanisms on animal health.

T9-02 Genomic Analysis of the Locus of Heat Resistance in *Escherichia coli*

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Introduction: The locus of heat resistance (LHR), a genomic island, confers heat resistance to *Escherichia coli,* which may lead to their survival of thermal treatment and cause food safety concerns.

Purpose: This study was to investigate the presence of LHR in *E. coli* of various serotypes and phylo-groups (Clermont method) and in generic (GEC) and Shiga toxin-producing *E. coli* (STEC).

Methods: All *E. coli* genomes (released until February, 2021) were downloaded from the public sequence database GenBank and serotyped using Ectyper v1.0. Each genome was screened for LHR and Shiga toxin genes using Abricate v1.0.1 with 70% and 90% as identity threshold, respectively. The phylo-group of each genome was determined via *in silico* PCR. Fisher's exact test was performed to determine the interrelation between LHR and serotypes/phylo-group and the effect was regarded significant if the *P*-value was < 0.05.

Results: A total of 18,987 *E. coli* genomes were analyzed, of which 2.8% harbored LHR (coverage >80%). One of 3,497 (0.03%) STEC was found to harbor LHR, which was likely due to a contamination issue. LHR was more prevalent in specific serotypes, for example, O-:H12 (10/55, 18.2%), O-:H30 (6/68, 9.0%), O9:H30 (8/101, 8.4%); on the contrary, LHR was less prevalent in serogroups including O157 (3/1009, 0.3%), O26 (6/770, 0.9%), O45 (2/200, 1%), O103 (0/249), O111 (0/239), O121 (0/151), O145 (0/257). Association was also found between LHR and phylo-groups of *E. coli*, with LHR bearing *E. coli* primarily belonging to phylo-group A (494/529, 93.4%), but being absent in phylo-groups B2, D, E, F or G.

Significance: The results show Shiga toxin genes do not seem to be compatible with LHR, and LHR is not uniformly distributed among different groups of *E. coli.* The findings would be useful for assessing food safety risks associated with LHR bearing *E. coli*.

T9-03 Target-Enriched Long-Read Sequencing (TELS): Metagenomic Profiling of Foodborne Pathogens, Antimicrobial Resistance, Virulence, and Mobilization Potential in Public Health Surveillance

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❖ ***Developing Scientist Entrant***

Introduction: Public health and food safety threats of antimicrobial resistance (AMR) and virulence are mediated by horizontal gene transfer of antimicrobial resistance genes (ARGs) and virulence factors (VFs) via mobile genetic elements (MGEs). Public health risks of such microbiome-level dynamics are difficult to predict and control, especially at the point of pathogen surveillance, due to limitations of current culture-based and culture-independent techniques to accurately reconstruct, colocalize, and contextualize ARGs with VFs, and MGEs in complex metagenomic samples.

Purpose: We demonstrate the use of a novel metagenomic sequencing approach– Target-enriched long-read sequencing (TELS) – to achieve accurate resolution of microbial resistomes, virulomes, mobilomes, and pathogen profiles from surveillance sampling in slaughter facilities. TELS metagenomic sequencing involves *in situ* enrichment using custom probes covering >7,000 ARGs, >6,000 VFs, >5,000 MGEs, as well as whole genomes of priority serovars of: *Salmonella*, *Escherichia coli*, *Campylobacter*, and *Enterococci*. Here we report on the first phase of this study: Molecular probe design for enrichment of pathogens from metagenomes.

Methods: Whole-genome-sequenced isolates (n=1000) for 2018–2021 were systematically abstracted from the NCBI pathogen detection system representing recent genomes and SNP-clusters of priority pathogen serovars. Following parameterization, these genomes served as the basis for probe design using the algorithm—CATCH— which was implemented in parallel to a new custom probe design algorithm. The performance of both algorithms to generate molecular probes was assessed.

Results: CATCH design coverage rate decays in a power fashion over time ($y = 2x10^{6x-0.198}$) before reaching equilibrium rate. For *Salmonella* serovars,

representing the largest proportion of genomes, polynomial probe synthesis ($y = 6784.2x^2 - 1x106x + 2x109$) converges on a theoretical completion of coverage in 3.4 months of algorithm runtime. In all cases the CATCH algorithm failed to converge on a probe design covering all genomes. This is contrasted with the custom algorithm producing 230,000 120-mer probes covering all intended genomes in minutes of runtime.

Significance: We extend bioinformatic techniques to enrich targets for purposes of metagenomic surveillance of foodborne pathogen threats.

T9-04 Multiplexing Long-Read Sequencing and Automated Analysis for *Salmonella* Serotype Prediction

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Introduction: Both short-read sequencing technology such as Illumina and long-read sequencing technology like Oxford Nanopore have been proven to provide reliable *Salmonella* serotype results. They have emerged as an alternative method to the gold standard: traditional serology using antisera. There are simple long-read library preparations for a single sample that can be combined with short sequencing time, however, the prices for consumables are high and the data analysis steps are relatively complicated for people without a bioinformatics background.

Purpose: This study evaluated the performance of the MinION sequencer when multiple samples were pooled into one library and sequenced in the same flow cell. The study also developed auto-analysis scripts for serotype prediction for singleplex and multiplexed samples.

Methods: Twelve *Salmonella* strains representing eleven serotypes were isolated on non-selective agar. Sample DNA was extracted using Qiagen Dneasy Ultraclean Microbial kit. The sequencing library for 12 samples was prepared using Oxford Nanopore Rapid Barcoding kit. The pooled library was sequenced 24 hours using the MinION sequencer and R9 flow cells. Scripts, which include porechop, Nanoplot, filtlong, Wtgdt2, and Seqsero 2, were used to automatically trim, filter, and assemble the sequencing data and predict the *Salmonella* serotype. Another 36 strains representing 30 serotypes were validated against the same workflow.

Results: The sequencing specifications passed the QC. The auto-script was able to process each sample from raw data to a serotype prediction with little hands-on time. Each strain had sufficient coverage from the multiplex sequencing run and received the correct serotype prediction from the automatic script.

Significance: Multiplex long-read sequencing decreases the price and the time to results for each sample. The auto-script enables people without bioinformatics background to perform WGS-based *Salmonella* serotype prediction.

T9-05 Deciphering the Transition of *Listeria monocytogenes* into Injury Using Fluorescent Microscopy and RT-qPCR

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❖ ***Developing Scientist Entrant***

Introduction: Exposure of *Listeria monocytogenes* to sub-lethal stresses related with food processing may induce injury, under the regulation of molecular mechanisms associated with dormancy.

Purpose: (i) To investigate, at population and single cell level, sub-lethal injury in *L. monocytogenes*, (ii) to describe the distribution of culturable, injured and dead cells during exposure to stress and (iii) to monitor the activation of the dormancy continuum associated molecular mechanism.

Methods: Acidic conditions (acetic and hydrochloric acid adjusted to pH 3, 2.7, 2.5 at 4 and 20 °C for 5 hours) were used to evaluate injury of *L. monocytogenes* Scott-A. To differentiate the resistant sub-population from the total, Tryptic Soy Agar with 0.6% Yeast Extract (TSAYE) supplemented or not with 5% NaCl were comparatively used. Sub-lethally injured cells were detected by comparing plate counts with fluorescent microscopy, using combinations of CFDA and Propidium-Iodide. Effect of acid stress on the relative transcription of *clpP, gadC, gadD, gadB, inlA, prfA, sigB*, *mazEF, relA, lmo0084, lmo0906* and *lmo0669* upon transition of total population into different physiological stages was evaluated through RT-qPCR. Ct values were normalized to *tpi* transcription and to reference samples.

Results: Acetic acid treated cells showed detectable logarithmic reduction of total population and an induction of injury and death, at single-cell level. *L. monocytogenes* retained its culturability after hydrochloric acid exposure, while cells remained metabolically active, exhibiting green fluorescence. Correlation analysis between genes with the same transcription profiles along time was performed. Six statistically significant ($P < 0.05$) down–regulated cases were revealed, *clpP*-AA pH 2.7 0 h, 2 h and 5 h, *gadB*-AA pH 2.7 3 h, *clpP* and *sigB*-HCl pH 3 5 h.

Significance: Assessing *L. monocytogenes* sub-lethal injury sheds light into risks of underestimation of a product's microbial status and potential resuscitation phenomena. Acknowledgment: *This project has received funding from the Hellenic Foundation for Research and Innovation (HFRI) and the General Secretariat for Research and Technology (GSRT), under grant agreement No [1788].*

T9-06 New Insights into Foodborne Outbreaks Caused by *egc* Enterotoxins from *S. aureus*

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❖ ***Developing Scientist Entrant***

Introduction: According to the European Food Safety Authority (2019), 77 out of 114 outbreaks caused by staphylococcal enterotoxin (SFPO) are weak evidence outbreaks. However, only five out of 27 enterotoxins can be analyzed using commercially available kits. Especially the presence of the so called "new enterotoxins," that have been described being involved in SFPOs, cannot be determined. A group of these new enterotoxin genes (*seg, sei, sem, sei, seo* and *seu*) is located on the same enterotoxin gene cluster (*egc*).

Purpose: The aim of the present study was to better understand the involvement of *egc* enterotoxin in SFPO. In addition, the results also give possible insights into the source of the involved strain.

Methods: The whole genome sequence of 80 *Staphylococcus aureus* strains from different origins (food poisoning outbreaks, human and animal) was investigated applying different bioinformatic methods. In addition, also a characterization of the different *v*Saβ phage, were the *egc* is contained, was performed. From a subset of the strains expression was measured (*seg, sei, sem, sen and seo*) by RT-qPCR as well as enterotoxin production (SEG, SEI) by using an internal ELISA method.

Results: The new insight from the genome analysis, as well as from the result from the enterotoxin expression and production, are showing that (1) Prediction of enterotoxin G and I can be performed by defining the clonal complex only; (2) The clonal complex can give an evidence of the possible source of the strain; and (3) With the new insight on the different *v*Saβ type analysis of the *egc* enterotoxin can be simplified.

Significance: The present study gives clear evidence on outbreaks where *egc* enterotoxins were involved. With the new knowledge and the simplified method, time and costs can be saved, giving on the same time more understanding and controlling of *egc* enterotoxin in foodborne outbreaks.

T9-07 Comparative Genomic Analysis of Persistent and Non-Persistent *Escherichia coli* O157:H7 Isolated from Cattle

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 is an important foodborne pathogen that causes outbreaks of hemorrhagic colitis and hemolytic uremic syndrome. Cattle are a major asymptomatic reservoir of STEC O157 which primarily colonizes the terminal recto-anal junction of the gastrointestinal system. Persistent STEC O157 strains found in animals and on farms are presumably well adapted to both hosts and environments and are responsible for a large part of O157 outbreaks. These environmentally persistent strains can potentially enable cattle to become bacterial super-shedders, secreting O157 at a rate of more than 10^4 CFU/g of feces. Previous studies have revealed that an *E. coli* O157:H7 subtype strain (FRIK2455) was predominant on a farm while another clonal variant was rarely isolated on the same farm (FRIK2533).

Purpose: The purpose of this study was to identify and understand genetic factors that may explain predominance of FRIK2455 in cattle through bioinformatics analysis and *in vitro* assay.

Methods: Whole genome sequencing of FRIK2455 and FRIK2533 strains was conducted using the PacBio sequencing technique. Comparative genome analysis was generated to compare whole-genome architectures of two strains. Also, metabolic capabilities were examined using genome-scale metabolic models. Adherence assay was performed to compare the adherence capacity between persistent and non-persistent strains.

Results: FRIK2455 and FRIK2533 strains share similar genetic composition and structure of chromosomes, but distinct features within plasmids. Only the predominant strain FRIK2455 carries a plasmid, pO157, which encodes many virulence factors. Adherence assay showed the differences on bacterial adherence mediated by pO157, indicating that pO157 is a critical factor for persistence of FRIK2455 in the gastrointestinal tract of cattle.

Significance: We suggest that pO157 plasmid in FRIK2455 provides an advantage for survival in hosts and environments by enhancing colonization of this pathogen.

T9-08 Transmission of Antimicrobial Resistant Genes at the Wildlife-Livestock Interface

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❖ ***Developing Scientist Entrant***

Introduction: The spread of antimicrobial resistance (AMR) is a major concern for animal and human health and leads to economic costs. It is increasingly postulated that wildlife could play an important role in the emergence and transmission of antimicrobial resistant microorganisms. However, the occurrence and transmission of AMR at the wildlife-livestock interface remain elusive.

Purpose: The purpose of this study was investigating the transmission of antimicrobial resistant genes (ARGs) at the wildlife-livestock interface to help gain perception of developing migration strategies of AMR.

Methods: We collected 364 fecal samples from cattle, feral swine and environments. Cefotaxime resistant bacteria (CRB) were isolated by plating on MacConkey agar containing cefotaxime (4 μg/mL). CRB carrying either CTX-M or CMY-2 gene were selected for whole genome sequencing to characterize extended-spectrum β-lactamase (ESBL) and AmpC β-lactamase-producing bacteria. Furthermore, microbiota transmission between cattle and feral swine was investigated using the 16S rRNA gene amplicon sequencing. The gut resistome and ARG transmission was detected using shotgun metagenomics sequencing.

Results: A high prevalence (33.5%) of ESBL- and AmpC β-lactamase-producing *Escherichia coli* was detected in feral swine. All isolates were multi-drug resistance, harboring various ARGs and robust virulence factors. Besides, similar microbiota structure was observed between cattle and feral swine reflected by bacterial phylum composition and co-occurring OTUs. Importantly, the proportion of ARGs that conferred tetracycline resistance was positively associated with the relative abundance of bacteria belonging to Bacteroidetes and Synergistetes in both cattle and feral swine. Moreover, clonal spread of AmpC-producing bacteria was observed between feral swine and cattle.

Significance: Our results provide critical knowledge to better understand the antimicrobial resistance bacteria and ARGs transmission at the wildlife-livestock interface which should be controlled to mitigate the potential spread of AMR.

T9-09 Genetic Diversity and Virulence of *Listeria monocytogenes* Recovered from an Artisan Cheese Facility over a Decade

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❖ ***Developing Scientist Entrant***

Introduction: Whole genome sequencing (WGS) and isolate genotyping are utilized during outbreak tracing; however, they are less often used to trace contamination events in food processing facilities. Here we describe a retrospective use of WGS to better understand *Listeria monocytogenes* (*Lm*) contamination in an artisan cheese facility over a decade.

Purpose: Assess diversity and properties of *Lm* recovered from an artisan cheese facility over 10 years.

Methods: *Lm* (*n* = 72) were recovered from 32 samples (13 cheese; 1 food contact; 18 non-food contact surfaces) collected during six sampling events in 2007-2016 (facility closed). Extracted DNA (Qiagen) was pair-end sequenced on Illumina HiSeq. Sequences were trimmed using Trimmomatic and *de novo* assembled with SPAdes. Multi-locus sequence types (STs), clonal complexes (CCs) and virulence profiles were assessed *in silico* using *Listeria* Pasteur database. Isolates were screened for antimicrobial tolerance-associated genes (*bcrABC, emrE*, *emrC*, *qacC*, *qacH, tetR*, *tnpABC*) with BLASTN (≥80% identity/alignment).

Results: Among the five STs and CCs recovered, the majority of isolates belonged to ST/CC11 (65/72), followed by ST/CC7 (3/72), ST/CC1 (2/72), ST/CC224 (1/72), and ST397/CC4 (1/72). Notably, ST/CC11 isolates were repeatedly recovered during 2007-2016, from drains and draining racks over multiple years, on mobile equipment (cart and table wheels, 2016), and in soft cheeses (2012). All isolates possessed full-length *inlA*, a known virulence factor, and *tetR*. *Listeria* pathogenicity island-3 (LIPI-3) was seen in four isolates, recovered from cheeses and a raw milk pump, while two *Lm* harbored *qacC*. Three isolates belonged to hypervirulent clones (CC1, CC4).

Significance: Results showed systemic and widespread contamination with some *Lm* clones (ST/CC11; ST/CC1), recovered from multiple food and environmental samples and sampling times, while other clones were transient. WGS also highlighted differences in virulence potential of recovered isolates. This information can be valuable in guiding recall decisions and assessing potential health risk to the consumer.

T9-10 Metagenomic Analysis of the Microbiome Associated with Single-Use Glove Manufacturing

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Introduction: Low counts and lack of effective protocols has limited microbial hazard identification on new single-use gloves (SUG) employed in food and healthcare (HC). In 2019 Eagle Protect PBC developed a methodology with rinse, count/enrichment, and genomic sequencing. Here we report the first large scale microbial survey of SUG brands, all claiming FDA food contact status and some for HC patient exam use.

Purpose: Metagenomic analysis has shown cross-contamination with identification of foodborne pathogens and indicators, but little study has taken place examining sufficient numbers/ unused SUGs employed in either venue.

Methods: Inside (I) and outside (O) plus I and O combined SUG rinse samples from 50-glove pools of 26 SUG brands (25 nitrile and 1 vinyl) were subjected to MPN determination with multiple enrichment/broths. Positive broths pooled/brand samples, with DNA extracted subjected to 16S/ITS1 amplicon and Shotgun WGS metagenomic sequencing.

Results: A total of 1,300 SUGs were tested w/ bacterial count range from 10^2 to ~2 x 10^5 CFU /100 gloves (a box) having geometric mean = 3.11 log. From >5.5 x 10^5 total CFU, >900 unique taxa, 229 genera and 245 species (+ >150 subsp., strains, serotypes/serovars) were identified. The taxa represented 21 phyla from, Archaea (2), Bacteria (14), Fungi (4) and Viruses (1). Differences in discrimination was seen between 16S and WGS methods w/ overlaps. Numerous food spoilage, frank and opportunistic pathogens and fecal indicator taxa were identified. Pathogenic genera/species included significant foodborne, dermal and respiratory pathogens. Based on knowledge of SUG production and early data, an extensive 16S inside/outside glove comparison examining 16 brands using multivariate cluster analysis determined that populations were significantly different inside from out.

Significance: This, a first in depth study examining SUG associated microbiome that due to flaws in manufacturing allows survival of pathogens and fecal indicators having public health and economic importance in food and HC applications.

T9-11 Comparative Whole Genome Analysis of MRSA to Understand Genetic Features Associated with Host Adaptation and Dissemination in Both Humans and Food Animals

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Developing Scientist Entrant

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a typical human and animal pathogen, causing various diseases. Importantly, certain MRSA clonal lineages have been found in both food animals and humans that pose great concerns to public health. Nevertheless, we still have limited understanding of how these MRSA clones successfully colonize in different hosts.

Purpose: We aimed to understand genetic features that enable MRSA for colonization in humans and animals.

Methods: We conducted whole genome sequencing (WGS) of 30 MRSA strains isolated from hospitalized patients, then compared with WGS of 10 MRSA strains, acquired from the NCBI database, which were isolated food animals. We performed comparative genome analysis for characterizing the phylogenetic relatedness, multi-locus sequence typing (MLST), antimicrobial resistance, virulence profile, mobile genetic elements, and Staphylococcal Cassette Chromosome mec of the closely related MRSA isolates.

Results: Seventeen, four, and two human isolates were belonging to ST ST8/CC8, ST5/CC5, and ST3309/CC5, respectively. WGS of MRSA strains from poultry, pork, beef, milk, and meat products, belonging to ST8 and ST5 clades, were compared with the human isolates. Interestingly, although isolates were belonging to the same lineage, with high similarity, they were genetically distinct based on the sources, indicating host adaptation may play a critical role to select host specific clones of MRSA.

Significance: Our study provides insights into genomic features, host adaptation, and clonal dissemination for MRSA, and highlights the importance of practicing One Health strategy to reduce MRSA burden in public health.

T9-12 Comparison of Pathogenic Characteristics for *Listeria monocytogenes* Isolated from Various Foods and Identification of Variations in Whole Genome Sequences

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Undergraduate Student Award Entrant

Introduction: *Listeria monocytogenes* causes foodborne illness with higher mortality than other foodborne pathogens, and the pathogenicity of *L. monocytogenes* is variant.

Purpose: This study analyzed the pathogenic characteristics of *L. monocytogenes* isolates from enoki mushrooms, smoked ducks and processed ground meat, and identified genetic variations in whole genome sequences (WGS).

Methods: *L. monocytogenes* isolates from enoki mushrooms (*N* = 23), smoked ducks (*N* = 7), and processed ground meat (*N* = 30) were analyzed for the hemolytic property, pathogenic genes, growth patterns, and heat resistance. Two *L. monocytogenes* isolates were then selected for highly pathogenic (*L. monocytogenes* SMFM201804 SD 5-3) and the low pathogenic (*L. monocytogenes* SMFM2019-FV43) isolates. WGS for the two isolates were obtained by PacBio® RS II platform, and analyzed with the CLC genomics workbench.

Results: All isolates (*N* = 60) showed *β*-hemolysis and possessed pathogenic genes (*hly*A, *inl*A, *inl*B, *plc*B, and *atc*A). D_{60} values of 3 isolates with high growth and 3 isolates with low growth at 4°C were 2.37-3.55 min. Subsequently, *L. monocytogenes* SMFM201804 SD 5-3 and *L. monocytogenes* SMFM2019-FV43 were selected for highly pathogenic and low pathogenic, respectively. WGS showed that two isolates possessed 45 toxic genes, 27 genes related to low temperature growth, and 21 genes related to heat resistance. Also, between two isolates, 41 single nucleotide variants (SNVs) and 4 single nucleotide polymorphisms (SNPs) for toxin genes, 18 SNVs and 4 SNPs for genes related to low temperature growth, and 16 SNVs for genes related to heat resistance were found.

Significance: The results indicate that SNVs and SNPs in genes related to toxin, low temperature growth, and heat resistance may induce the high pathogenicity of *L. monocytogenes* SMFM201804 SD 5-3.

T10-01 Withdrawn

T10-02 Frontiers in Pressure-based Treatment of Bacterial Spores and Pressure-Stressed Pathogens of Public Health Concern

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Introduction: Utilization of elevated hydrostatic pressure for elimination of microorganisms had been first proposed more than a century ago. However, due to advancements in engineering of commercially available units, application of this technology is gaining increasing popularity in the food commerce. Inactivation of bacterial spores and pressure-stressed sessile bacterial pathogens are important challenges associated with this re-emerging technology.

Purpose: This presentation provides an overview of recent microbial challenge studies performed in Public Health Microbiology laboratory for pressure-based inactivation of bacterial spores and pressure-stressed sessile pathogens. Effects of elevated hydrostatic pressure is additionally discussed in synergy with bacteriocin and bactericidal compounds to improve sustainability and cost optimization of pressure-treated commodities.

Methods: Presented studies are complete randomized block designs with two biologically independent repetitions as blocking factors, investigating effects of elevated hydrostatic pressure of up to 650 MPa in various temperatures and in presence of naturally-derived bacteriocin and bactericidal compounds. Inactivation of important indicator spores including *Geobacillus stearothermophilus*, *Bacillus amyloliquefaciens*, *Bacillus atrophaeus*, and *Alicyclobacillus acidoterrestris* as well as pressure-stressed and wild-type Gram-positive and Gram-negative bacterial pathogens will be discussed. Studies are statistically analyzed using analysis of variance followed by Tukey and Dunnett's-adjusted means separations.

Results: Elevated hydrostatic pressure lead to reductions (P <0.05) of >2 log CFU/mL of the bacterial spores. The decontamination efficacy of the treatment was augmented (P <0.05) by use of natural bacteriocin and bactericidal compounds including nisin, lysozyme, carvacrol, and lactic acid. Pressure-stressed and wild-type phenotypes exhibited comparable ($P \geq 0.05$) sensitivity to vast majority of pressure-based treatments.

Significance: Results of the studies indicate that an optimized pressure-based treatment could lead to >99% reduction of bacterial endospores. Pressure-stressed phenotypes of the bacterial pathogens exhibited comparable sensitivity to the wild-type cells. This indicates that a validated process against wild-type cells could almost certainly eliminate the pressure-stressed phenotypes as well.

T10-03 Inactivation of *Salmonella*, Shiga Toxin-producing *E. coli* and *Listeria monocytogenes* in Raw Diet Pet Foods Using High Pressure Processing

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Introduction: Raw diet pet food formulated with raw meat proteins can improve pet health or correct conditions thought to be caused by conventional pet foods. However, raw meat use can pose significant health risks to both pets and humans and outbreaks have occurred.

Purpose: The study evaluates HPP to achieve a 5-log reduction of *Salmonella*, *E. coli* STEC and *L. monocytogenes* in various commercial raw diet pet foods and to maintain a 5-log reduction throughout post-HPP storage.

Methods: Eight raw diet pet foods, three formulations (A-, S- and R- diets) containing beef or chicken proteins and two formulations (A- and S- diets) containing lamb proteins, were inoculated with 7 log CFU/mL cocktails of *Salmonella*, *E. coli* STEC and *L. monocytogenes*, vacuum sealed in high barrier bags and cooled to 4ºC. Samples were HPP treated at 586 MPa for 1-4 min at 4ºC and stored at either refrigeration or frozen for 21 days with microbiological analyses conducted at various time intervals.

Results: All A-diet formulations inoculated with *Salmonella* and *E. coli* STEC and treated at 586 MPa for 2 and 3 min, respectively, achieved a 5-log reduction and the 5-log reduction was maintained during storage. Freezing of the product post-HPP was better at maintaining the 5-log reduction than refrigerated storage. *L. monocytogenes* inactivation, in particular lamb-based formulations, behaved differently to *Salmonella* and *E. coli* STEC inactivation and formulations containing beef or chicken. No *L. monocytogenes* inoculated samples stored refrigerated post-HPP (4.10 ± 0.19 log reduction) achieved a 5-log reduction and only 30% of frozen samples (6.45 ± 0.52 log reduction) achieved a 5-log reduction.

Significance: HPP treatment (586 MPa, 3 min for beef and lamb; 4 min for chicken) inactivate 5-log of *Salmonella* and *E. coli* STEC and microbial reduction was maintained by freezing post-HPP treatment. *L. monocytogenes* inactivation, at least in this study, was variable and impacted by formulation.

T10-04 Lower Levels of Lipoprotein YhcN Likely Contribute to *Bacillus subtilis* Spores High Pressure Superdormancy

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❖ ***Developing Scientist Entrant***

Introduction: Bacterial spores are extremely resistant and challenging to inactivate in their dormant state. One approach to facilitate inactivation is by first germinating them. This strategy is called germination-inactivation strategy and has received widespread interest in the food science community due to its potential to eradicate spores while retaining food quality. High pressure (HP) of 150 MPa can trigger germination, however, germination is extremely heterogenous and some spores, termed superdormant (SD) spores, do not germinate or only extremely slowly.

Purpose: The aim of this study was to identify potential protein(s) involved in HP superdormancy.

Methods: *B. subtilis* spores were HP treated at 150 MPa, 37°C for 4 min. SD spores were quantified by flow cytometry and isolated by buoyant density centrifugation. To identify potential protein(s) involved in superdormancy, the proteins present in the initial dormant population were compared to the isolated SD spores. A label-free LC-MS/MS proteomics approach was applied, and protein identification and quantification were performed using the *MaxQuant* software and *Andromeda* search engine (n = 4 samples/category). Proteins with a log2(fold change) ≥ |1| and P-value <0.05 were considered as significantly different regulated. To validate the role of selected proteins in superdormancy, the germination capacity of mutants lacking the specific proteins was compared to that of the wildtype (150 MPa, 37°C, 3 min, $n \geq 3$).

Results: Approximately 1,600 proteins were identified of which 52 were significantly different regulated between dormant and SD spores. Of special interest is the lipoprotein YhcN with reported function in germination, which was approx. 2.5x less abundant in SD spores (adj. P-value = 0.022, n = 4). The germination capacity of the *yhcN* mutant was reduced compared to the wildtype (28 ± 5% SD spores compared to 5.0 ± 0.5% in the wildtype, $n \geq 3$).

Significance: Identifying proteins involved in superdormancy and understanding their function is the first step to eventually overcome the bottleneck of SD spores in germination-inactivation strategies.

T10-05 Biofilm Formation of Wild-Type and Rifampicin-Resistant O157 and Non-O157 Shiga Toxin-producing *Escherichia coli* and Their Inactivation by Bactericidal Compounds

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Introduction: Various epidemiologically significant serogroups of Shiga toxin-producing *Escherichia coli* continue to be important concerns in primary and further processing of an array of commodities and particularly meat products.

Purpose: The current study investigated the effects of sodium hypochlorite, 5% lactic acid, and quaternary ammonium compounds against planktonic cells and biofilms of wild-type and rifampicin-resistant Shiga toxin-producing *Escherichia coli* on stainless steel coupons.

Methods: The current study investigated effects of the above-mentioned sanitizers (one-minute submersion) on day 0 (planktonic cells) and against one-, two- and three-week mature biofilms of the pathogen. A six-strain mixture of wild-type and rifampicin-resistant *Escherichia coli* O157: H7 and a six-strain mixture of wild-type and rifampicin non-O157 serogroups (O26, O45, O103, O111, O121, and O145) were used in the current study (total of four replications per pathogen/day/treatment). Results were statistically analyzed using ANOVA followed by Tukey-adjusted means separation.

Results: The tested antimicrobials were efficacious for >2 log CFU/mL reductions (P < 0.05) of wild-type and rifampicin-resistant O157 and non-O157 Shiga toxin-producing *Escherichia coli* at planktonic state (day 0 treatments). Same treatments left behind in excess of >1,000 CFU/cm^2 when tested against

one-week, two-week, and three-week mature biofilms. Cell reduction of wild-type and rifampicin-resistant phenotypes of planktonic and sessile cells were similar ($P \geq 0.05$) in vast majority of testing days and antimicrobial treatments.

Significance: Our results indicate that the tested sanitizers are efficacious to eliminate the pathogen at planktonic state while exhibiting low decontamination efficacy against sessile cells. Additionally, we observed that wild-type and rifampicin-resistant phenotypes of Shiga toxin-producing *Escherichia coli* have comparable biofilm formation capability and sensitivity to sanitizers and thus could be used interchangeably in microbiological hurdle validation studies.

T10-06 Characterization and Application of Bacteriophages for Biocontrol of Shiga Toxin-producing *Escherchia coli*

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Introduction: Currently, there are no effective approaches to eradicate Shiga toxin-producing *Escherichia coli* (STEC) O157 on fresh produce post-harvest—representing a need for alternatives. Phages, viruses that specifically target and utilize bacterial hosts for replication, are a sustainable, natural, and cost-effective method to control contamination in the food industry.

Purpose: The purpose of this study was to probe the diversity of *E. coli* O157 phages and evaluate their efficacy in liquid media.

Methods: Whole-genome sequencing and annotation were employed to probe the lytic abilities and potential pathogenic genes of three phages capable of lysing STEC O157. Their individual abilities to suppress four O157 strains *in vitro* were assessed in biological triplicates at a multiplicity of infection of 100 PFU/CFU at 10°C over 72 hours using 10 mL of Tryptic Soy Broth and compared to a positive control containing STEC only. Enumeration of STEC was completed in technical duplicates using spread plating on Tryptic Soy Agar, while phage populations were enumerated using a soft-agar bacterial overlay. Changes in STEC and phage populations were analyzed using a two-way analysis of variance and Tukey's Honestly Significant Difference was utilized to further differentiate significant results ($\alpha = 0.05$).

Results: Phage genome annotation revealed a lack of integrase genes, indicating their putatively lytic nature. All three phages lacked resistance and virulence genes. *In vitro* examination revealed phages achieved up to a 5-log CFU reduction in STEC populations when compared to the control ($P < 0.05$). Overall, treatment of all STEC strains maintained phage populations within the range of 5.62-7.39 log PFU/mL ($P > 0.05$). The potential of these bacteriophages for biocontrol will be further explored by testing their efficacy on Romaine lettuce.

Significance: This research signifies contribution to the emerging field of phage therapy in food safety control which will combat STEC contamination and reduce economic and health burden.

T10-07 Dynamic Changes in Bacterial Communities during Seafood Decomposition at Low Temperature

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Introduction: Bacterial communities drive the development of chemical and sensory attributes during seafood decomposition. Therefore, understanding the dynamic changes in bacterial communities as they relate to chemical/sensory attributes produced during spoilage is important to develop and assess methods for analyses of seafood decomposition.

Purpose: The objective of this study was to examine the changes in bacterial communities associated with seafood decomposition at a low storage (0°C) temperature.

Methods: Seafood products (shrimp, snapper, and Spanish mackerel) were harvested from the Gulf of Mexico. Headed shrimp and gutted whole fish were immediately placed on ice and stored up to 42 days. Triplicate samples (25 g of shrimp or right anterior 1/3 fillet tissue) were tested weekly, and total bacterial counts and metagenomic analyses were performed. Samples were spread plated on TSA and incubated at 4°C for 14 days. DNA was extracted from fish samples using commercially available kits. 16S rRNA gene amplicons were sequenced using the Illumina MiSeq V3 platform. Data were analyzed using the QIIME2 pipeline.

Results: Total bacterial counts increased from 4.9 to 8.8 log CFU/g in shrimp after 20 days and <2.4 to 7.3 log CFU/g in snapper and Spanish mackerel after 42 days. The diversity of bacterial communities within samples decreased significantly ($P < 0.01$) during storage of all seafood types. Initially the seafood samples were mainly composed of *Vibrio*/*Flavobacterium*/*Shewanella*, *Staphylococcus*/*Escherichia*/*Cutibacterium,* and *Pseudomonas*/*Psychrobacter*/*Staphylococcus* for shrimp, snapper, and Spanish mackerel, respectively. However, at the end of the storage period the communities were mainly composed of *Shewanella*/*Pseudoalteromonas*, *Pseudomonas*, and *Pseudomonas*/*Janthinobacterium*/*Flavobacterium* spp. for shrimp, snapper, and Spanish mackerel, respectively.

Significance: Identifying and characterizing the bacterial communities responsible for producing chemical metabolites and sensory attributes will help us understand the impact of storage time and temperature with respect to seafood decomposition and aid in developing methods for their analysis.

T10-08 Effect of Acidified Fish Nutrients and Physiological State of *Vibrio cholerae* in the Biofilm Formation Capacity on Food Contact Surfaces

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Introduction: *Vibrio cholerae* is a foodborne pathogen associated with the consumption of contaminated seafood causing a severe disease. The microorganism is capable to form biofilm on food-contact surfaces and persist in the food-processing environment becoming a contamination source. The exposure to factors such acids used during food preparation might affect the biofilm formation.

Purpose: The purpose of this study was to evaluate the effect of physiological state of *V. cholerae* cells in biofilm formation on food contact surfaces with acidified fish nutrients.

Methods: Two strains of *V. cholerae* in logarithmic and stationary growth-phase were exposed to trypticase soy broth-2% NaCl (TSBN) acidified with citric acid (pH 5) per 60 min. Cells were inoculated on plastic and stainless-steel surfaces, submerged in TSBN (pH 5) and fish extract (10%, pH 5), and incubated at 25°C/4 h. Surfaces were rinsed to remove non-attached cells and placed in a 97% relative humidity chamber and incubated at 25°C/72 h. Bacterial population and biopolymers were quantified by spread plating and crystal violet stain/optical density measurements, respectively. Data were analyzed by ANOVA test.

Results: Acid exposed *V. cholerae* cells populations increased during incubation for up to 72 h (from 3.5 ± 1.0 to 6.5 ± 1.2 log CFU/cm^2); population on acidified TSBN were significantly lower than acidified fish extract ($P < 0.05$). The type of surfaces and growth-phase culture did not have effect on the growth ($P > 0.05$). Biopolymers also increased during storage (from 0.4 ± 0.1 to 0.6 ± 0.1 $DO_{595\ nm}$) independently of growth-phase culture. Biopolymers production were higher on plastic surfaces using TSBN as vehicle ($P < 0.05$).

Significance: *V. cholerae* is able to grow and produce biofilms on food contact surfaces containing acidified fish residues, representing a risk for cross-contamination in kitchens.

T10-09 Microbial Safety and Quality of Public Drinking Water in Ethiopia: Lessons Learned from Eleven Years Retrospective Data

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Introduction: Although the UN has declared that safe and readily available drinking water is a human right, by the year 2017, 785 million people still lack a basic water service. Ethiopia has declared that 57% of the population has access to safe drinking water. Despite the efforts, access to safe water still remains a public health threat.

Purpose: To assess the bacteriological safety and quality of drinking water and also to look for the influence of season on safety and quality of drinking water.

Methods: This is a retrospective study, 2008–2018 for eight years, whereby drinking water samples from different parts of the country were received from different customers across the nation. A total of 5,024 samples of drinking water results was considered for this study.

Results: From the total samples tested for thermotolerant coliforms, 78% and 85.3% met both the national standard and the WHO guideline value of <1 CFU/100 mL, respectively. Declining compliance of fecal coliform and E. The couple was observed during the main rainy season, particularly in June with compliance of 73.3% for fecal coliform and 80% for *E. coli*, consecutively. While the highest compliance was observed during the dry season particularly from November to January whereby only 91.9% and 85.6% for fecal coliform and *E. coli*, consecutively. Of the total samples, 37.8% (1,296) of the samples were microbiologically non-potable for not complying with one or more of the microbiological parameters, while 62.2% (2,351) of the samples were microbiologically potable.

Significance: The percentage availability of improved and safe water, from the retrospective data, is in agreement with a previous study performed. The study also indicated the impact of seasonal change on the quality of drinking water in particular to compliance of fecal coliforms and *E.coli.* This work estimates the progress being made in safe drinking water for the public.

T10-10 Comparison and Evaluation of Methods for Monitoring Agricultural Water to Meet FSMA Produce Safety Rule Requirements

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Introduction: Fruit and vegetable growers need to understand their agricultural water quality to make food safety decisions about water use as well as to meet regulatory and buyer requirements, but water testing laboratories are often not located close to farms, nor do they offer the required water analysis methods, making compliance and well-informed decision-making difficult.

Purpose: The objective of this study was to compare the variability of microbial test results of agricultural surface water sources from two accredited laboratories and one non-accredited university laboratory.

Methods: Surface water samples (n = 451) from 9 sites were collected using sterile technique during 3 consecutive growing seasons and analyzed using IDEXX Colilert 24 (university laboratory and commercial lab #1) and Colilert 18 (commercial lab #2) for MPN of generic *E. coli*. Each sample was divided in half; one half was held for at least 24 hours under 40°F refrigeration (i.e., hold-sample) and the other half was divided into thirds for analysis within 8 hours at the two commercial laboratories and the university laboratory. After the 24 hours, each hold-sample was divided and sent to the laboratories for analysis. Microbial water quality profiles (MWQPs) were created for each site.

Results: There was a statistically significant (P < 0.001) effect of hold-time on the microbial test results. Additionally, commercial lab #1 (least squares mean= 3.203) yielded statistically significant (P < 0.001) lower results than the university laboratory (least squares mean = 3.413) and commercial lab #2 (least squares mean = 3.402). The variation in microbial test results between labs and impact of 24-hour hold could change whether a water source is in compliance with water standards, as currently written, in Subpart E of the Produce Safety Rule.

Significance: These sources of variation (e.g., lab, method, hold-time, accreditation) may impact compliance status of an agricultural water source, which could impact growers' ability to meet market and regulatory requirements.

T10-11 The Effect of Rainfall on Spatiotemporal Patterns of *Salmonella enterica and Listeria monocytogenes* Survival in Irrigation Water Sources Using Empirical Orthogonal Functions

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Introduction: Levels of *Salmonella enterica* and *Listeria monocytogenes* in agricultural ponds and rivers, potential irrigation water sources, can be affected by environmental factors. These pathogens have caused previous outbreaks of fruits and vegetables. Empirical Orthogonal Function (EOF) analysis can provide spatial and temporal patterns of variability to assess the effect of specific environmental factors on pathogen levels in water.

Purpose: To examine the role of rainfall in affecting *Salmonella* spp. and *L. monocytogenes* levels in irrigation water using spatiotemporal pattern analysis by EOF.

Methods: *S. enterica* and *L. monocytogenes* (MPN/L) values were determined from two river (MA04, MA05), two pond (MA10, MA11) and 1 treated wastewater pond (MA06) irrigation water sources in a previous study (Sharma et al., 2020). EOF analysis was used to interpret spatial and temporal patterns of pathogen levels in water based on rainfall data during that period. EOF analysis generates a sequence of spatial patterns EOF1, EOF2, etc., which account for the progressively decreasing portions of the variance of the dataset.

Results: The EOF1 pattern of the one-day and seven-day rainfall totals had similar patterns to the EOF1 for *L. monocytogenes* at pond water sites, indicating that rainfall events increased levels of *L. monocytogenes* in ponds; however, *L. monocytogenes* EOF patterns in river water had a lower correlation with EOF rainfall patterns. EOF1 and EOF2 values for *L. monocytogenes* explained 84.4% and 9.7% of the total variance, respectively, in all water sources. EOF1 and EOF2 values for *S. enterica* explained 50.8% and 45.0% of the total spatial variance, respectively; *S. enterica* EOF2 patterns from MA04, MA05, and MA11 were similar to EOF2 patterns for rainfall, indicating a strong relationship between rainfall and *Salmonella* at these sites.

Significance: The effect of rainfall on bacterial pathogen level in irrigation water is dependent on the pathogen and water type.

T10-12 *E. coli* O157:H7 Viability after Storage within Ultrafilters or Ultrafilter Backflush

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Introduction: In the last five years, environmental assessments following foodborne outbreaks have heavily relied upon the collection of 10 L or more of irrigation or surface waters using ultrafilters intended to capture and concentrate bacteria and other microbes but the impact on viability of *E. coli* O157:H7 held for extended periods of time on these filters or backflush is largely unknown.

Purpose: To assess the viability of *E. coli* O157:H7 following up to weeklong refrigerated storage within inoculated ultrafilters and in the solution used to backflush these filters.

Methods: Pre-conditioned ultrafilters were inoculated with ca. 10^7 CFU of a GFP-labeled *E. coli* O157:H7 strain to achieve a final concentration of more than 10^2 CFU/mL in the backflush solution. Triplicate sets of filters and backflush were either processed immediately or after storage at 4°C for processing at Day 0, 1, 2, 3, 5, or 7 days, before enumeration of O157:H7 by plating aliquots of backflush onto non-selective agar.

Results: Viability of *E. coli* O157:H7 was not significantly reduced in either refrigerated stored ultrafilters or backflush solutions even after 7 days post-inoculation. On the filters, an overall reduction of 0.60 log CFU ± 0.10 was observed after 7 days of storage with the largest decrease observed between Day 2 (0.12 log CFU ± 0.10) and Day 3 (0.48 log CFU ± 0.10). In the backflush, loss of viability was negligible until Day 5 (0.34 log CFU ± 0.16). A 0.49 log ± 0.19 CFU decline was noted at Day 7.

Significance: Regularly, the downstream analysis of ultrafiltered water is delayed due to shipping or laboratory-related issues. These results demonstrated that belated processing of either the filters or backflush should not have detrimental effects on O157:H7 viability.

T11-01 Reference Method Selective Media Differ Significantly in Their Ability to Support *Listeria* spp. Growth

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Introduction: Commonly used reference method selective enrichment broths may not be suitable for the detection of all *Listeria* species.

Purpose: The objective of this study was to evaluate the detectability of 19 *Listeria* species using the selective media from three reference methods, (i) FDA BAM, (ii) USDA-MLG, and (iii) ISO.

Methods: Pure cultures of each species were diluted and inoculated into the reference method selective enrichment broths. The media evaluated include (i) BLEB, (ii) Demi Fraser, (iii) Fraser, (iv) MOPS-BLEB, and UVM. A BHI control was concurrently inoculated. Enrichments were incubated as described in the method and enumerated post incubation. Three biological replicates were completed with each media-species combination. The data were analyzed using two-way ANOVA and pairwise comparisons were performed using Tukey's test. Further analysis with BHI as the control group was performed using Dunnett's to assess inhibition.

Results: Only the FDA BAM BLEB enrichment recovered all species with no significant difference ($P > 0.05$) between species and had no inhibitory effect. The USDA-MLG UVM enrichment yielded the highest level of inhibition and showed potential for *L. seeligeri* to be masked by several newly described species. To a lesser degree, the ISO Demi Fraser and Fraser enrichments as well as the USDA-MLG's MOPS-BLEB also showed inhibitory effects as well as the potential not to detect *L. seeligeri* or *L. ivanovii*. *L. grayi* did not grow in UVM, Fraser or MOPS-BLEB.

Significance: The detection of *Listeria* non-*monocytogenes* species is important to food safety as these species serve as indicators for the risk of *L. monocytogenes* (LM) contamination. While the newly described *Listeria* species are not recognized as indicators for LM, our data showed mixed populations with these species may mask the presence of indicator species (e.g., *L. grayi, L. seeligeri* and *L. ivanovii*) when using USDA or ISO enrichments.

T11-02 Validation of the EnviroX Assay for the Detection of *Listeria*, *Listeria monocytogenes* and *Salmonella* in Environmental Surface Samples

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Introduction: The PathogenDx Enviro^X is a novel, multiplexed diagnostic technology that uses PCR coupled to DNA microarray to detect dozens of organisms simultaneously from food, water and environmental samples without the need for enrichment. The advent of this technology allows the user to detect and speciate multiple organisms in under 8 hours allowing same shift turnaround.

Purpose: The purpose of this study was to validate the Enviro^X Environmental Screening Assay according to the AOAC INTERNATIONAL *Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces, Appendix J*.

Methods: The *Performance Tested Method*^{SM} evaluation was performed in two parts by the method developer and independently through Q Laboratories. The study included the evaluation of the Enviro^X assay for the detection of *Listeria, Listeria monocytogenes* and *Salmonella* species (inclusivity/exclusivity study), four different matrices (stainless steel, sealed concrete, rubber, and plastic), a consistency/stability study, instrument variation study and robustness testing. The reference method for the matrix studies was the U.S. Food and Drug Administration *Bacteriological Analytical Manual* (BAM) Chapter 5 for *Salmonella* and BAM Chapter 10 for *Listeria* and *Listeria monocytogenes*.

Results: According to AOAC Appendix J, statistical analysis was performed using Probability of Detection (POD) statistical model. Fractional positive results for the low inoculum level, (25-75% positives) 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 *Performance Tested Method*^{SM} evaluation concluded that the Enviro^X assay is able to detect *Listeria, Listeria monocytogenes* and *Salmonella* species on multiple surfaces in accordance with AOAC standards.

T11-03 Verification of Quantification and Limits Testing of *Salmonella* in Finely Textured Beef (FTB) Using Hygiena's BAX® System Real-Time PCR Assay for *Salmonella*

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Introduction: In order to make rapid decisions for beef products, prevalence does not provide enough information within an adequate time period. Using a quantitative method would provide a tool for a deeper understanding of consumer risk to make product release decisions.

Purpose: The purpose of this study was to perform a verification of enumeration of *Salmonella* in FTB samples using the BAX® System SalQuant™ to quantify between 1 – 10,000 CFU/g and SalLimits™ testing for LOD1 and LOD10 threshold levels.

Methods: Sixty-five grams of each FTB sample ($N = 547$) of was combined with 260 mL of pre-warmed BAX® MP media and homogenized. A 30 mL aliquot of solution was combined with 30 mL of BAX® MP plus supplement in a separate container, then incubated at 42°C for 5 h (LOD10) and 6 h timepoints (LOD1 & SalQuant™). For all testing, the BAX® System Real-Time PCR Assay for *Salmonella* was utilized.

Results: SalQuant™ and SalLimits™ accurately estimated 99% of all positive (72/79) and negative (468/468) samples. Only 22/79 positive samples were greater than 1 CFU/g, but less than 10 CFU/g with LOD1 positive, LOD10 negative, and verified with SalQuant™. Only 50/79 samples were greater than 1 CFU/g and 10 CFU/g, and verified SalQuant™. Only 7 samples (8.9%) were positive at LOD10 and estimated to be less than 10 CFU/g, with SalQuant™ ranging from 2.76-7.57 CFU/g.

Significance: Verification of SalLimits™ and SalQuant™ in finely textured beef provides a rapid tool that is precise and accurate to make data-driven decisions for product release and diversions.

T11-04 A Rapid Assurance® Gds Method for Quantitative Estimation of *Salmonella* Contamination Level in Raw Beef

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Introduction: *Salmonella* is a leading cause of foodborne illness in the US and approximately 38% of cases are attributed to meat and poultry. Beyond prevalence testing, *Salmonella* enumeration is required to assess risk levels in a holistic strategy to reduce *Salmonella* contamination.
Purpose: Develop a limit-based approach and a quantitative method to rapidly estimate *Salmonella* contamination level in beef trim, ground beef (GB), finely textured beef (FTB), and carcass cloths using the time-to-positive (TTP) and post-enrichment PCR cycle threshold (Ct), respectively.
Methods: To determine the TTP for 10 CFU/g, 30 samples of each matrix were spiked with a cocktail of *S.* Enteritidis, *S.* Newport, *S.* Dublin, and *S.* Typhimurium, then enriched in mEHEC® (1:5, 200 mL for cloth) at 42°C for 3-6 h. Enrichments were tested every 30 min to determine recovery. To establish the quantitative equation, a cocktail of *S.* Enteritidis, *S.* Newport, *S.* Dublin and *S.* Montevideo were tested in trim, FTB, and carcass cloths. Standard curves were established by linearly correlating Mean Ct post enrichment to the pre-enrichment Most Probable Number (MPN). Quantitative equations were verified by comparing quantification results to MPN.
Results: The TTP of 10 CFU/g (500 CFU/cloth) was determined to be 4 hours for trim and carcass cloth and 4.5 hours for FTB and GB. GDS standard curves in trim, cloth and FTB were established for 5 h of enrichment. The quantification of cocktail strains from 1 to 1,000 CFU/g (for cloth, 0.5 to 500 CFU/g or 187.5 to 187,500 CFU/cloth) was comparable to MPN estimation ($R^2 \geq 0.997$, RMSE 0.19-0.22 log CFU/g).
Significance: GDS *Salmonella* quantitative and limit testing methods provide rapid options to accurately estimate the contamination level in beef matrices. The limit testing method identifies product lots containing over 10 CFU/g *Salmonella*; the quantitative method provides estimates of initial *Salmonella* cell count over a wide range of concentrations.

T11-05 Rapid Identification and Molecular Characterization of *Escherichia coli* Isolates from Food and Environment through Whole Genome Sequencing

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❖ ***Developing Scientist Entrant***

Introduction: Whole genome sequencing is becoming the tool for detection and characterization of foodborne pathogens for various applications.
Purpose: This study was conducted to evaluate the performance of Minion sequencing for rapid identification and molecular characterization of *E. coli*.
Methods: Eleven *E. coli* isolates obtained from pecan orchards were sequenced using Minion and Illumina NextSeq 500. As Minion allows real-time reads analysis, the reads were time-based subsampled to determine the earliest identification turnaround time for each isolate.
Results: Species-level identification was achieved at 15 min of sequencing run. In 8 hours of sequencing run, O antigen prediction was available, whereas the antigen H could not be determined, not even after the whole sequencing process was completed (48 hours). Variants of the virulence genes *eae* and *stx* were detected in seven isolates at 24 hours of sequencing run. The presence of these virulence genes was confirmed by multiplex PCR. Also, assemblies obtained from the subsampled reads were compared with assemblies obtained from the full reads dataset, as well as hybrid assemblies of Minion and Illumina reads. The results showed that the best values of continuity were obtained in just 4 hours of sequencing run. Finally, with a stringent BLASTp search using the Comprehensive Antibiotic Resistance Database (CARD) and Virulence Factor Database (VFDB) against the annotated proteins from each of the assembled genomes, none of the complete reads datasets were sufficient to generate significantly similar profiles to those obtained from the hybrid assemblies. Nevertheless, it was possible to obtain an average of 87.25% and 75.51% of the hits obtained from the hybrid assemblies using the CARD and VFDB, respectively; whereas no significant changes were observed after 4 hours compared to the complete datasets ($P < 0.05$).
Significance: These results demonstrated that Minion can offer an effective tool for the rapid identification and partial characterization of *E. coli* isolates.

T11-06 Using an Impedance Cytometer for the Enumeration of Bacteria Commonly Found on Food Production Surfaces and Foodstuff

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Introduction: Bacteria are the most common source of food poisoning. This makes the reduction of bacteria load on production surfaces a central concern for food producers. To verify such procedures, traditional cultural methods are very common. Impedance cytometry is an alternative way to count bacteria and has the potential to significantly speed up hygiene verification processes, which usually last days, to a matter of minutes.
Purpose: This study compares the ability of traditional petri dish-based methods to count bacteria commonly found in foodstuffs and in food production areas with that of an innovative impedance cytometer designed for this purpose.
Methods: Frozen (-20°C) bacterial cultures were incubated overnight (16-22 hours) and serial dilutions were made. The pure bacterial cultures were diluted to achieve a final concentration of between $10x10^2$ and $10x10^3$ colony forming units per mL. One hundred µl of each dilution were spread on tryptic soy agar (TSA) plates in 3 replications. The same cultures were also analyzed, measuring 10 replications for each concentration (between $10x10^4$ and $10x10^6$ cells/mL) using an impedance cytometer.
Results: Statistical comparisons were performed with a significance level of 0.05. Out of the tested bacteria only *Escherichia coli* (ATCC #11775), *Pseudomonas aeruginosa* (ATCC #27853) and *Enterococcus faecalis* (ATCC #29212) showed no significant differences in results. Nevertheless, all calculated concentrations both of the plates and of the cytometer were within the same order of magnitude, which is an acceptable deviation for hygiene monitoring applications. The detection limit of the impedance cytometer (with a measurement duration of 30 seconds) was at $1.5x10^4$ cells/mL.
Significance: The results of the study suggest that impedance cytometry can provide food producers with insights into the hygienic conditions of their production facilities significantly faster than traditional methods within an acceptable range of deviation.

T11-07 Metabolomics of Foodborne Pathogenic Fungi and Their Derivatives

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Introduction: High precision detection methods (such as metabolomics) required to profile pathogenic microorganisms and their metabolites in foods and other biological systems are gaining popularity as recent analytical approaches to determining the presence of microorganisms and their metabolic by-products in food systems.
Purpose: This study examined the different metabolomic approaches that are currently used to determine the presence of pathogenic fungi and their metabolites, especially mycotoxins, in foods to understand their characteristics and functionality.
Methods: Published works on the use of different mass spectrometric approaches and their tandem enhancements (such as Gas Chromatography/Mass Spectrometry (GC/MS), Liquid Chromatography/Mass Spectrometry (LCMS) and Matrix Assisted Laser Desorption Ionization-Time of Flight/Mass Spectrometry (MALDI-TOF/MS)), nuclear magnetic resonance (NMR, 2D-NMR and H-MNR) and other methods such as Fourier Transform Infra-red spectros-

copy (FTIR) were reviewed to understand the presence of pathogenic fungi, their toxins and other metabolites in various food systems.

Results: Fungal pathogens and their metabolites have been considerably studied. Potentials for the production of biomarkers from the toxins of pathogenic fungi identified exist and should be explored for a more rapid detection. Also, other important metabolites, apart from mycotoxins were detected and could be linked to the functionality and metabolism of these pathogenic fungal strains.

Significance: The study expands the potentials for rapid detection of pathogenic fungi and their metabolites in foods and food systems.

T11-08 Custom Baits with Mitochontrakr to Detect and Identify Insects in Food

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Introduction: Next Generation Sequencing (NGS) technologies and bioinformatics have not been used to identify insects in foods. Targeted enrichment NGS approaches such as capture baits have shown to increase read coverage of regions of interest from genomic DNA (gDNA) extracted from foods. It is possible that targeted enrichment of insect mitochondrial genomes (mitogenomes) might improve matching of query sequences to k-mers (k = 30) derived from mitochondrial DNA fragments in an in-house database (MitochonTrakr) containing >11K mitogenomes of animal and plant origins.

Purpose: To evaluate the use of a custom target capture bait design to enhance insect detection and identification of the MitochonTrakr.

Methods: NGS libraries with and without targeted enrichment with a custom bait were prepared per triplicate from gDNA of 30 insect species combined at equal and unequal DNA concentrations and from gDNA extracted from wheat flour samples spiked with Indian meal moth (IMM) fragments at 1, 10, 100, and 1,000 ppm. Targeted enrichment was performed by solution-based hybridization capture. The designed custom bait contains ~260K probes (110 nt long) targeting over 3,600 mitogenomes from 2,160 insect species. NGS was performed using the Illumina Miseq system after normalizing pooled libraries to 4 nM. Sequence reads were queried against k-mers in the MitochonTrakr database.

Results: We observed accurate taxonomic identification of all 30 insect species when assigning sequence data from libraries with and without targeted enrichment with a custom bait in the MitochonTrakr database. Using the custom bait decreased background sequence reads from the wheat flour, making the insect detectable at 1 and 10 ppm, and increasing read coverage at other insect concentrations.

Significance: Using custom target capture baits along with the MitochonTrakr database increases read coverage of insect regions and the sensitivity of insect detection. This sequence-based approach may be a useful tool for detecting and identifying insects in foods.

T12-01 Multi-Serovar Salmonella Populations Hide Antimicrobial Resistance Reservoirs in Food Animals

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Introduction: *Salmonella* can exist in food animals as multi-serovar populations. Since conventional culturing allows only the most abundant *Salmonella* serovars to be isolated and characterized, low frequency serovars within a mixed *Salmonella* sample are not detected. Where underlying serovars exhibit unique patterns of antimicrobial resistance (AMR), this also remains undetected, and could contribute to the overall *Salmonella* AMR reservoir.

Purpose: Our goal was to assess *Salmonella* populations in cattle before and after antibiotic treatment using CRISPR-SeroSeq, and to determine whether underlying serovars exhibited differential, functional AMR.

Methods: CRISPR-SeroSeq, an amplicon-based next-generation sequencing tool that profiles multiple *Salmonella* serovars in individual samples, was used to assess serovar populations in feedlot cattle before (55 samples) and after (11 samples) in-feed chlortetracylcine (CTC) treatment. An *in vitro* tetracycline (16 µg/mL) challenge on enriched fecal cultures was performed, followed by serovar-specific qPCR to assess changes in serovar abundance after selection.

Results: Population analyses revealed that 60% (40/66) of samples harbored multiple serovars, including five serovars in one sample. Before CTC treatment, 11% fecal samples contained *Salmonella* ser. Reading. In each instance other serovars (ser. Mbandaka, Give, and Montevideo), were more abundant than ser. Reading. The low frequency of ser. Reading within samples where it was present (0.2-37%, relative to other serovars), resulted in this serovar not being detected by culture-based *Salmonella* isolation. After CTC treatment, ser. Reading was found in 100% fecal samples, concurrent with culture analyses and also antibiotic susceptibility testing that confirmed Tetracycline resistance. Serovar-specific qPCR analyses performed following *in vitro* tetracycline challenge revealed that the ser. Reading present in cattle at low levels were resistant to tetracycline while ser. Mbandaka, Give, and Montevideo were susceptible.

Significance: This study highlights the importance of surveillance in multi-serovar populations in identifying low abundance AMR serovars that can be selected for by antimicrobial use.

T12-02 Preliminary Analysis of the Role of the Noncoding RNA Rli47 in the *Listeria monocytogenes* Response to Lactic Acid Stress

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❖ ***Developing Scientist Entrant***

Introduction: Transcriptomic data indicate that the noncoding RNA Rli47 is important in the *Listeria monocytogenes* stress response, hypothetically by limiting growth under harsh conditions through its known function of suppressing isoleucine biosynthesis.

Purpose: Our research seeks to elucidate the role of Rli47 in the survival of *L. monocytogenes* following lactic acid stress exposure.

Methods: We utilized allelic exchange to delete *rli47* from the chromosome of the *L. monocytogenes* ST121 strain 6179. Growth curves of 6179 and 6179*Δrli47* were conducted at 20°C in tryptic soy broth (TSB) and TSB with final concentrations of 0.15% or 0.2% lactic acid. Percent survival assays were conducted by inoculating tubes of 1% lactic acid TSB with either 6179 or 6179*Δrli47* cells grown to logarithmic phase at either 30°C or 20°C. Tubes were incubated for two hours, and colony forming units were determined via plate counts.

Results: Our previous analysis demonstrated that *rli47* expression increases 355-fold following 1% lactic acid stress. Growth curve analysis demonstrated no differences between 6179 and 6179*Δrli47* under any of the conditions tested. Additionally, 6179 and 6179*Δrli47* did not display a significant difference in lactic acid survival when the cells used for inoculation were grown to early-logarithmic phase at 30°C ($P > 0.05$). However, 6179*Δrli47* had a higher rate of survival than 6179 when the cells used for inoculation were grown to early-logarithmic phase at 20°C ($P < 0.05$).

Significance: Our results indicate that *rli47* has a temperature-dependent effect on *L. monocytogenes* lactic acid survival. Interestingly, deletion of *rli47* confers increased lactic acid tolerance in contrast to what would be expected from our earlier transcriptomic data. Our findings provide a foundation for future efforts to understand Rli47 and its relationship to *L. monocytogenes* stress survival.

T12-03 Impact of *Lactobacillus*-Originated Metabolites on EHEC in Collected Rumen Fluid

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❖ ***Developing Scientist Entrant***

Introduction: Rumen is the most enriched microbial ecosystem and ruminants specifically cattle harbor miscellaneous microorganisms which are considered as 'normal flora of cattle'. This microflora including zoonotic pathogens can be disseminated to the environment and passed to humans indirectly and/or directly through contaminated farm waste, dairy, and meat products. Controlling the colonization of major cattle-originated pathogens e.g., enterohemorrhagic *Escherichia coli* (EHEC) is a crucial challenge to animal husbandry.

Purpose: Positively stimulating rumen microflora using live probiotics, synbiotic, and their metabolites to suppress the pathogens specifically EHEC.

Methods: Rumen fluid (RF) was collected from a healthy cow to simulate rumen ambiance and ~10^3 CFU/mL of EHEC was introduced experimentally. The RF with EHEC was incubated up to 72 h separately with live wild type *Lactobacillus casei* (LC_{wt}), LC_{wt} with 0.5% peanut flour (LC_{wt+PF}), an engineered LC capable of overexpressing linoleate isomerase (LC_{CLA}), and their metabolites collected in cell-free culture supernatants (CFCS). Metagenomic analysis of the samples at different time points was performed by targeting 16S rRNA genes. Student's *t*-test was applied to determine statistical significance.

Results: Culture-based technique revealed the significant ($P < 0.05$) growth stimulation of *Lactobacillus* spp. by all CFCSs while the growth of EHEC was found to be continuously suppressed. Whereas, only LC_{wt+PF} at 72 h was able to reduce EHEC by 2.68 logs ($P < 0.05$). Metagenomic analysis indicated a significant ($P < 0.05$) reduction in Bacteroidetes and Proteobacteria (8.96%, 23.66%, and 23.28% by $CFCS^{wt}$, $CFCS^{wt+PF}$, and $CFCS^{CLA}$) by the metabolites at 48 h; and increase in Firmicutes at 48 h in presence of CFCSs as compared to control. This trend was also visible at genus level where we found a significant ($P < 0.05$) stimulation on *Lactobacillus* growth and suppression on *Escherichia* from CFCSs at both 24 and 48 h of treatment.

Significance: Probiotic-originated metabolites modulate rumen microbiota positively which can deploy to control the transmission of cattle-borne pathogens specifically EHEC.

T12-04 Effects of Electrolyzed Water Treatment on the Metabolic Responses of "Big Six" in Vegetable Sprouts

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Introduction: "Big six" causes safety concern related to vegetable sprouts. However, the bacteria's response upon sanitation treatment is not much understood.

Purpose: To understand the metabolomic response of "Big six" upon low concentration electrolyzed water thus better developing green non-thermal processing technologies to control the pathogens.

Methods: Pea sprouts were selected with 10 min, 4 mg/L electrolyzed water treatment against *E. coli* O26 (ATCC BAA-2196), O45 (ATCC BAA-2193), O103 (ATCC BAA-2215), O111 (ATCC BAA-2440), O121 (ATCC BAA-2219), O145 (ATCC BAA-2192) along with O157:H7 (ATCC 43895) and non-pathogenic ATCC 25922. Plate count and NMR were tested for the bacterial counts and metabolite responses of the bacteria, respectively. Pathway analysis via MetaboAnalyst 4.0 were applied and at least independent triplicates were conducted and analyzed with one-way ANOVA ($P < 0.05$).

Results: Sensitivity to 4 mg/L electrolyzed water and metabolic variation showed positive correlation: low reduction of O103 and O45 (approximately 0.72 log CFU/g reductions) was less affected than O26 and O145 (greater than 1.44 log CFU/g reductions). Disturbed pathways were mainly involved in amino acid metabolism as decarboxylation and deamination were enhanced, while natural osmoprotectants decreased. O111 and O157:H7 demonstrated similar metabolic alteration patterns while O121 showed an increase in multiple metabolites. ATCC 25922 presented a special pattern, indicating that non-pathogen bacteria might apply different defense mechanisms.

Significance: The result elucidates the metabolic response of "Big six" upon sanitization treatment, which is helpful for finding better approaches to control the hazard in vegetable sprouts.

T12-05 Purified Plant-derived Phenolic Acids on *Salmonella* Typhimurium and in *Ex Vivo* Simulated Gut Microbiota

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella enterica* serovar Typhimurium (ST) remains a prevalent pathogen because of its colonization in poultry and increasing antibiotic resistance, warranting novel antimicrobials. Purified phenolics, gallic acid (GA), protocatechuic acid (PA) and vanillic acids (VA) have demonstrated antimicrobial activity. However, there are questions regarding their impact within complex microbial ecosystems over time, and how they could modulate the microbiota.

Purpose: Simulate chicken gut ecosystem with *ex vivo* model and evaluate their role in eliminating ST and modulating the microbiota.

Methods: Cecal fluid was collected from chickens and supplemented with bactericidal concentrations of GA, PA and VA, compared to an untreated control at 0, 24 and 48 h. ST was quantified with plating on XLD. Taxonomic analysis was performed through pair-end (2x300 bp) sequencing of V3 and V4 variable 16S rRNA regions on Illumina MiSeq.

Results: CFU/mL increased by 4.21 log from initial concentration at 24 h but decreased by 0.24 log at 48 h. GA reduced ST by 3.28 and 2.78 log at 24 and 48 h ($P < 0.05$). PA decreased ST by 1.27 and 1.89 log at 24 and 48 h ($P < 0.05$). VA reduced ST by 4.81 and 5.20 log at 24 and 48 h ($P < 0.05$). Significant changes in the relative abundance of cecal microflora at the phylum level were observed at 24 h for samples with GA and VA as Firmicutes levels increased 8.30% and 20.90%, while Proteobacteria decreased 12.86% and 18.48%, respectively ($P<0.05$). Changes at the genus level were seen in significant reduction of *Acinetobacter* (3.41% in GA) and *Escherichia* (13.53% in VA) with an increase in *Bifidobacterium* (3.44% in GA) ($P < 0.05$) and no significant change in *Lactobacillus* for any treatment ($P > 0.05$).

Significance: Purified phenolic acids can be used to control colonization of ST in complex gut microbial systems without negatively affecting normal bacteria population.

T12-06 Investigation of Microbiome Shift by Plant Probiotic in Strawberry Plants

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Introduction: Plant growth promoting bacteria (PGPR) behave as a plant-probiotic in soil by increasing productivity and efficiency in the variety of agricultural crops. Understanding the effect of PGPR in the soil microbiota is important to study the potential functions of PGPR on strawberry plants.

Purpose: The purpose of this study was to evaluate the effectiveness of PGPR to strawberry plants as a plant-probiotic to enhance productivity, efficiency, and safety aspects.

Methods: The commercial PGPR including *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Pseudomonas monteilli* are used in this study. Two different concentrations of PGPR, low (0.24%) and high (0.48%), and control (no PGPR) were treated biweekly on the soil of strawberry plants at the farm in Dayton,

Oregon. The soil samples were collected monthly from August, 2020 to November, 2020. From each sampling, 45 samples were collected (15 samples from each group), a total of 180 soil samples were collected. Genomic DNA isolated from all the soil samples were subjected for microbiome sequencing based on the V4 region of the 16S rRNA gene via a MiSeq platform (Illumina) to investigate the bacterial population differences. Ammonium levels of the soil samples were measured to examine the nitrogen fixation efficiency of PGPR.

Results: A total of 13,033,279 sequencing reads generated from 180 samples. The mean value of the frequency of sequences per sample was found to be 72,407. *Bacillus* and *Pseudomonas* (in genus level) exhibited an increase in abundance (21.5%) in the November PGPR treated soil samples compared to the August samples. The ammonium level between the PGPR treated and untreated group of the September soil samples showed statistically significant difference (*P*-value, 0.03). Also, the average level of ammonium (in ppm) increased in the high concentration of PGPR treated group (Control, 7.3; low concentration, 10.7; and high concentration, 18.7).

Significance: Evaluating the effectiveness of PGPR will contribute on enhancing the competitiveness of agricultural crops by increasing productivity and efficiency.

T12-07 Romaine Lettuce Phyllosphere Microbial Communities Shift as the Crop Develops and in Relation to Irrigation Water Type By the Time of Harvest

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Introduction: The microbial safety of leafy green crops may be affected by irrigation water quality. How various water types and irrigation application methods influence leafy green crop phyllosphere microbiota in the field throughout the growing cycle remains poorly understood.

Purpose: To investigate how the lettuce crop microbiome is influenced by overhead irrigation with recycled and pond water from transplant to harvest.

Methods: Greenhouse-grown Romaine lettuce cv. 'Sparx' seedlings were transplanted in the field in a randomized complete block design and drip-irrigated with municipal water. Lettuce received overhead irrigation with pond (PW), recycled (RW), sterile (SW) or no water (NO), on a weekly basis and 52 lettuce samples were collected on days 0, 11, 18 and 32. Four water samples (2 pond, 2 recycled) were also collected. DNA was extracted from leaf washes and water samples using the Qiagen Power Water kit. Leaf phyllosphere microbial community analysis was done by metagenomic sequencing on the MiSeq platform and data analyzed using R4.0.

Results: Higher observed species richness was detected on Day 32 lettuce samples regardless of water used for overhead spraying compared with Day 0 samples ($P < 0.05$). However, α-diversity indices (ACE, Shannon, Simpson and Fisher) did not identify differential diversity. Based on β-diversity, all Day 11 and Day 18 samples as well as Day3 2 SW and NO samples clustered together in data ordination. ANOSIM showed they differed ($P < 0.05$) from Day 0 and Day 32 PW and RW lettuce samples and all irrigation water samples. Potential foodborne pathogens *Mycobacterium, Bacillus, Clostridium, Aeromonas* spp. dominated in D 32 RW lettuce and RW water samples. Multiple virulence and antimicrobial resistance genes were detected from D 32 RW lettuce and RW water samples. No significant weather events were recorded.

Significance: Human pathogens and antimicrobial resistance and virulence genes were detected on at-harvest lettuce sprayed during cultivation with recycled but not pond and sterile water, or on plants receiving no spraying.

T12-08 Expansion of *Salmonella* Infantis pESI Plasmid to Additional *Salmonella* Serotypes

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Introduction: Multi-drug resistant *Salmonella* Infantis have increased in poultry since 2015. A pESI (plasmid for emerging *S*. Infantis) plasmid imparting multi-drug resistance was first described in U.S. *S.* Infantis food isolates in 2015 and was recently reported to be carried by 89% of poultry Infantis isolates. Through this study, the authors identified $bla_{CTX\text{-}M\text{-}65}$ and 12 additional genes that indicated the likely presence of the pESI plasmid. A recent surveillance study revealed the pESI plasmid only in Infantis but not in additional *Salmonella* serotypes. Plasmid transmission to other serotypes remains possible and warrants surveillance.

Purpose: The objective of this study was to examine 2019-2020 FSIS verification testing isolate data for the presence of pESI plasmid in *Salmonella* serotypes in addition to Infantis.

Methods: Assembled whole genome sequencing (WGS) data from ~10,000 *Salmonella* isolates collected in CY2019 and CY2020 were investigated for the presence of the pESI plasmid. Isolates were considered positive for the plasmid if pESI *repA* and at least 5 additional pESI targets were identified.

Results: Of the 13,060 sequences analyzed, 1,714 isolates were positive for the presence of pESI (*repA* positive and at least 5 additional pESI genes detected). Of the 1,714 isolates with pESI, >99% were serotype Infantis, however four non-Infantis isolates were positive for the pESI plasmid. The serotypes were Senftenberg (3) and Alachua (1), and all were isolated from ground or comminuted turkey products. One of the Senftenberg isolates had the pES1 plasmid but no $bla_{CTX\text{-}M\text{-}65}$. Additionally, all four isolates were multi-drug resistant on antibiotic susceptibility testing panels.

Significance: This is the first finding of the pESI plasmid in a *Salmonella* serotype other than Infantis from FSIS products. As this plasmid first emerged in Infantis, communicating this emerging finding in additional *Salmonella* serotypes is necessary to build an effective mitigation strategy.

T13-01 Combined Effect of Protein and Fat on Thermal Resistance of Salmonella *enterica* Enteritidis PT 30 in Low-Moisture Foods

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Introduction: Microorganisms in low-moisture foods (LMFs) exhibit prolonged survivability and high heat resistance. Various external factors (water, food texture, nutritional compounds, etc.) influence the microbial heat resistance in LMFs, yet the degree of influence of each factor (fat, protein, water activity, etc.) was not fully understood.

Purpose: Here we reported the combined effect of fat and protein on thermal resistance parameters of pathogen *Salmonella enterica* Enteritidis PT 30 (*S*. PT 30) at water activity ($a_{w,25°C}$) 0.30 and temperatures (80, 85, and 90°C).

Methods: Three forms of egg powders (egg white powder, egg yolk powder, and whole egg powder) and four egg white and yolk powder mixtures (1:1, 2:1, 4:1, and 1:5 v/v) were chosen as the model foods due to their naturally different ratios of fat and protein (e.g., fat content from 0% to 56.7%). Primary and secondary models were built from isothermal inactivation kinetics of *S.* PT 30.

Results: Isothermal inactivation kinetics of *S*. PT 30 fitted better to Weibull-type model than log-linear model. The combined model, which exponentially scaled both temperature and fat effects on thermal inactivation rates, predicted *Salmonella* lethality significant better ($P < 0.05$) than did the other secondary models examined. High ratio of fat can proportionally increase the bacterial heat resistance. For instance, $D_{80°C}$ of *S.* PT 30 at egg powders with fat ratios of 0, 19, 41, and 56% were 19.35, 23.28, 39.36, and 97.48 min, respectively.

Significance: At constant moisture content, the effects of fat and protein on thermal resistance of bacteria were quantified in this study.

T13-02 Comparing Efficacy of Neo-Temper and Lactic Acid in Reducing *Enterococcus faecium* on Wheat Applied in Tempering Stage of Milling

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Introduction: Consumption of raw flour comes with a risk of foodborne illnesses due to potential presence of pathogens. Previous studies had found that *Enterococcus faecium* NRRL B-2354 is a viable surrogate for STEC and *Salmonella*. This study was conducted to compare impact of lactic acid and Agri-Neo's proprietary solution, Neo-Temper, which is a hydrogen peroxide and peracetic acid based organic solution, added to tempering water, in reducing *E. faecium* on wheat kernels.
Purpose: To address the global food safety issue related to wheat-flour by reducing its microbial load during tempering stage of milling process.
Methods: Hard wheat kernels were inoculated with overnight *E. faecium* NRRL B-2354 culture and dried back to initial moisture content in a fluid bed dryer. Six solutions were prepared where either the hydrogen peroxide (20%) and peracetic acid (2%) based organic solution (at 5, 8, 10 L) or the 88% lactic acid (at 0.57, 1.14, 2.85 L) were mixed with water to make total of 50 L. For each treatment, a 25-mL aliquot from each solution was applied on individual 500 g inoculated wheat batches while mixing for 1 minute. After 16 hours, 45 g samples (n = 10) were enumerated from both treated and untreated samples.
Results: Proprietary solution resulted in 2.37 ± 0.15, 3.07 ± 0.28 and 3.18 ± 0.15 log reductions on *E. faecium* at 5, 8 or 10 L per 50 L total solution, respectively. Whereas 0.57, 1.14 and 2.85 L lactic acid per 50 L total solution were only able to reduce population of *E. faecium* by 0.70 ± 0.13, 1.17 ± 0.09, and 1.36 ± 0.05 log CFU/g, respectively.
Significance: Integrating proprietary sanitization solution into tempering stage provides 2-3 log reduction on population of *Salmonella* and STEC surrogate and would result in improving food safety of wheat flour.

T13-03 Effect of Steam Conditioning on Microbial Safety and Quality of Pecans

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❖ ***Developing Scientist Entrant***

Introduction: Steam conditioning of pecans is one of the post-harvest practices done to facilitate kernel separation. Although less frequently used, it could be an efficient alternative conditioning method to achieve >5-log reduction of pathogens besides minimizing kernel breakage. However, heat treatment can affect its quality.
Purpose: This study evaluated the application of steam treatment for inactivating *Enterococcus faecium* on in-shell pecans and effect on kernel quality.
Methods: In-shell pecans inoculated with *Enterococcus faecium* (7.28 ± 0.17 log CFU/g) were treated with steam in a custom-designed chamber at 70°C, 80°C or 90°C for 0-300 s. Log-linear model was fitted to obtain *D*-value. Pecans were then treated with steam at respective time-temperature combination giving 5-log reduction; kernels were packaged in metallized film bags and placed in accelerated storage conditions (35°C, 65% RH and 450-500lx for 25 days) simulating 4 months storage at 22°C. Samples were tested at 5-day interval for peroxide value, moisture, water activity (a_w), color and texture as quality indices using standard methods. Experiments were conducted in triplicates.
Results: At 80°C and 90°C, >5-log reduction was observed within 120 s and 25 s, respectively, whereas, only 4.28 ± 1.0 log CFU/g reduction was observed within 300 s at 70°C. The *D*-value at 70°C, 80°C and 90°C was 77 s, 26 s and 5 s, respectively. The steam treated pecans' peroxide value were lower (P < 0.05) than of control (0.7 ± 0.2-2.1 ± 0.26 meq/kg pecan oil) throughout storage (until day 25). Among all, 90°C treatment significantly lowered the lipid oxidation (0.8 ± 0.16-1.4 ± 0.16 meq/kg) during storage. Steam treatment slightly increased (P > 0.05) moisture and a_w but significantly increased (P < 0.05) the hardness (N). Steam treatment had no significant effect on the color of pecans, but kernels became significantly darker over storage time, irrespective of the treatments (indicated by L* value).
Significance: The findings of this study indicate that steam conditioning of in-shell pecans is a promising approach to help ensure microbiological safety while maintaining its quality.

T13-04 Thermal Inactivation of *Salmonella* and *Enterococcus faecium* during Walnut Toasting

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Introduction: Consumers often toast walnut kernels prior to consumption to modify texture and flavor. The impact of toasting, typically at 350°F (177°C) for 8-10 min, on microbial populations is unknown.
Purpose: This study was undertaken to determine the reduction of *Salmonella* and surrogate *Enterococcus faecium* NRRL B2354 during simulated home toasting of walnut kernels.
Methods: Walnut kernels were co-inoculated with a rifampicin-resistant *Salmonella* (5 strain) and *E. faecium* NRRL B2354 (single strain) cocktail prior to drying at room temperature for 7 days. Inoculated walnuts were toasted for 8-12 min in a countertop household oven on a baking sheet at 300, 325, 350, and 375°F (149, 163, 177, and 191°C). Air temperatures in the oven were recorded; moisture content of walnuts was measured before and after toasting. Samples (n = 6 at each time point) were stomached in tryptic soy broth and plated onto tryptic soy agar supplemented with rifampicin, CHROMagar Salmonella, and Slanetz and Bartley agar.
Results: The moisture content of walnuts decreased (from 3.59 to ~0.34%) as toasting temperature and time increased. *E. faecium* did not form colonies on tryptic soy agar supplemented with rifampicin or CHROMagar Salmonella and *Salmonella* was unable to form colonies on Slanetz and Bartley agar. Reductions of *Salmonella* and *E. faecium* were not significantly (P < 0.05) different at any time point. *Salmonella* and *E. faecium* declined by mean of ~3 log CFU/g (12 min at 149°C or 8 min at 163°C), mean of ~5 log CFU/g (10 min at 163°C or 8 min at 177°C), and >7 log CFU/g (10 min at 177°C and 8 min at 191°C).
Significance: Current directions for toasting walnuts should result in significant reductions of *Salmonella*. *E. faecium* NRRL B2354 may be a useful surrogate for *Salmonella* in thermal validation studies on walnut kernels.

T13-05 Potassium Lactate as a Strategy for Sodium Content Reduction without Compromising Salt Associated Antimicrobial Activity in Salami

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❖ ***Developing Scientist Entrant***

Introduction: Reformulating recipes of ready-to-eat products such as salami to reduce salt content can mitigate the negative health impacts of a high salt diet.
Purpose: To evaluate the potential of potassium lactate (KL) as a sodium chloride (NaCl) replacer during salami production.
Methods: Four *Listeria innocua* food isolates were compared to seven reference outbreak-related *L. monocytogenes* strains based on stress tolerance (4 and 6% NaCl, 2.8% NaCl plus 1.6% KL) to validate their suitability as *L. monocytogenes* surrogates. Using the selected *L. innocua* strains, challenge tests were carried out in meat simulation broth (MSB), beef, and salami supplemented with high salt (4% NaCl) or low salt (2.8% NaCl plus 1.6% KL). *Listeria* and starter culture growth profiles were monitored through periodic plate counts on selective media. Furthermore, salami pH, water activity, proximate composition,

and Warner–Bratzler measurements were determined. Data were evaluated using ANOVA.

Results: The *L. innocua* strains selected are appropriate *L. monocytogenes* surrogates (growth rate under osmotic stress 0.39 vs 0.31 OD_{600}/hour, respectively). MSB and beef salami-ripening simulation models showed that the low NaCl plus KL combination retained similar to superior anti-*Listeria* activity compared to the high salt concentration treatment [growth potential on beef -0.2 (2.8% NaCl plus 1.6% KL) vs 1 log CFU/g (4% NaCl)]. Salami challenge tests showed that NaCl plus KL combination had significantly ($P < 0.05$) superior anti-*Listeria* activity as high NaCl concentration during ripening and storage (growth potential -1.73 vs -1.14 log CFU/g). No significant differences were detected in product characteristics and starter culture growth profiles between 4% NaCl and 2.8% NaCl plus KL treated salami. For instance, water activity was 0.894 in 4% NaCl vs 0.892 in the 2.8% NaCl-KL treated salami.

Significance: KL is an effective NaCl replacer allowing 30% NaCl reduction without compromising product quality and antimicrobial benefits of high NaCl concentration.

T13-06 Viability, Membrane Integrity and Metabolic Activity of Salmonella enterica in Conventionally and Osmotically Dehydrated Coconut Flakes during Storage

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Introduction: Multiple outbreaks involving *Salmonella enterica*-contaminated dehydrated coconut flakes have been reported. Little is known about *S. enterica* metabolic activity and membrane integrity on dehydrated coconut flakes during storage.

Purpose: We assessed *S. enterica* viability, membrane integrity, and metabolic activity on conventionally and osmotically dehydrated coconut flakes stored at 25°C.

Methods: Coconut flakes (4 x 1 mm) were inoculated (~7.0 log CFU/g) with a cocktail of *S.* Muenster, *S.* Bredeney, *S.* Agona, *S.* Enteritidis and *S.* Typhimurium. Coconut flakes were submitted to conventional (CD; 55ºC/6 h) or osmotic (OD; 40% sucrose/50ºC/3 h followed by 55ºC/6 h) dehydration. Coconut samples (a_w 0.34) were kept in a desiccator at 25°C over a saturated magnesium chloride salt solution to minimize a_w variations. Cells were collected by centrifugation (4500 *g*, 10 min, 4ºC) immediately after dehydration and after 90 days of storage, resuspended in PBS, and labeled with propidium iodide and bis-1,3-dibutylbarbituric acid and 5-cyano-2,3-ditolyl tetrazolium chloride. Damaged subpopulations were measured using flow cytometry. Cells were also enumerated on Tryptic Soy Agar after dehydration and over 90 days of storage.

Results: CD compromised enzymatic activity and depolarized/permeabilized membrane of ~90 and 18% of *S. enterica* cells on coconut flakes, respectively. OD compromised enzymatic activity and damaged the membrane in 82% and 10% of *S. enterica* cells, respectively. The OD-damaged subpopulation did not change after 90 days of storage, while the CD-damaged subpopulation decreased ($p < 0.05$) by ~10%. *S. enterica* counts decreased ($p < 0.05$)~3.5 log CFU/g in OD coconut flakes over 90 days. Counts in CD coconut flakes remained stable (~10^6 CFU/g) over the storage.

Significance: This research shows that OD is a less risky method of preparing dehydrated coconut flakes (verses CD) and this appears be due to ongoing damage to cell membranes and enzymatic activity loss during storage.

T13-07 Neo-Pure Achieves >1-Log CFU/g Reduction in Aerobic Plate Counts (APC) on Crude Dehydrated Onion Flakes

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Introduction: One of the key metrics for dehydrated onion quality is microbial load, measured by aerobic plate counts (APC), which can increase with the number of processing stages the product travels through. Increase in APC load on dried onion flakes results in value degradation since it is an indicator for quality and safety of crude onion flakes. Efficacy of Neo-Pure in reducing APC and its impact on sensory attributes of onion flakes was investigated in this study.

Purpose: To identify a suitable novel technology that can ensure a >1-log CFU/g reduction in APC counts on dried onion flakes.

Methods: Crude dried onion flakes (45 kg) were treated with Neo-Pure solution at 95 L/tonne in a pilot scale continuous applicator. Treated onion flakes were immediately conveyed and dried at 260°F in a pre-warmed pilot scale continuous fluidized-bed dryer (3 MT/h throughput) to return moisture content (%) to its original value. Post treatment, samples were collected inside organza bags and cooled in the fridge for 3 min. Samples (22.5 g, $n = 6$) of treated and untreated product were enumerated for APC using Health Canada Method (MFHPB-33) on 3M™ Petrifilm™ AC Plates. Additionally, moisture content and water activity tests were conducted within 24 h of treatment.

Results: The average CFU/g counts for untreated and treated samples were 5,640,000 CFU/g and 208,667 CFU/g, respectively. Neo-Pure treatment process was able to produce a 96% or 1.31 log CFU/g reduction on APC. Average moisture content values for untreated and treated flakes were 6.55% and 5.58%, and their water activities were 0.27 and 0.20, respectively.

Significance: The Neo-Pure organic solution applied in a continuous pilot scale applicator and dryer improved quality and safety of dried onion flakes by delivering >1-log CFU/g reduction on APC levels.

T13-08 Neo-Pure Achieves >4-Log Reduction in Salmonella surrogate *Enterococcus faecium* NRRL B-2354 on Dehydrated Onion Flakes

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Introduction: Dehydrated onion product is prone to contamination due to the way it is cultivated, harvested, and processed. In July 2020 alone, the outbreak of *Salmonella* Newport contaminated onions led to a widespread recall of products including bulk and processed onions. It spanned across 48 US States and 7 Canadian provinces, causing 1,642 illnesses involving 246 hospitalizations. An intervention approach to ensure pathogen control in onions is imminently needed to prevent such outbreaks. Efficacy of Neo-Pure in reducing *Enterococcus faecium* NRRL B-2354, a *Salmonella* surrogate used on low moisture foods, was investigated on dried onion flakes in this study.

Purpose: To identify a suitable novel technology that can ensure a >4-log CFU/g reduction in *E. faecium* population on dehydrated onion flakes.

Methods: Inoculated dried onion flakes (45 kg) were treated with proprietary Neo-Pure solution at 75 L/tonne in a pilot scale continuous applicator, followed by drying at 260°F in a pre-warmed pilot scale continuous fluidized-bed dryer (3 MT/h throughput) to return moisture content (%) to its original value. Post treatment, samples were collected inside organza bags and cooled in the fridge for 3 min. Samples (22.5 g, $n = 10$) of treated and untreated product were enumerated for *E. faecium* on *Enterococcus* agar plates. Additionally, moisture content and water activity tests were conducted within 24 h of treatment.

Results: Post Neo-Pure treatment, population of *E. faecium* dropped from 7.60 log CFU/g to 3.12 log CFU/g, producing a 4.65-log reduction. Average moisture content values for untreated and treated flakes were 6.60% and 6.33% and their water activities were 0.38 and 0.31, respectively.

Significance: The Neo-Pure organic solution applied in a continuous pilot scale applicator and dryer demonstrated the safety of dried onion flakes by delivering >4-log CFU/g reduction on *E. faecium* NRRL B-2354 levels.

Poster Abstracts

P1-01 Switchgrass Extractives Against Salmonella enterica Serovar Typhimurium Populations in Vitro and in Planta

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❖ ***Developing Scientist Entrant***

Introduction: Outbreaks of *Salmonella enterica* linked to produce, including lettuce, sprouts, onions, and peaches, continue to occur. Novel, biobased approaches are being investigated as alternatives to chlorine washes. Switchgrass extractives generated during bioenergy production contain nonstructural components with antimicrobial potential.

Purpose: The objective of this research was to determine the ability of switchgrass extractives to decrease *Salmonella enterica* serovar Typhimurium populations on lettuce leaves *in vitro* and *in planta*.

Methods: For *in vitro* studies, 100 µL of *S*. Typhimurium LT2 (~7 log CFU/mL) was inoculated onto 3 × 3-cm lettuce leaves, which were treated with 200 µL of either 195X switchgrass extractives for 3, 30, and 45 min or 3-min with 10% bleach and replicated thrice. For *in planta* studies, Romaine lettuce grown in the greenhouse (3 separate plants thrice) was treated with switchgrass extractives prior and post *S.* Typhimurium LT2 (~7 log CFU/mL) contamination for 0, 3, and 24 h. Bacteria were recovered by stomaching leaves, and then ten-fold serially diluted, surface-spread plated onto Tryptic Soy Agar and incubated at 37°C overnight. Counts were converted to log CFU/g and analyzed using mixed model analysis of variance (adjust=Tukey, SAS v 9.4).

Results: *S.* Typhimurium was nondetectable (≥6-log CFU/g reduction) after a 3-min treatment of lettuce leaves with 10% bleach, and by 2.95 ± 1.15, 3.43 ± 1.20, and 5.46 ± 1.4 log CFU/g, after treatments with switchgrass extractives for 3, 30 and 45 min, respectively. For *in planta* studies, *S.* Typhimurium was significantly ($P < 0.05$) reduced by 2.49 ± 0.67 log CFU/g using switchgrass extractives as a pre-treatment method after 3 h. No significant reduction was obtained as a post-treatment application at 0 or 3 h after contamination of lettuce leaves *in planta.*

Significance: Switchgrass extractives show potential for use as antimicrobial washes against *S.* Typhimurium to prevent contamination of Romaine lettuce leaf surfaces and as a pre-treatment option.

P1-02 Inhibitory Activity of Aqueous Extracts of Pomegranate Peel Products and Juice Powder Against *Salmonella enterica*

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❖ ***Developing Scientist Entrant***

Introduction: Pomegranate is a nutrient dense fruit rich in polyphenols including a high concentration of ellagitannins that have antimicrobial properties. Inedible pomegranate peel is reported to have even higher amounts of polyphenols than the edible arils, which makes pomegranate peel a promising source of natural preservatives.

Purpose: This study evaluated the inhibitory effect of aqueous extracts of different pomegranate products, including three dry peel powders, a whole peel, and a juice powder against two different strains of *Salmonella enterica* in tryptic soy broth (TSB) or phosphate buffered saline (PBS).

Methods: *Salmonella* cells (10^5 CFU) were added into either TSB or PBS containing either 9% or 23% of the extracts prepared by two different methods. Surviving *Salmonella* cells were enumerated after treatment for 5, 10, or 24 h at 25°C, and the reduction of *Salmonella* population in relation to the control was subsequently determined.

Results: Results showed that the reduction of *Salmonella* population in TSB was significantly higher ($P \leq 0.05$) than that in PBS. *Salmonella* Enteritidis had a significantly lower ($P \leq 0.05$) reduction than *Salmonella* Tennessee in PBS and a numerically lower ($P > 0.05$) reduction in TSB. The extracts from the three powdered peels were significantly more effective ($P \leq 0.05$) in reducing the *Salmonella* population (1.67 - 2.03 log CFU/mL) than the extract from the whole peel (1.05 log CFU/mL) and juice powder (0.94 log CFU/mL). Higher dose of extracts resulted in a greater reduction in *Salmonella* population in TSB (3.02 log CFU/mL) or PBS (0.66 log CFU/mL) than media with lower dose of extracts. The level of *Salmonella* population reduction correlated positively with the polyphenolic content (R^2 0.66 – 0.99) and titratable acidity (R^2 0.72 – 0.98) in the treatment systems.

Significance: The study suggests that the pomegranate peels have the potential to be used as preservatives in human food or animal feed to control pathogens like *Salmonella*.

P1-03 Antimicrobial and Chemical Assessment of Two Green Tea Extracts

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Introduction: Catechins (flavonoids) and their gallates are the major polyphenolic components of green tea and proposed to be the main chemical moieties responsible for the antimicrobial activity of green tea extracts (GTE), with the substances having epi- configuration being the most active components.

Purpose: This study aims at evaluating the chemical composition and antimicrobial activity of two GTEs, a water extracted GTE and an ethanol and water extracted GTE.

Methods: The chemical composition of catechins in GTEs were evaluated using reversed phase high-performance liquid chromatography and 1H Nuclear magnetic resonance and reported as average of three individual measurements. The antimicrobial efficiency of GTEs was evaluated in duplicate against more than 30 isolates of spoilage or foodborne pathogenic bacteria (30°C or 37°C, in MRS or CASO) and fungi (25°C, YM broth) in vitro using an automated high-throughput robotic system.

Results: The chemical composition of the two GTEs differed in content of epigallocatechin (EGC), epigallocatechin gallate (EGCg), gallocatechin gallate (GCg), and epicatechin gallate (ECg), with the ethanol and water extracted GTE having 10 w/w% higher content of EGC and EGCg and 6 w/w% lower content of GCg and ECg than the water extracted GTE, and overall higher content of epi-catechins.

Minimum inhibitory concentrations (MICs) were measured to determine the antimicrobial efficiency of the GTEs, and results indicated that the ethanol and water extracted GTE, in general, had higher activity than the water extracted GTE with average MICs for Gram-positive bacteria: 1355 mg/L and 1812 mg/L, Gram-negative bacteria: 3029 mg/L and 3406 mg/L, yeast: 2393 mg/L and 3332 mg/L, and mold: 1101 mg/L and 2321 mg/L, respectively.

Significance: These data suggest that the increased activity of the ethanol and water extracted GTE with high epi-catechins content could be due to the higher occurrence of EGC and EGCG or synergy between individual GTE components.

P1-04 The Antimicrobial Activity of Two Phenolic Acids Against *E. coli* O157:H7 and *L. monocytogenes* and Their Effectiveness in a Meat System

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Introduction: Ready-to-eat meats are vulnerable to contamination during their production, distribution and sale and they are associated more with foodborne illnesses.
Purpose: This study evaluated the antimicrobial effects of two phenolic acids (caffeic and ferulic acids) against foodborne pathogens in cold-cut meat at low-temperature conditions.
Methods: The individual and combined antibacterial activities of caffeic and ferulic acids against *Escherichia coli* O157:H7 ATCC 43888 and *Listeria monocytogenes* ATCC 7644 were determined by diffusion disk assay, in broth media and thereafter antibacterial activities were carried out in cold-cut meat. Broth media and meat samples already inoculated with *E. coli* and *L. monocytogenes* were treated with caffeic acid, ferulic acid, and their combination at the concentrations of 150 ppm and 200 ppm stored at 4°C. Microbial growths were monitored at 0, 24, 48, and 72 h.
Results: Caffeic acid at 200 ppm exhibited a zone of inhibition of 12.33 mm on *E. coli,* and ferulic acid revealed a zone of inhibition of 11.00 mm inhibition on *L. monocytogenes*. The combination of caffeic-ferulic acid at a concentration of 200 ppm was most effective against *E. coli*, demonstrating a synergistic effect over 72 h at 4°C in both broth media and meat. For meat samples, the combination of caffeic acid and ferulic acid exhibited a log reduction of 3.63 CFU/g at 150 ppm and 2.51 CFU/g at 200 ppm against *E. coli* O157:H7 at the end of cold storage. Moreover, caffeic acid also singly alone exhibited an overall log reduction of 2.48 CFU/g at 150 ppm and 2.75 CFU/g at 200 ppm against *L. monocytogenes*.
Significance: These results indicate the potential of caffeic and ferulic acids, individually and in combination, to reduce pathogenic contamination and improve safety of cold cut meats.

P1-05 Catfish Gelatin Coating on Shrimp as an Antimicrobial Agent

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Introduction: Fresh Gulf of Mexico shrimp is prone to rapid spoilage once harvested, limiting its availability on the market. In order to optimize shelf life, seafood products are often treated with various organic acids and phosphates. Catfish skin gelatin coating infused with chemical treatments has been shown effective in extending the shelf life of shrimp.
Purpose: The aim of this study was to determine the efficacy of treatments on fresh, wild caught shrimp protected by a catfish skin gelatin coating in combination with other antimicrobials.
Methods: Fresh, wild caught shrimp samples were treated with combinations of 4-Hexylresorcinol (4H), lactic acid (LA), and sodium tripolyphosphate (STP). Once treated, the shrimp were dipped in a gelatin (G) solution. Treatment groups were observed over 28 days for microbiological, physical, and chemical changes.
Results: Up to day 12, samples treated with LA and STP presented a significantly lower Aerobic Plate Count, 3.05 ± 0.08 and 3.18 ± 0.30 log CFU/g, respectively, compared to 4H and G, 4.11 ± 0.16 and 4.04 ± 0.7 log CFU/g, respectively. The effect of LA was also observed for yeast and psychrophilic bacteria, with no significant difference between treatments for *E. coli*, *Staphylococcus aureus*, or *Pseudomonas*. TBARS values for all treatments were significantly lower than the control. Throughout the study, the color shifted to a more red and yellow hue with no significant difference between treatments ($P > 0.05$).
Significance: Based on the results, the use of gelatin did not improve the shelf life of the shrimp compared to the control.

P1-06 Enhancement of Fresh Catfish Fillets Quality by the Application of a Catfish Skin Gelatin and Antimicrobial Coating

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❖ ***Developing Scientist Entrant***

Introduction: Catfish is the main aquatic species commercially raised in the U.S. Catfish dressing yields from 45-70%; the skin byproduct is 6%. Gelatin can be extracted from the catfish skin and used in the food industry to improve shelf life.
Purpose: The purpose of this study was to evaluate the effect of catfish skin gelatin in combination with antimicrobials in the shelf life of fresh catfish fillets.
Methods: Fresh catfish fillets were purchased at a local market and separated into 4 groups. Fish was treated with catfish skin gelatin alone and in combination with potassium sorbate (PS) and lactic acid (LA); and DI water. Samples were randomly assigned to the treatments, immersed for 60 seconds, placed in plastic bags and stored in ice in a refrigerated unit. Samples were tested every 3 days for 30 days. Physical/chemical and microbial activity were analyzed during the study.
Results: The initial Aerobic Plate Count (APC) of the samples treated with gelatin in combination with LA was significantly lower than the control. Initial APC for the control group was 4.50 ± 0.21 log CFU/g, compared to LA with 3.55 ± 0.03 log CFU/g. Through the shelf life, PS presented a significantly lower APC compared to other treatments. At day 30, APC was 7.17 ± 0.10, 5.77 ± 0.28, and 4.40 ± 0.34 log CFU/g for control, LA, and PS, respectively. The pH started below 7, with no significant differences during the first 24 days for the control and gelatin groups. However, PS and LA pH decreased after day 3. TBARS was significantly higher for LA (1.54 ± 0.17 MDA equivalent/kg) than other groups at day 30.
Significance: Gelatin in combination with other antimicrobials increased 3 days the shelf life of fresh catfish fillets compared to the control.

P1-07 Comparison of Antimicrobial Activities of Essential Oil Vapors Against *Salmonella enterica* as Affected by the Types of Diluents

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❖ ***Developing Scientist Entrant***

Introduction: Antimicrobial activities of vaporized essential oils (EOs) have been reported but diluents suitable for the generation of EO vapors have received meager research attention.
Purpose: This study was conducted to determine diluents suitable for the spontaneous generation of EO vapors.
Methods: *Salmonella enterica* (*ca*. 5 log CFU) was inoculated on nutrient agar containing 1% dextrose and 0.025% bromocresol purple and exposed to 82 types of EO vapors at 0.3125 µL/mL for 24 h. After incubation, EO vapors that inhibited the growth of *S. enterica* were selected. The selected EO vapors were serially diluted in two-fold using jojoba oil, dimethyl sulfoxide (DMSO), or ethanol followed by determining minimal inhibitory concentrations (MICs) against *S. enterica* using vapor disc assay.
Results: Among 82 EO vapors tested, cinnamon bark, oregano, tea tree, thyme thymol showed strong antimicrobial activities against *S. enterica*. It was observed that EO vapors had the lowest MICs when EOs were diluted in ethanol compared to DMSO or jojoba oil. For example, MIC of cinnamon bark EO vapor was 0.1563 µL/mL when diluted in ethanol but those became 0.3125 µL/mL when diluted in DMSO or jojoba oil.
Significance: This study showed that vaporization rate of EO is significantly affected by the types of diluent. The result of this study would provide useful information in developing a decontamination method using essential oil vapors.

Poster

P1-08 Comparison of Antimicrobial Activities of Organic Acid Vapors Against *Bacillus cereus* and *Shigella flexneri*

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❖ ***Developing Scientist Entrant***

Introduction: Organic acids have received attentions as natural antimicrobial agents, but antimicrobial activities of vapor forms of organic acids have not been intensively studied.

Purpose: The aim of study was to select organic acid vapors inhibitory to *Bacillus cereus* and *Shigella flexneri*, and to determine their minimum inhibitory concentrations (MICs) and minimum lethal concentrations (MLCs) on a laboratory medium.

Methods: Airtight cylindrical vials (1 mL) which contain tryptic soy agar (TSA) in the upper part and paper disc in the lower part were constructed. *B. cereus* or *S. flexneri* cells (5 log CFU) were inoculated on TSA and 14 different types of organic acids (3000 µg) were individually deposited on the paper discs. After tightly sealing the vials with parafilm, they were incubated at 30°C (*B. cereus*) or 37°C (*S. flexneri*) for 24 h. After incubation, organic acid vapors that inhibited the growth of pathogens were screened. For screened organic acid vapors, organic acids were serially diluted in 2-folds using distilled water and the MICs and MLCs of organic acid vapors were determined using vapor disc assay in the airtight cylindrical vials.

Results: Acetic, formic, and propionic acid vapor showed inhibitory activities against *B. cereus* and *S. flexneri* at a concentration of 3,000 mg/mL. For *B. cereus*, MICs of acetic, formic, and propionic acid vapor were 375, 375, and 750 µg/mL, respectively, and MLCs were > 3,000 µg/mL regardless of types of organic acid vapors. For *S. flexneri*, MICs of acetic, formic, and propionic acid vapor were 375, 375, and 750 µg/mL, and MLCs were 1,500, 750, and 3,000 µg/mL.

Significance: The results of this study may provide useful information in developing a method to inactivate foodborne pathogens using organic acid vapors.

P1-09 Chemical Disinfection of Sessile Cells of *Listeria monocytogenes* Under Single- and Dual-Species (with *Lactobacillus* spp.) Conditions

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Introduction: *Listeria monocytogenes* biofilms are a major concern because they serve as contamination sources and are often resistant to disinfectants. Different interactions that may contribute to increased resistance occur between *L. monocytogenes* and other populations in biofilms. These relationships are an important aspect of studies dealing with biofilm-associated food safety.

Purpose: This study was conducted to investigate the possible influence of bacterial interspecies interactions on the resistance of sessile cells to chemical disinfection.

Methods: The response to hypochlorite and quaternary ammonium compounds (QACs) of single and dual culture biofilms of three *L. monocytogenes* strains (Lm5, Lm7, and LmC) and two *Lactobacillus* species (Lb, and Lp) was evaluated. Survival rate was determined using standard microbiological methods and biofilm characterization was studied by confocal laser scanning microscopy (CLSM) with fluorescence staining methods and image analysis software (COMSTAT 2) for qualitative and quantitative data. Statistical analyses were performed using ANOVA.

Results: When treated with hypochlorite, Lm5 had greater survival in binary biofilms. Lm5 increased its survival up to 1.8 log CFU/well when in coculture with Lb ($P < 0.05$). Binary biofilms had thicker structures, and produced more protein matrix, hence the greater share of intact (live) cells within treated biofilms. However, hypochlorite reacted with the biomass fast enough to disrupt some cell aggregates, altering their three-dimensional organization. When treated with QACs, LmC had only 0.6 log CFU/well greater survival when grown with Lp ($P > 0.05$). CLSM revealed that QACs had difficulty penetrating the binary biofilm since cells encased deeply were intact and thus remained largely protected.

Significance: This study highlights the impact of microbial interactions in mixed-culture biofilms on disinfection resistance and the importance of elucidating *L. monocytogenes* persistence in the food processing environment.

P1-10 Biofilm Formation and Phytochemical Disinfection of Sessile Cells of *Listeria innocua* from Processing Surfaces

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Introduction: Surface disinfection is of great importance in the control of *Listeria* on food processing equipment. Several studies have reported the use of plant secondary metabolites (phytochemicals) as sanitizers, however the impact on biofilms has yet to be demonstrated. A better understanding of biofilm behavior in response to various plant constituents is essential to design an effective control strategy.

Purpose: This study was undertaken to investigate the ability of *Listeria innocua* (*L. monocytogenes* surrogate) to form biofilms on processing surfaces and the subsequent resistance of sessile cells to selected phytochemicals.

Methods: The biofilm formation on various abiotic surfaces: silicone, stainless steel, polypropylene, polytetrafluoroethylene, polyurethane, copper, borosilicate glass, and polyethylene terephthalate (PET) was evaluated. To quantify biofilm growth two methods were applied: direct epifluorescent filter technique and plate count method. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined for eugenol, citronellol, terpineol, and cinnamaldehyde. Finally, we evaluated the effects of selected compounds on preformed biofilms. Statistical analyses were performed using ANOVA.

Results: Biofilms grown on silicone had the largest live cell count of 9.0 ± 0.1 log cells/cm^2 and cell count of 8.1 ± 0.4 log CFU/cm^2, but biofilms grown on copper had the smallest population of culturable cell count of 5.5 ± 0.9 log CFU/cm^2 ($P < 0.05$). On copper, almost 80% of the total cells had perturbed cell membranes. The selected phytochemicals were effective at concentrations of 5–10 mM (MIC) and 8-13 mM (MBC). They interfered with biofilms too, and cinnamaldehyde was the most effective treatment against biofilms grown on silicone and PET with cell reductions of 5.3 and 6.2 log CFU/ cm^2, respectively ($P < 0.05$). Biofilm cells were non-recoverable after phytochemical disinfection of copper surfaces.

Significance: This study highlights the impact of surface type on biofilm formation and disinfection resistance and may help developing better strategies for the effective use of phytochemicals against *Listeria*.

P1-11 Effect of Anthocyanins in Okinawan Sweet Potato on Growth and Physicochemical Properties of Salmonella Typhimurium and Listeria monocytogenes

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Introduction: Anthocyanins from various sources have been proved to pose antimicrobial properties against foodborne pathogens. Finding new antimicrobial agents is urgent as many bacteria exhibit drug resistance against common antibiotics.

Purpose: The objective of this study was to extract anthocyanins from Okinawan sweet potato and evaluate their effect on the growth and physicochemical properties of *Salmonella* Typhimurium and *Listeria monocytogenes.*

Methods: Anthocyanins were extracted from Okinawan sweet potato by an aqueous two-phase extraction method. The extract was filter sterilized before every experiment. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the anthocyanin extract against *S.*

Typhimurium and *L. monocytogenes* were determined. Physicochemical properties of both treated bacteria, including autoaggregation, swimming and hydrophobicity, were measured using spectrophotometric methods.

Results: MBC/MIC ratios of Okinawan sweet potato anthocyanin extract were 1 and 2.49 for *S.* Typhimurium and *L. monocytogenes*, respectively. The extract had no effect on autoaggregation of tested bacteria. However. motility was significantly reduced by 92,93% and 72.89% for *S.* Typhimurium treated by extract concentrations of 4.12 and 2.06 mg/L, respectively, and by 80.11% and 74.97% for *L. monocytogenes* at 4.71 and 2.36 mg/L, respectively. Hydrophobicity of *L. monocytogenes* was significantly reduced when treated with 4.71 mg/L of sweet potato extract (4.41% vs 10.48%), but no hydrophobicity change was found in treated *S.* Typhimurium.

Significance: This study showed the bactericidal properties of anthocyanins extracted from Okinawan sweet potato and their effects limiting bacterial functions related to attachment and colonization at sublethal concentrations. This extract can be potentially used as a natural preservative to enhance food safety.

P1-12 Antibacterial Effect of Plant-based Essential Oil Against *Salmonella* spp. in Hummus

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✦ ***Undergraduate Student Award Entrant***

Introduction: Hummus is a traditional Middle Eastern food typically made of mashed chickpeas, tahini, lemon juice, and spices. In 2019, over 80 types of hummus were recalled due to *Salmonella* contamination. As a ready-to-eat food product, hummus is not further cooked after purchasing, which makes it particularly dangerous if contaminated during or after processing. The incorporation of essential oils, such as carvacrol, has been considered as a post-processing strategy that meets consumer demands for the use of natural antimicrobials.

Purpose: The present study was designed to determine the effectiveness of carvacrol against *Salmonella* spp. in hummus stored at 4°C or 10°C.

Methods: Commercial hummus was bought from a local market. The hummus was divided into five treatment groups: 2.0% carvacrol, 1.0% carvacrol, 0.5% carvacrol, negative control (no carvacrol or *Salmonella*), and positive control (*Salmonella* only). Ten-gram samples were placed into sterile stomacher bags and inoculated with a 3-strain cocktail of *Salmonella* spp. (~ 4.0 log CFU/g), dependent on treatment group. Samples were stored at either 4°C or 10°C and microbial analysis was taken intermittently on days 0, 1, 4, 7, and 10. A pH meter was also used to determine the pH of non-inoculated samples.

Results: Results showed that there was no growth for all 2% groups by Day 4 at both temperatures, and that there was no growth for all 1% carvacrol samples by Day 10 at both temperatures. This translates to a 4.5-log reduction for both 2.0% and 1.0% groups by Day 10. By comparison, positive control groups on average did not exceed 1.0-log reduction by Day 10. No significant pH changes were found after the addition of carvacrol.

Significance: Carvacrol could be a potential natural preservative incorporated into hummus to prevent the growth and survival of *Salmonella*.

P1-13 Application of Cinnamon Oil Nano-Emulsion to Inhibit *Salmonella* spp. on Alfalfa Seeds and Sprouts

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Introduction: Seed Sprouts have become a fresh produce item commonly linked to foodborne illness. There have been foodborne outbreaks linked to consumption of alfalfa seeds and sprouts associated with *Salmonella* spp. Surface sterilizing with antimicrobial agents like cinnamon oil may reduce the attachment of microbes without effecting the nutrient value and germination of the alfalfa seeds. Nano-emulsion of cinnamon oil prepared by sonification which has stable configuration in attaching on the surface of the seeds and sprouts have shown effective antimicrobial activity.

Purpose: The purpose of the study is to test the effectiveness of Cinnamon oil Nano-emulsion on three different strains of *Salmonella* spp. for the treatment of alfalfa seeds and sprouts.

Methods: Alfalfa Seeds and sprouts were treated with control, 0.75% and 1% Cinnamon oil Nano-emulsion after artificially inoculating the seeds with the bacterial cocktail made from three different strains of *Salmonella*. The 5% Cinnamon oil Nano-emulsion was prepared using sonication method by combining Tween 80, DI water and pure cinnamon oil. Three batches of seeds are then treated with DI water, 0.75% and 1% of Cinnamon oil Nano-emulsion for 1 minute and left for drying in the drying hood for 24 hours. The treated samples were enumerated at the intervals of 0 h, 24 h and 48 h.

Results: The 0.75% and 1% Nano-emulsion treated seeds showed significant amount of reduction in *Salmonella* compared with the control. Greatest reduction was seen after 24 hours of treatment with the Nano-emulsion with 1.16-log reduction with 0.75 % and 1.32-log reduction with 1% after 24 hours. The sprouts did not show a significant amount of reduction compared to seeds. Future studies will evaluate higher concentration of Nano-emulsions for antimicrobial activity on sprouts.

Significance: These data suggest that Nano-emulsions of cinnamon oil may be effective as an antimicrobial treatment for alfalfa seeds and sprouts.

P1-14 Use of Novel Antimicrobials to Mitigate Risk of E. coli in Cake Mixes

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❖ ***Developing Scientist Entrant***

Introduction: Wheat flour contaminated with Shiga Toxigenic *Escherichia coli* (STEC) O26 and O121 was responsible for recent outbreaks in the United States and Canada. Out of the 40 individuals sickened, 11 claimed to have consumed raw dough.

Purpose: The aim of this study was to test the efficacy of novel powdered antimicrobial formulations against STEC O26, O121 and O157:H7 as additives to dough and batter.

Methods: The MIC (Minimal Inhibitory Concentration) of vinegar (P1) and 3 citrus-based (P2, P3 and P4) powdered antimicrobial formulations individually (1 g/10 mL) and in combination with maltodextrin encapsulated pelargonic acid (MEPA) (0.5 g of P1/P2/P3/P4 + 0.5 g, EPA/10 mL) was evaluated against STEC O26, O121 and O157:H7. A 96 well plate resazurin based assay was used to determine the MIC. The antimicrobial stocks used were tested ranging from a concentration of 1 g/10 mL to 0.07 g/10 mL. The lowest concentration of antimicrobial that restricted pathogen growth was determined by the absence of color change of resazurin from blue to pink in the wells. All wells contained 5 log CFU/mL of the pathogen and Iso-Sensitest broth to support bacterial growth. Water was used as a control.

Results: Antimicrobial P1 had an average (n = 3) MIC of 23 mg/mL against all three strains of *E. coli*; when combined with MEPA, the MIC was significantly lowered to 7.8 mg/mL ($P > 0.05$). Similarly, the MIC of 27 mg/mL of P2 was reduced to 12.5 mg/mL with MEPA ($P < 0.05$), while MIC of 26 mg/mL for P3 was reduced to 12 mg/mL ($P < 0.05$). P4 had an MIC of 21.87 mg/mL and did not have synergistic antimicrobial activity with MEPA. The combinations of antimicrobials that were most effective will be evaluated in cake mixes (n = 3) under ambient and abuse storage conditions.

Significance: Antimicrobial additives could prevent the survival and growth of STEC in batter and dough.

Poster

P1-15 Plant-based Microemulsions Inactivate *Escherichia coli* O157:H7 and *Pseudomonas fluorescens* on Iceberg Lettuce and Improve Visual Quality during 28-Day Storage

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❖ ***Developing Scientist Entrant***

Introduction: Current industry practices include the use of chemical-based sanitizers such as chlorine to wash vegetables post-harvest. The occurrence of produce-related outbreaks poses serious concerns on food safety and suggests that better intervention methods need to be implemented. Due to a growing consumer demand for natural products, alternatives for wash sanitizers have been considered.

Purpose: Investigate the antimicrobial activities of plant-based microemulsions in the wash water against *Escherichia coli* O157:H7 and *Pseudomonas fluorescens* on Iceberg lettuce during 28 days of refrigerated storage.

Methods: Wash microemulsions that were evaluated included oregano oil, lemongrass oil, and cinnamon oil along with a plant-based emulsifier for improved solubility of the oil in water. Iceberg lettuce was inoculated with either *E. coli* O157:H7 or *P. fluorescens* (6.0 log CFU/g) and dip treated in a phosphate buffered saline (PBS) control, 50-ppm chlorine, 3% hydrogen peroxide or 0.1%, 0.3%, or 0.5% of one of the antimicrobial microemulsion solutions for 2 min. The treated leaves were stored at 4°C, visually observed, and analyzed for surviving bacterial populations by dilutions and plating on days 0, 3, 7, 10, 14, 21, and 28.

Results: The efficacies of the antimicrobials were concentration- and storage-time dependent. The microemulsions exhibited a 1.95-4.86 log CFU/g reduction in *E. coli* O157:H7 population during the 28 days. The microemulsions were also effective against *P. fluorescens* resulting in a 0.32-2.35 log CFU/g reduction during storage at days 0-28. The visual observation of treated leaves indicated that the 0.3% lemongrass microemulsion showed the best visual appeal in Iceberg leaves inoculated with *E. coli* O157:H7 and 0.1% oregano oil showed improvement in the quality of Iceberg leaves inoculated with *E. coli* O157:H7 and *P. fluorescens*.

Significance: Essential oil microemulsions have the potential to provide natural, eco-friendly, and effective alternatives to chemical sanitizers for leafy green decontamination against *E. coli* O157:H7 and *P. fluorescens*.

P1-16 Behavior of *Listeria monocytogenes* upon Exposure to Muscadine Extract and Chlorine Dioxide *in vitro* and on Leafy Greens

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Introduction: *Listeria monocytogenes* was linked to 8 produce-related outbreaks between 2008-2016 in the United States. With mortality rates of ~20%, it represents a food safety challenge for the produce industry. Therefore, there is a need to find effective antimicrobial treatments to apply on produce. Chlorine dioxide (CLO2) and muscadine extract (ME) are safe alternatives to chlorine. CLO2 has been effective at reducing *L. monocytogenes* on lettuce, while ME contains phenolic compounds capable of disrupting bacterial cells.

Purpose: The purpose of this study was to determine the antimicrobial activity of ME and CLO2 against *Listeria monocytogenes* in broth and on spinach.

Methods: Tryptic soy broth with yeast extract (TSBYE) and spinach were inoculated with a 3-strain cocktail of *L. monocytogenes.* For the in-vitro study, minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations were calculated after 24 h incubation at 37°C. For the spinach study, microbial reduction was evaluated after exposure to various treatments (water as control, 3 ppm CLO2, 300 mg/mL ME, and combinations of both) for 10 min + 30 min dry time + 24 h storage at 10°C.

Results: MIC and MBC values were 1 and 3 ppm for CLO2, respectively, and ME above 100 mg/mL reduced *Listeria monocytogenes* to undetectable levels in TSBYE. ME (300 mg/mL) had comparable ($P > 0.05$) surface microbial reduction on spinach to CLO2 (3 ppm), ~ 4.2 log CFU/g reduction after 24 h storage. A synergistic effect (2 ppm CLO2 x 200 mg/mL ME) was observed on spinach, achieving 4.4 log CFU/g reduction. Both treatments could be used as intervention methods to reduce *L. monocytogenes* on leafy greens.

Significance: The potential use of muscadine extract as antimicrobial may lead to a non-synthetic chemical alternative to treat produce. When used in combination with chlorine dioxide, it can help to reduce the amount of chemicals utilized resulting in a synergistic effect.

P1-17 Application of Nanobubble-Antimicrobial Solutions to Remove Fresh *Listeria monocytogenes* Biofilms on Stainless Steel Food Surfaces

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Introduction: Foodborne pathogens, such as *Listeria monocytogenes*, can attach to food contact surfaces and form biofilms, limiting the ability of sanitizers to effectively disinfect food contact surfaces. Nanobubble technology is a novel concept in food safety that can improve the potency of commercial sanitizers against biofilms.

Purpose: This proof-of-concept study was conducted to determine the impact of gas (air, CO_2, or N_2) nanobubbles incorporation in 100 and 200 ppm chlorine (Cl_2) solutions to inactivate fresh *L. monocytogenes* biofilms on stainless steel.

Methods: Fresh biofilms of *L. monocytogenes* were grown on stainless steel coupons through static incubation at 37°C for 72 h by immersing in *L. monocytogenes* inoculated brain heart infusion (BHI) broth. Random pre-determined areas on each coupon were swabbed into D/E neutralizing broth before and after treatment. The coupons were treated by dipping in water or Cl_2 solutions with or without gas nanobubbles for 1 min. *L. monocytogenes* were enumerated using BHI agar. This study was designed as a completely randomized design with three replications. All data were analyzed using ANOVA, and Tukey's test was used to determine significant differences among the mean values at $P \leq 0.05$ using Minitab®19.

Results: Incorporation of any gas nanobubbles in water and 200 ppm Cl_2 did not have any significant effect on the biofilm inactivation. However, incorporating air and CO_2 nanobubbles in 100 ppm Cl_2 resulted in significantly greater log reductions (5.0 and 4.9 log CFU/cm^2, respectively) in *L. monocytogenes* biofilms compared to that of 100 ppm Cl_2 without any gas nanobubbles (3.7 log CFU/cm^2).

Significance: The findings from this study showed that air and CO_2 nanobubbles increased the potency of 100 ppm Cl_2 against fresh *L. monocytogenes* biofilms on stainless steel, suggesting that the impact of different gas nanobubbles incorporation in various sanitizer solutions should be evaluated using more detailed experimental designs.

P1-18 Functional Qualities, Antimicrobial Activities and Geospatial Investigation of Retailed Nigerian Honey

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Introduction: Increasing severe intestinal infection caused by antibiotic-resistant enteric bacilli pathotypes in several southwest communities in Nigeria intensify use and consumption of various honey including adulterated products as a local treatment regimen with little or poor outcome.

Purpose: The study focused on the evaluation of the retailed honey functional qualities, microbial contamination, antimicrobial potential against prevalent resistant enteric bacilli, and geospatial investigation of functional properties and safety.

Methods: Biotyped enteric bacilli obtained from randomly collected fecal samples were investigated for multi-antibiotic resistance and phylo-diversity. A total of 112 locally and branded honey products were profiled for physico-chemical and phytochemical properties, assayed for bacteria and fungi con-

taminants, cidal activity, time-kill kinetics, and geo-spatial analysis.

Results: More than 30% of enteric biotypes including *Escherichia coli, Klebsiella oxytoca,* and *Pseudomonas aeruginosa* significantly showed high antibiotic resistance having two unrelated cluster complexes with diverse antibiotic resistance indices. Among the honey, physico-chemical properties and phyto-chemical compounds significantly differed with more than 23% locally retailed honey showing *Bacillus subtilis*, *Enterococcus faecalis* and *Aspergillus flavus* contaminants compare to branded products. Of resistant enteric bacilli, 24.3% were susceptible to branded honey at MIC90 500 mg/mL and 8.1% (MBC90 1,000 mg/mL) with evidence of 14.85% and 5.94% significant viable reduction ($P < 0.05$). Only alkaloids significantly regressed ($P = 0.028$) with susceptibility of resistant bacilli and correlated bacteria inhibition rate ($r = 0.534$, $P = 0.049$). Acidic pH, phenol, and flavonoids were significant in identified antibacterial honey from coaster locations, at 3°10'0-4°40'0E and 6°30'0-7°00'0N of southwest Nigeria with significant alkaloid biomarker than branded products.

Significance: Both branded and locally packaged honey possessed adequate antimicrobial properties but local products were unsuitable for consumption or therapeutic use due to high level bacteria and fungi contaminants. Spatial mapping reveals coastal water honey to possess suitable bioactive compounds as functional food ingredient rich in alkaloid.

P1-19 Antimicrobial Efficacy of Photosensitizer Curcumin on Food Contact Surfaces in Cold-Smoked Fish Industry

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* is the number one cause for recall of cold-smoked fish. In addition, 58% of foodborne illnesses are caused by norovirus closely followed by *Salmonella*. These pathogens are transferred by infected individuals or through contact with contaminated products and surfaces.

Purpose: The aim of the study is to evaluate the antimicrobial efficiency of water-soluble photosensitizer curcumin (PSC) against *Listeria monocytogenes* (6 strains), *Salmonella* (3 strains), and MS2 (surrogate for norovirus) on food-contact surfaces typical of the smoked seafood industry.

Methods: The absorption spectrum of photosensitizer curcumin was measured using Cary 50 UV-Visual spectrophotometer to identify the maximum absorption. Depending on the maximum absorption, an LED light box was constructed. Currently, the minimum inhibiting concentrations and minimum bactericidal concentrations are being evaluated for the pathogens of interest. Furthermore, the incubation time of inoculated surface in curcumin and exposure duration to light will be evaluated in combinations for determining the optimum combination for significant log reduction in pathogens. Thereon, the photosensitizer efficacy on inoculated food-grade stainless steel coupons equating to food contact surfaces will be evaluated.

Results: The absorption maximum of photosensitizer curcumin was found to be 414.98 nm. A LED light source of wavelength 430 nm was constructed and was found to have a wavelength of 111W/m^2. The *Salmonella* strains had a 3-log reduction at 200 ppm when it was incubated in curcumin for 5 minutes and exposed to light at an intensity of 66.6KJ/m^2. Further studies are being done to determine the best combination of incubation time versus light exposure.

Significance: Photosensitizer curcumin has shown to be a strong antimicrobial agent. Furthermore, it is a naturally occurring compound making it an attractive method of sanitation.

P1-20 Efficacy of Citrus Essential Oil Applications on the Shelf Life of Strawberries

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Introduction: The food industry loses billions daily due to the loss of food products from spoilage and pathogenic bacteria. Strawberries are subject to a limited shelf life due to microbial deterioration, high respiration rates, and soft texture. Due to its perishability, natural preservatives would help extend shelf life addressing consumer demand for the replacement of synthetic chemicals.

Purpose: The objective of the study was to determine the effect of three citrus essential oil applications on the shelf life of strawberries.

Methods: Strawberries (10-30 g) were purchased from a local retailer, randomly separated, and subjected to various concentrations (0.5% and 1.0%) of lime oil, lemon oil, and limonene to evaluate the growth response of background microflora and yeast and mold (YM). The samples were weighed for moisture pickup post-treatment application and stored individually at room temperature. Microbial analysis was conducted in triplicate for total plate count and YM on days 1, 3, and 5.

Results: The control had a 1.39 log CFU/g growth and .91 CFU/g death over the testing period. On average the oils displayed a bacterial growth of .93 log CFU/g from day 1-3, and death of .81 log CFU/g from days 3-5. YM counts spiked in growth on day 5, with lime oil acting as a food source for growth. Moisture loss in the control group was 2.5 g on day 3 and 1.7 g on day 5, respectively. The treatments on average lost 4.4 g on day 3 and 2.9 g on day 5, leading to shriveling and significant ($P < 0.05$) quality losses.

Significance: The treatments were not effective as the moisture loss, discoloration, and overall quality degradation would not render them a suitable alternative due to consumer acceptability. The treatments effectively slowed bacterial and YM growth from day 1-3, however, the YM values were consistently higher on day 5.

P1-21 Survival of *Escherichia coli* (STEC) *In vitro* and on Leafy Greens Exposed to Natural Antimicrobials and Chlorine Dioxide

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❖ ***Developing Scientist Entrant***

Introduction: Essential oils and plant extracts are natural alternative antimicrobials to synthetic chemicals. These substances are rich in phenolic compounds and have exhibited growth inhibition of foodborne pathogens. Chlorine dioxide up to 3 ppm is an effective, safe alternative to chlorine in the produce industry.

Purpose: To determine the antimicrobial activity of emulsified thymol (THY), muscadine extract (ME) and chlorine dioxide (CLO2) against *E. coli* O157:H7 in broth and on spinach.

Methods: For the spinach study, microbial reduction was evaluated after exposure to treatments for 15 min + 24 h storage at 8°C. MIC and MBC values were 2 mg/mL (both) for THY and 1 and 3 ppm for ClO2, respectively, in broth. Muscadine extract above 150 mg/mL reduced *E. coli* O157:H7 to undetectable levels in TSB. The inoculum level was ~6 log CFU/mL or /g.

Results: The ME (300 mg/mL) had comparable surface microbial reduction ($P > 0.5$) to that reached with ClO2 (3 ppm) on spinach, approximately 1.2 log CFU/mL reduction. No antimicrobial effect was achieved with emulsified thymol (up to 6 mg/mL). A synergistic effect was observed in the broth (150 M x 0.1 THY and 150 M x 0.15 THY) but not on spinach.

Significance: The ME and CLO2 antimicrobial interventions could be used as consecutive intervention methods to decrease *E. coli* STEC on leafy greens. There was no sensory testing of the spinach.

P1-22 Temperature Dependent Antimicrobial Activity of Menhaden Fish Oil *In Vitro* and on Pet Food Kibbles Against *Salmonella* spp.

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Introduction: Fish oil inclusion into a dry pet food provides a source of long-chain omega-3 fatty acids. Polyunsaturated fatty acids in fish oil have antibacterial activity against various foodborne pathogens such as *Salmonella* and *Escherichia coli.*
Purpose: To determine the effect of temperature applied to dry pet food kibbles on the antimicrobial activity of Menhaden fish oil against *Salmonella* spp.
Methods: Sterile Menhaden oil was inoculated with ~8 logs of *Salmonella* cocktail (~3 % moisture; *S.* Enteritidis, Heidelberg, and Typhimurium) and incubated at 25°C, 37°C, and 45°C. Microbiological evaluation of the water phase was done after 2 h on TSA agar. Sterile kibbles were coated with fish oil (7.0 % w/w). Canola oil coating was kept as a control. One hour after coating, the kibbles were inoculated with ~9 logs of *Salmonella* and incubated at their respective temperature. The microbiological evaluation was conducted at 0 h, 2 h, 6 h, 12 h, and 24 h. Both experiments were evaluated in triplicate.
Results: The oil phase of the fish oil system was negative for *Salmonella* after 2 h of incubation and confirmed by enrichment and PCR. From the water phase, 8.1 and 7.3 logs were recovered at 25°C and 37°C, respectively, and no *Salmonella* was detected at 45°C. On the kibble, Menhaden oil had higher antimicrobial ($P \leq 0.05$) activity after 12 h at 25°C, and throughout the experiment at 37°C. At 45°C, the fish oil had a superior antimicrobial activity against *Salmonella* cocktail after 2 h. When the fish oil alone was compared at different temperatures a higher antimicrobial activity was observed at 37°C and 45°C across all time points.
Significance: The results indicate antimicrobial activity of the Menhaden oil increases with temperature. This is an important finding to the pet food industry; wherein a higher temperature (~ 45ºC) during fat holding the application process may help mitigate *Salmonella* on the kibbles.

P1-23 Application of a Natural Bioactive Glycolipid to Control *Listeria monocytogenes* Biofilms and as Post-Lethality Contaminants in Milk

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* can form persistent biofilms on food processing surfaces, resulting in cross-contamination of food products. Natural glycolipids are a promising intervention to control undesirable microbes due to their antimicrobial activity and low toxicity.
Purpose: This study aimed to determine the antimicrobial activity of a natural glycolipid to control *L. monocytogenes* biofilms as well as growth in milk.
Methods: The tested commercial glycolipid, Nagardo™, is obtained via fermentation of glucose by an edible jelly fungus. Sub-inhibitory, minimum-inhibitory, and minimum-bactericidal concentrations (SIC, MIC, MBC) of the glycolipid against a 6-strain cocktail of *L. monocytogenes* were determined. Glycolipid at SIC and 6 log CFU/mL *L. monocytogenes* were then incubated on polystyrene and stainless-steel surfaces at 37°C for 7 days to determine the potential against biofilm formation. Glycolipid at 1xMBC, 10xMBC, or 100xMBC was also added to mature biofilms formed on both surfaces for 1 and 4 h at 37°C to determine the inactivation on biofilm-associated *L. monocytogenes*. Varying concentrations of the glycolipid were also added to commercial UHT milk inoculated with 4 log CFU/mL *L. monocytogenes* and stored at 7°C for 21 days.
Results: The SIC, MIC, and MBC of the glycolipid were determined as 1.6, 3.5, and 4.5 mg/L. The glycolipid significantly reduced biofilm-associated *L. monocytogenes* on both surfaces at concentrations as low as 45 mg/L ($P < 0.01$). When added to UHT skim milk, a concentration of 1,000 mg/L inhibited *L. monocytogenes* growth through 7 days at 7°C ($P < 0.001$), whereas application of 1,300 and 1,500 mg/L reduced counts to levels below the enumeration limit at day 21 ($P < 0.001$). However, 2,000 mg/L were necessary to inhibit growth through 7 days in whole milk ($P < 0.001$).
Significance: Natural glycolipids have the potential as a natural alternative for the removal of biofilms and as an antimicrobial to control *L. monocytogenes* in milk.

P1-24 Antimicrobial Effects of a Bioactive Glycolipid on Spore-forming Spoilage Bacteria in Milk

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❖ ***Developing Scientist Entrant***

Introduction: Milk spoilage often results from the growth of psychrotolerant aerobic spore-forming bacteria during refrigerated storage resulting in off-flavors and curdling. Natural glycolipids are a promising intervention to control undesirable microbes due to their antimicrobial activity and low toxicity.
Purpose: This study aims to determine the efficacy of a commercial glycolipid product to inhibit spore germination, spore outgrowth, and the growth of vegetative cells of *Paenibacillus odorifer*, *Bacillus weihenstephanensis*, and *Viridibacillus arenosi*, which are the predominant spore-forming spoilage bacteria in milk.
Methods: The tested commercial glycolipid, Nagardo™, is obtained via fermentation of glucose by an edible jelly fungus. For spore germination and outgrowth assays, varying concentrations (25 - 400 mg/L) of the glycolipid product were added to commercial UHT whole and skim milk inoculated with ~ 4 log spores/mL of each bacteria and incubated at 30°C for 5 days. The effect of glycolipid addition on vegetative cell growth with inoculum level ~ 4 log CFU/mL was also determined in UHT whole and skim milk over 21 days of storage at 7°C.
Results: Inhibition of spore germination in UHT whole milk was only observed for *V. arenosi*, and only when glycolipid was added at 400 mg/L. However, concentrations of 400 and 200 mg/L markedly inhibited the outgrowth of vegetative cells from spores of *P. odorifer* and *B. weihenstephanensis*, respectively. For vegetative cell inhibition, glycolipid addition at 50 mg/mL was bactericidal against *P. odorifer* and *B. weihenstephanensis* in skim milk through 21 days of storage, whereas 100 mg/L was needed for similar control of *V. arenosi*. Concentrations of 100 mg/L and 200 mg/L inhibited the growth of vegetative cells of *B. weihenstephanensis* and *P. odorifer*, respectively, in whole milk; 200 mg/L was also bactericidal to *B. weihenstephanensis.*
Significance: Natural glycolipids have the potential to inhibit the growth of dairy-spoilage bacteria and extend the shelf-life of milk.

P1-25 Use of White Mustard Essential Oil-based Solutions in Natural Produce Washes Against E. coli

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Introduction: *Escherichia coli* is an important pathogen closely related to fresh produce. The industry reduces this pathogen on fresh produce using chlorine washes, however many consumers desire fewer chemicals to be used.
Purpose: The purpose of this study was to evaluate the efficacy of white mustard essential oil (WMEO) based solutions in combination with organic acids and other plant extracts in produce wash against *E. coli* compared to the industry standard of 150 ppm chlorine.
Methods: Lettuce was stored at 4°C for 24 hours and dip inoculated with *E. coli* BAA-2196. Inoculated pieces and uninoculated control samples were washed in these treatments: tap water, 150 ppm chlorine, 0.84% WMEO, 0.01% Lauric arginate (LAE), 0.1% Carvacrol, 0.84% WMEO + 2% Acetic acid, 0.84% WMEO + 0.01% LAE, 0.84% WMEO + 0.1% Carvacrol, 0.84% WMEO + 0.01% LAE + 2% Acetic acid and 0.84% WMEO + 0.1% Carvacrol + 2% Acetic acid. After rinsing in sterile water, the pH of solutions, color change and effect on levels of *E. coli* were evaluated. Bacterial reductions of the treatments were compared using ANOVA, the color was compared using *t*-test.
Results: All treatments caused a significant ($P < 0.05$) decrease in *E. coli* numbers compared to the positive control with no significant change of color

and smell. WMEO + LAE + Acetic Acid and WMEO + Carvacrol + Acetic acid were as effective as 150 ppm chlorine ($P > 0.05$). For instance, the average log reduction of WMEO + Carvacrol + Acetic acid and chlorine were 2.3 and 2.2, respectively. The pH of the chlorine solution was the highest at 8.4, while the solutions including WMEO ranged from pH 2.5 to 5.3, being lower when acetic acid was added.

Significance: These data suggest that the WMEO-based produce wash may reduce *E. coli* levels on leafy greens as efficiently as chlorine solution.

P1-26 Impact of Enhanced Organic Acids-based Leavening Ingredients in Shelf-life Extension of Baked Cupcakes

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Introduction: Organic acids are well known for their antimicrobial properties and increasing shelf life. They have their potential to cross the cell membrane, modify the proton and anion concentrations in the cytoplasm and cause decrease in pathogenic cell viability. Organic acids in combination showed effective reduction of spoilage microorganisms.

Purpose: To evaluate the synergic antimicrobial effects of citric, lactic and malic acids with acetic acid (vinegar) to extend the shelf life of baked cupcakes.

Methods: Buffered dry vinegar was blended with dry citric, malic and lactic acids separately and their pH were adjusted to neutral by sodium bicarbonate. These ingredients were incorporated individually at 1% in the cupcake recipe by replacing the conventional leavening agent, baking powder, and cupcakes were baked at 175°C for 20 minutes; control was prepared using baking powder. Baked cupcakes were tested for their physical properties (color, texture) and shelf life at room temperature (18-20°C); antimicrobial potential of organic acids blends was measured against aerobic plate count (APC) and mold growth.

Results: *F*-test showed that acetic acid blended citric, lactic and malic acids have no significant difference in controlling the mold and APC growth in the cupcakes, stored for 17 days. All controlled the mold and APC growth equally. There is no significant difference in their physical properties. Control and tested leavening agents were significantly different with $P < 0.01$ on both mold and APC growth control. Mold and APC counts were more than 6 log CFU/g in control sample after 8 days of storage.

Significance: Shelf-life results showed that the tested organic acids blends have good control in microbial growth. Therefore, they can be used as shelf life extending leavening agent in baking by replacing the baking powder.

P1-27 Preventing Pathogen Outgrowth and Extending Shelf Life of Ready-to-Eat Convenience Meal Kit Products Using a Secondary Inhibitor

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Introduction: Increasing popularity in the use of meal kits during COVID-19 pandemic and delivery of such meals through overnight transportation poses food safety risk of pathogen outgrowth due to any potential storage temperature deviation in delivery supply chain.

Purpose: To evaluate the efficacy of buffered vinegar at controlling *Listeria monocytogenes* and extending shelf life of meal kit components.

Methods: Single components such as meatloaf, steak, grilled chicken, sweet potato, green beans & carrots, and seasoned risotto that can form a part of a meal kit were treated with 1.25% liquid buffered vinegar. A five-strain (ATCC 19111, 19112, 19115, 19118 and 13932), cold-adapted *Listeria monocytogenes* cocktail at 2-3 log CFU/g was used to inoculate the control and treated samples. After allowing 30 min inoculum attachment time, samples ($n = 2$) were MAP packaged with 70:30, Nitrogen:Carbon dioxide, gas mix and stored at 39 ±1.5°F for 15 days and enumerated for *L. monocytogenes*, total aerobic count, lactic acid bacteria and yeast and mold.

Results: The results indicated that buffered vinegar at 1.25% was effective in controlling outgrowth of *Listeria monocytogenes* and reducing the bacterial populations in various meal kit components. Single factor ANOVA analysis showed that control and all treated samples were significantly different with $P < 0.01$ over 15-day period of the study. Other shelf-life indicators such as total aerobic count, lactic acid bacteria and yeast & mold counts remained stable below 6 log CFU/g throughout the study.

Significance: Results suggest that buffered vinegar was effective at controlling *Listeria monocytogenes* and extending shelf life of meal kit components; improving safety in case of probable temperature fluctuations in supply chain.

P1-28 Natural Disinfectant to Reduce *Listeria monocytogenes* Contamination on Food Contact Surfaces

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Introduction: *Listeria monocytogenes* has been implicated in several outbreaks linked to consumption of ready-to-eat (RTE) sliced deli meats. Food contact surfaces like meat slicers in retail delis provide ideal conditions for the bacteria to colonize and grow. Sliced meats can become contaminated with *L. monocytogenes* during slicing and may pose a serious public health concern. Effective interventions are needed to control this pathogen and prevent cross-contamination on food contact surfaces.

Purpose: To evaluate the effect of natural surface spray disinfectant at reducing *Listeria monocytogenes* population on food contact surfaces.

Methods: Combined vinegar and citrus extract emulsion was evaluated for its antimicrobial effect as natural disinfectant by direct exposure of surface inoculated stainless-steel coupons ($n = 30$) with a five-strain *L. monocytogenes* cocktail at 6 log CFU/mL into the emulsion for 30 s. Polyethylene surfaces ($n = 3$) were inoculated with *L. monocytogenes* cocktail, with an attachment of 3-4.5 log CFU/cm^2 on the surfaces and treated with antimicrobial emulsion using spray method for 10 s, 3 min and 60 mins of exposure time. Stainless steel surfaces ($n = 5$) were inoculated, treated and reinoculated for 3 times to mimic potential re-contamination at a deli slicer. Pathogen enumeration was performed before and after treatments by dislodging attached cells from surfaces in a solution using sponge sampling method and plated using selective media.

Results: Direct exposure of antimicrobial to inoculum showed efficacy at 30 s ($P < 0.001$). Treated, inoculated polyethylene surface showed the reduction of 1.21, 1.36 and 1.98 log CFU/ for 10 s, 3 min and 60 min of exposure time, respectively. The effect of natural disinfectant on polyethylene surface was significant at 90% confidence level compared to control. Stainless steel surfaces showed significant reduction at each level with $P < 0.05$.

Significance: Natural surface spray disinfectant showed positive reduction of *Listeria monocytogenes* on selected surface under various simulated conditions.

P1-29 Control of *Clostridium perfringens* Outgrowth Under Abusive Conditions Using Buffered Vinegar as a Secondary Inhibitor in a Simple Food Model

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Introduction: *Clostridium perfringens* is a common food poisoning pathogen that produces heat-resistant spores. Inadequate cooling or abuse storage temperature provide favorable conditions for *C. perfringens* to grow and sporulate in various food products.

Purpose: To determine the efficacy of buffered vinegar as a secondary inhibitor in preventing the outgrowth of *Clostridium perfringens* in a simple food model.

Methods: Cooked vegetable broth was used as a food model to test the efficacy of vinegar in inhibiting outgrowth of *C. perfringens.* Vegetable broth was mixed together with fluid thioglycolate medium for a target pH of 6.50. Broth media mix was treated with vinegar at four usage levels from 1.0-1.75% (T1-T4). Each treatment mix was inoculated with 4-5 log CFU/g of *C. perfringens* inoculum (cocktail of vegetative cells and spores) and held anaerobically at

elevated temperature of 43 ± 2°C for 14 h. Enumeration of pathogen was performed at time 0, 4, 8, and 14 h.

Results: Single factor ANOVA analysis indicates treatments T2 (1.25%), T3 (1.5%) and T4 (1.75%) showed same level of effective growth inhibition with no difference among them ($P > 0.45$). However, T1 at 1.0% usage rate, the growth inhibition was lesser when compared to other treatments ($P < 0.001$). So, the vinegar at minimum usage of 1.25% and above could effectively control the outgrowth of *C. perfringens* at elevated temperature storage conditions.

Significance: Results showed that buffered vinegar can be used as an effective secondary inhibitor to control growth *C. perfringens* under abusive temperature conditions.

P1-30 Effects of Cranberry Extract on Conditioning Films and Bacterial Biofilm Formation

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Introduction: Bacterial persistence and growth in food processing environments has many undesirable impacts. Of particular concern are species that form biofilms on surfaces and that are pathogenic to humans, such as *Listeria*. Various factors influence biofilm formation including the presence of inhibitory substances. Among these, cranberry extract (CE) has demonstrated antimicrobial properties.

Purpose: Biofilm formation is a multi-step process that begins with formation of a conditioning film. Conditioning films form when molecules in the surrounding liquid are absorbed onto substrates and alter bacterial adhesion. In this study, we examined whether CE would inhibit bacterial adhesion and biofilm development via the formation of conditioning films.

Methods: To create conditioning films, acid-washed, sonicated, and rinsed glass beads were incubated for 24 h in 12.5% neutralized CE; controls had water without CE. Subsequently, beads were thoroughly rinsed and inoculated with *L. innocua* (5 logs/mL) grown in brain heart infusion broth. Beads were incubated ($n = 3$ for CE and controls) for 48 h, rinsed in deionized water and sonicated to remove biofilm. Samples were filtered, stained with DAPI, and enumerated via epifluorescence microscopy.

Results: Bacterial cell counts were nearly 3-times lower in the CE treated beads compared to controls. However, due to high variability in biofilm surface coverage, there was not a statistically significant difference (t-test, $P > 0.05$). Qualitatively cells in the CE treatment appeared less well stained and smaller in size. Biofilm matrix was observed in both treatments and controls.

Significance: This study demonstrated inhibition of *L. innocua* attachment and biofilm formation. Previously, we have documented the physiological stress caused by the antimicrobial properties of CE. CE may be developed as an addition to cleaning and sanitation regimes for *Listeria* control. Future studies will compare the direct physiological effects of CE in combination with the indirect effects of biofilm inhibition.

P1-31 Effect of Organic Acids-Enhanced Nano Size Ice Slurry Chilling Techniques on the Microbial and Physicochemical Properties of Black Drum (*Pogonias cromis*)

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Introduction: Enhanced chilling methods with organic acids were aimed to improve shelf life and profitability in the highly perishable fish market. Nano size ice slurry is a phase-changing system which consists of tiny ice particles in crystalline form suspended in a solution of brine at sub-zero temperature.

Purpose: Evaluate the benefits of using organic acid enhanced nano size ice slurry as chilling method for seafood in the Gulf of Mexico.

Methods: Four treatments namely, nano size ice slurry (SI), SI with lactic acid (SLA), SI with citric acid (SCA), and SI with acetic acid (SAA) were employed for this study. Sixty-four (64) freshly caught black drums were immersed in a 1% W/V aqueous SI solution of organic acids for 20 minutes, filleted, pack in Ziploc bags, placed in ice chest filled with flake ice and stored in a refrigerated cooler at ≤4°C for 28 days. At intervals of 4 days, fillets were analyzed for microbial stability, and physico-chemical quality. Mean separation was analyzed using ANOVA and Tukey's studentized range test at $\alpha = 0.05$.

Results: The treatments employed maintained quality within the first 8 days. No significant difference ($P > 0.05$) was observed between treatments throughout the study. At day 8, the Aerobic Plate Counts recorded were 4.78 ± 0.40 log CFU/g for the control SI, while others were 3.62 ± 0.34 log CFU/g, 3.23 ± 0.11 log CFU/g and 3.23 ± 0.21 log CFU/g for SAA, SCA, and SLA treatments, respectively. Although nano size ice slurry extends the shelf life of black drum over time, the result shows limited benefit when combined with organic acids at the chilling step.

Significance: Findings present fish harvesters with a cost-effective alternative for fish preservation to minimize post-harvest loss of product, amidst information on the properties of the preserved product.

P1-32 *Salmonella* spp. and *Listeria monocytogenes* Behavior with Chitosan Application on Pig Carcasses Samples

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Introduction: Contamination of pig carcasses by pathogenic microorganisms often occur during the slaughter process.

Purpose: This study aims to evaluate the behaviour of Salmonella spp. and Listeria monocytogenes by chitosan application as a decontaminant in pig carcasses samples.

Methods: *Salmonella* spp. mix was prepared with *Salmonella* Typhimurium ATCC 14028 and Salmonella Derby. Listeria monocytogenes mix was prepared with Listeria monocytogenes ATCC 4b and Listeria monocytogenes environmental. The bacterial suspensions were inoculated in samples (n=210, each mix) of rind about 25 cm2 at two different concentrations (Salmonella spp. mix - 1.42 × 105 CFU/cm2 (A) and 4.92 × 106 CFU/cm2 (B); Listeria monocytogenes mix - 1.74 × 105 CFU/cm2 (C) and 6.38 × 106 CFU/cm2 (D). Samples (n=180, each mix) were decontaminated by spraying chitosan at 0.2 and 0.5%, stored at 7 ºC and analyzed at 30 minutes, 6, 12, 24 and 48 hours.

Results: For *Salmonella* spp., significant differences were observed between control samples without and with chitosan for both suspensions and chitosan concentrations, particularly after 24 hours. During the 48 hours, a bacteriostatic effect was observed for Salmonella spp. mix for chitosan 0.2%, and a bactericidal effect at 0.5%, decreasing approximately 0.49 and 1.46 log CFU/cm2 for suspension A and B, respectively. Listeria monocytogenes was able to grow at 0.2 and 0.5 % chitosan. However, compared with control samples, chitosan showed better results with significant differences observed during time. During 48h at 0.2%, counts were 0.94 and 2.23 log CFU/cm2 lower for suspension C and D, respectively. At 0.5%, counts were 1.29 and 2.66 log CFU/cm2 lower for suspension C and D, respectively.

Significance: Chitosan has good bioactive properties that can be used in the food industry. The behaviour of *Salmonella* spp. and *Listeria monocytogenes* demonstrates the possibility of using this compound in meat preservation.

P1-33 Impact of Antimicrobial Application Sequence on Destruction of *Salmonella* and *Campylobacter* in Raw Poultry

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Introduction: Despite numerous interventions throughout processing, *Salmonella* spp. and *Campylobacter* spp. continue to proliferate in raw poultry products, leading to illnesses and recalls. New application methodologies may alleviate this challenge.

Purpose: Determine reductions of *Salmonella enterica* or *Campylobacter* spp. following sequential or individual dip application of common antimicrobials.

Methods: Chicken breasts were trimmed to 5 cm^2 surface area with even thickness and inoculated with a cocktail containing *Salmonella enterica* subsp. Enteritidis (ATCC 13076 and 31194), Typhimurium (ATCC 13311 and 14028), and Heidelberg (ATCC 8326), or a cocktail containing *Campylobacter jejuni* (ATCC 33560) and *Campylobacter coli* (ATCC 43483) to achieve a starting population of ca. 5 log CFU/g. Cells were permitted to attach for 30 min post-inoculation in a biosafety cabinet. Post-attachment, samples were dipped (30 s dip with agitation by forceps) in one of the following treatments: 5% buffered lactic acid (BLA; pH 3.5) alone, 200 ppm lauric arginate ethyl ester (LAE) alone, BLA followed by LAE, or LAE followed by BLA (15 s each application). Samples were placed on a clean wire rack to dry in a biosafety cabinet for 10 min. Samples were then transferred to sterile bags, diluted 1:1 in Dey-Engley neutralizing buffer, and stomached (230 rpm; 30 s). Dilutions were performed in Butterfield's buffer and plated on xylose-lysine-tergitol 4 agar (XLT-4; 35°C for 24 h) or Campy-Cefex agar (41°C for 48 h). Samples were enumerated and reductions compared to control.

Results: For *Salmonella* and *Campylobacter*, application order of BLA and LAE was not significant ($P > 0.05$); however, the co-application of antimicrobials resulted in lower ($P < 0.05$) counts than untreated control (average 1.3 and 2.3 log CFU/g reduction compared to control in *Salmonella* and *Campylobacter*, respectively).

Significance: Application sequence of two common antimicrobials may not impact destruction of *Salmonella* and *Campylobacter*.

P1-34 Ultrasonic Formulation of Bergamot Oil and Linalool Nanoemulsions and Their Bactericidal Activity

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❖ ***Developing Scientist Entrant***

Introduction: Bergamot essential oil (BEO) and Linalool (LO) exhibit a broad spectrum of antimicrobial activity. However, their low water solubility limits their application in food systems.

Purpose: The objectives of this study were to formulate ultrasonic assisted nanoemulsions (NEs) with high stability, to investigate their antibacterial activities, and to explore their potential applications on fresh produce.

Methods: NEs were prepared using BEO or LO as organic phase, water as continuous phase, and Tween 80 as emulsifier. Water was added to the organic phase containing oil and surfactant at 1:3 (v/v) ratio using a magnetic stirrer at 1,000 rpm for 10 min to form a coarse emulsion containing 6% BEO or LO. The coarse emulsion was subjected to ultrasonic emulsification using a 20 kHZ ultrasonicator (120 W) for 5 min (30 s pulses on, 30 s off). Serial two-fold dilutions of BEO or LO NEs were used to determine MIC by the broth microdilution and agar diffusion methods against *Salmonella enterica*, *E. coli* O157:H7, and *L. monocytogenes* strains.

Results: The prepared emulsion showed stability for at least 2 months even when stored at ambient conditions, without any visual evidence of creaming and phase separation. NE-LO exhibited higher bactericidal effects than NE-BEO, confirming that LO was the most effective anti-bacterial component of BEO. In NE containing 0.75% LO, *Salmonella* population was reduced by 3.5 log, *E. coli* by 2.9 log, whereas no immediate effect was observed for *Listeria* strains. The MIC of LO was 3.23 mg/mL for *E. coli*, 6.46 mg/mL for *Salmonella*, and 12.92 mg/mL for *Listeria*.

Significance: The stable formulated BEO and LO NEs have potential for application as natural antimicrobials in the food industry.

P1-35 Antimicrobial Effects of Corn Zein Impregnated with Nisin as Edible Coating for Mangoes Stored at Different Temperatures

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❖ ***Developing Scientist Entrant***

Introduction: Pathogen growth during food transport and storage can be addressed with coating fruits with natural antimicrobials. A promising strategy would be to use zein's ability to form tough coatings to support nisin's antimicrobial effectiveness.

Purpose: The objective of this study was to evaluate the potential of corn zein-nisin coatings against major foodborne pathogens on mangoes stored at different temperatures.

Methods: Serial two-fold dilutions of nisin up to 2^{10} (from 8.0 to 0 mg/mL) was performed to determine the MIC by the agar diffusion and broth microdilution methods. Zein-based coatings containing nisin (0, 0.25, 0.5, 1.0, and 2.0 mg/mL) was spread at individual areas of 15 mm diameter mango skin and dried for 1 h. The inoculation level was 10^4 CFU *L. monocytogenes*/area. Inoculated areas uncoated were used as controls. Samples were processed on days 0, 1, 3, and 7. Mangoes were stored at 22°C and 6.5°C. The skin piece was sonicated (1 min in 5 mL PBS), and vortexed (60 s). Cell suspension was spiral platted onto Harlequin agar plates. On day 7, samples were also enriched with 10 mL LEB (15 h, 120 rpm, at 37°C) followed by a loop-full streak.

Results: The MIC was 0.25 mg/mL for *L. monocytogenes*, whereas no evident effect was observed for *E. coli* O157:H7 or *Salmonella* Typhimurium. Immediately upon coating, 1 log CFU/mL reduction was observed when coating with 2.0 mg/mL of nisin. Mangoes stored for 3 and 7 days, coated with 1.0 and 2.0 mg/mL, led to lower microbial concentration than the detection limit (1 log CFU/mL) regardless of the storage temperature. After 7 days, no cell recovery was obtained for fruits coated with 2.0 mg/mL nisin even after enrichment.

Significance: These findings indicate that corn zein-nisin coatings can be a natural and relatively low-cost option for preventing growth of *L. monocytogenes* at refrigerated and room temperatures.

P1-36 Development of Applied Antimicrobial Intervention to Control *Salmonella* spp. during Wheat Milling

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Introduction: Raw flour has been linked to multiple flour-associated illness outbreaks and recalls due to *Salmonella* spp. contamination. In response, resources are being invested to understand this emerging food safety issue and to develop practical risk reduction options to mitigate this public health threat.

Purpose: Quantify the antimicrobial effectiveness of 5% lactic acid compared to water applied at different temperatures to inoculated wheat kernels during the tempering phase of milling.

Methods: Cleaned and dried HRW kernels were inoculated with a 6-strain cocktail of *Salmonella* to 10^4 or 10^6 log CFU/g and dried at 37°C to original moisture level. Then kernels were subjected to ten treatments corresponding to a factorial combination of either water or 5% lactic acid and five temperatures (ambient, 120°F, 130°F, 140°F, 150°F). Samples were collected at 0, 4, 12, 18 and 24 h post-treatment and residual *Salmonella* populations determined. The study was designed as randomized complete block with repeated measurements.

Results: Ambient water treatment resulted in a 0.4 log CFU/g reduction over 24 h of tempering at both inoculation levels, whereas ambient LA tempering reduced the *Salmonella* population by 1.2 and 1.4 log cycles at T0 for both inoculation levels. By 12 h of ambient tempering of low-level inoculated wheat, only 1 of 3 replications indicated residual *Salmonella*, while two were negative by enrichment. For the high-inoculation, a 2.4-log CFU/g reduction was observed after 12 h and no further reductions were noted up to 24 h of tempering. For high inoculation, increasing tempering temperature for LA application to 130°F resulted in >5 log CFU/g reductions by 4 h, and no *Salmonella* was detected by enrichment at 140 and 150°F at any time. LA showed significantly higher reduction than water ($P < .005$), when comparison were made.

Significance: An effective antimicrobial application for wheat taking advantage of the pre-milling tempering stage of processing would provide millers with a preventive control step to increase the safety of raw flour.

P1-37 Effects of Liquid Smoke Preparations on Shelf Life and Growth of Wild-Type Mold and *Aspergillus flavus* in a Model Semi-Moist Pet Food

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❖ ***Developing Scientist Entrant***

Introduction: Liquid smoke is a naturally derived flavor component and preservative with known antimicrobial properties. There is no available information on its antimycotic potential against storage fungi like *Aspergillus flavus* which produce mycotoxins in human and pet foods. Semi-moist pet food with high moisture content (20-30%), is susceptible to mold contamination and requires intervention.

Purpose: To determine the effects of liquid smoke preparations on wild-type mold and *Aspergillus flavus* growth in semi-moist pet food in a shelf-life study model.

Methods: Semi-moist pet food was formulated with liquid smoke preparations (S1 to S8) at 0%, 0.5%, 1%, 2% and 4% (w/w). A positive control consisted of 1% potassium sorbate and no smoke. Shelf life was estimated by storing the samples at 28°C and 65-70% RH over 30 days and recording the number of days to the appearance of visible wild-type mold. In another experiment, samples were spot inoculated with *A. flavus* (~4 log, CFU/mL), incubated at 25°C, and analyzed for fungal growth by enumeration on potato dextrose agar, at sampling intervals of 2 days over a 35-day period.

Results: Liquid smoke at 4%, 2%, 1% and 0.5% concentration extended the shelf life of samples by 16.4, 9.5, 4.8 and 3.9 days, respectively, when compared to the untreated (7.7 days). Smoke preparations S3 and S6 (high carbonyl, medium/low phenol) were the most effective in prolonging the number of days to visibly mold (26-28 days). In the challenge study with *A. flavus,* S3 and S8 (high carbonyl, low phenol) reduced ($P < 0.05$) mold counts by 2.5 logs, 1.7 logs and 1 log when compared to the untreated at 4%, 2% and 1% concentrations, respectively. Addition of smoke at 0.5% did not reduce mold counts.

Significance: The carbonyl preparations of liquid smoke were the most effective at enhancing shelf life of semi-moist pet food, and at inhibiting *A. flavus* growth.

P1-38 Effectiveness of Natural Antimicrobials for Control of Mold Growth on Artificially Inoculated Shredded Cheddar Cheese Held at 7ºC

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❖ ***Developing Scientist Entrant***

Introduction: Preservatives such as potassium sorbate and natamycin have traditionally been used to inhibit mold growth in cheese. Despite their high antifungal activity, certain limitations such as degradation of sorbate by some mold species, and poor solubility and distribution of natamycin, have led cheese manufacturers to seek alternative methods for mold control in cheese.

Purpose: The purpose of this study was to evaluate the antifungal effectiveness of selected natural antimicrobials for preventing mold growth on refrigerated (7°C) shredded cheddar cheese.

Methods: Samples of shredded cheddar cheese (pH 5.6) were mixed with the anticaking agent, vitacel FL-600 cellulose (2% wt/wt). The following natural antimicrobials were added to portions of the cheese: i) 0.5, 1, or 2% cytogard ultra (CYTO); a fermentate ii) 1, 2, or 4% inhibit violet (IV), iii) 1.5, or 3% *Yucca schidigera* extract (YEX), and iv) 20 ppm natamycin. The cheese samples were spot inoculated with conidia from *Penicillium* mold to obtain an initial viable count of ~4.0 log CFU/g and stored at 7°C for 40 days. Inoculated cheese with anticaking agent alone served as the control. During storage, cheese samples were observed every 5 days for visible mold. Viable counts of mold were determined by surface plating diluted (10-fold) samples of cheese homogenate on dichloran rose bengal chloramphenicol agar (DRBC) followed by counting mold colonies after incubation (25°C, 5 days).

Results: Mold growth was visible after just 10 days in the control cheese and in cheese treated with 20 ppm natamycin, 1% IV or YEX (1.5, 3%). In contrast, all concentrations of CYTO or IV (2, 4%) completely inhibited mold growth even after 40 days ($P < 0.05$).

Significance: CYTO, even at the lowest concentration tested (0.5%) and IV (2 and 4%) show great potential for controlling mold growth and extending the microbial shelf life of refrigerated shredded cheddar cheese.

P1-39 Exploring the Antimicrobial Efficacy of Spearmint, Peppermint, and Dill Essential Oils and Fumes

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Introduction: Characterization of behavior of foodborne pathogens in essential oil systems can support manufacturers in developing food safety plans.

Purpose: Our objectives were: 1) quantify reduction of *Salmonella* and *Listeria monocytogenes* in peppermint, spearmint, and dill oils; 2) determine minimum inhibitory concentration (MIC) of oils; 3) determine antimicrobial activity of oil fumes to provide foundational data for food safety plans in essential oil industry.

Methods: Cocktails of *Salmonella* or *Listeria* (~9 log CFU/mL) were inoculated in Tryptic Soy Broth (TSB) with 1.2% essential oil. Inoculated oils were incubated (25°C, 7 days) with enumeration by spread plating on Tryptic Soy Agar. ANOVA and Tukey's HSD were used to test for differences between oil types. For MIC determination, oil:ethanol (1:1, 0.08-4.80%) was combined in a 96-well plate with inoculated TSB (5 log CFU/mL). Plate was incubated in the FilterMax F5 (25°C, 24 hours) with optical density measured (595 nm). For oil fumes tests, filter paper was inoculated (4-6 log CFU/g) and suspended in the headspace of a 50-mL tube containing essential oil (35 mL) at ambient temperature (24 hours). Filter paper was transferred onto a TSA plate (37°C, 24 hours) and observed for growth.

Results: Spearmint and peppermint (1.2%) significantly reduced *Salmonella* and *Listeria* after 1 hour (P-value < 0.05), whereas dill (1.2%) did not cause a significant reduction until day 3 (*Salmonella*) or day 7 (*Listeria*). Populations of both pathogens recovered from mint oil exposure with continued incubation. Spearmint had the lowest MIC (0.6% *Salmonella;* 1.2% *Listeria),* whereas MICs for both pathogens were 1.2% (Dill) and 4.8% (peppermint). Exposure to oil fumes resulted in complete inactivation (4-6 log CFU/filter) on at least one filter for each pathogen-oil combination.

Significance: Dill, peppermint and spearmint essential oils and oil fumes have antimicrobial properties against bacterial pathogens*;* however, efficacy is dependent on application method and incubation conditions.

P1-40 Product Depth and Air Velocity Impact Microbial Reduction during Hazelnut Roasting

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Introduction: *Salmonella* is a primary pathogen of concern for the tree nut industry; therefore, processors must demonstrate that processes, including roasting, are effective at reducing this hazard.

Purpose: This project consisted of three objectives: 1) quantify reduction of *Salmonella* during hazelnut roasting; 2) determine relative contribution of air velocity and product depth on microbial inactivation during roasting; 3) evaluate suitability of *E. faecium* NRRL B-2354 as a surrogate for *Salmonella* for hazelnut roasting.

Methods: Hazelnuts (25 g) were inoculated with *Salmonella* and *E. faecium* (6-7 log CFU/g) and roasted at 127°C or 188°C for 6 minutes in a bench-scale oven (Cuisinart Model #TOA-60) with a convection option (air velocity: 0.44-0.94 m/s). Hazelnuts were roasted as a single layer or with a product depth of 6-10 cm. After roasting, samples (n = 5/trial) were diluted in 0.1% peptone water and spread plated on Tryptic Soy Agar. Plates were incubated at 37°C for 2 hours prior to overlaying with Hektoen Enteric Agar (*Salmonella*) or m-Enterococcus Agar (*E. faecium*) and incubation was continued to 72 hours.

Results: At 127°C, the most effective condition (single layer, high air velocity) resulted in limited inactivation: 2.23 ± 0.05 (*Salmonella*) and 1.65 ± 0.07 (*E. faecium*) log CFU/g reductions. Decreasing air velocity resulted in lower log reductions for both *Salmonella* (1.55 ± 0.07) and *E. faecium* (1.16 ± 0.07). At 188°C, inactivation was significantly improved and resulted in reductions of 4.67 ± 0.97 (*Salmonella*) and 4.79 ± 1.3 (*E. faecium*) log CFU/g. Product depth significantly reduced efficacy of thermal treatment, resulting in 0.75 ± 0.36 (*Salmonella*) and 0.58 ± 0.22 (*E. faecium*) log reductions.

Significance: Product depth and air velocity have a significant effect on the microbial reduction on roasted hazelnuts and demonstrates the importance of commercial hazelnut roasters to consider these variables when validating their process.

P1-41 Mature and Immature Biofilms of *Listeria monocytogenes* Isolated from Vermont Dairy Production Environments are Susceptible to Sodium Hypochlorite

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* is found in biofilms on surfaces and equipment in the food processing environment, particularly on stainless steel. Sodium hypochlorite (SH) is a readily available and commonly used sanitizer. However, it is inactivated by organic material, so killing cells within a biofilm may be a challenge.

Purpose: Our objective was to determine tolerance of 24-hour, 72-hour, and 10-day old biofilms from six strains to sodium hypochlorite and compare with planktonic cultures' tolerance.

Methods: Sanitizer MIC: cultures were incubated statically in 1x or 1/20x Brain Heart Infusion broth (BHI) in polystyrene microtitre plates for 24 hours (22°C) with serial dilutions of sodium hypochlorite from 6.5-1,600 ppm to determine minimum inhibitory concentrations. Sanitizer efficacy on biofilms: isolates were grown on one cm stainless steel coupons in 1x or 1/20x BHI. Coupons were incubated statically (22°C) for 1, 3, or 10 days. Media was replaced every 48 hours to prevent nutrient depletion. Coupons were rinsed 3 times with phosphate buffered saline, and placed into 0, 50, 100, or 200 ppm sodium hypochlorite for 60 seconds. Sanitizer was neutralized with Dey-Engley broth, and adherent cells were removed by vortexing with beads for enumeration. Significant differences for biofilm survival were assessed using Analysis of Variance in R (v.3.6.1).

Results: MIC for isolates grown in nutrient poor (1/20X BHI) conditions ranged from 25-400 ppm, and in nutrient rich conditions (1X BHI) from 25-1,600 ppm. In both biofilm growth conditions SH was significantly more effective at 200 than 50 ppm ($P < 0.05$). SH was more effective at day 1 than 10 at 1x BHI (2.6 vs. 1.7 log decrease), and more effective on day 3 than 10 at 1/20x BHI (3.9 vs. 3.1 log decrease) ($P < 0.05$).

Significance: Sodium hypochlorite reduces *L. monocytogenes* biofilm on stainless steel, but MICs for planktonic cells are higher than the recommended working concentration.

P1-42 Evaluating the Effect of Organic Load on Peroxyacetic Acid Measurement in a Model Flume Tank

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Introduction: Peroxyacetic acid (PAA) is an organic-based sanitizer used the treatment of produce flume water to prevent cross-contamination. Unlike chlorine-based sanitizers, PAA's organic composition can affect measurements such as chemical oxygen demand (COD) used to determine water quality. The excessive organic load measured as a result of PAA utilization could overestimate the contamination in a flume system.

Purpose: Accurately monitoring sanitizer concentration in flume tanks is of great interest to produce packers. Understanding the effect that PAA has on COD measurement will help further the use of this sanitizer and improve produce safety.

Methods: Experiments to determine the effect of organic load on PAA measurement were performed in a 35 x 58 cm laboratory model flume system. Target PAA levels of 0, 20, 40, 60, and 80 ppm were tested in a model flume system under organic loading conditions of 0, 100, and 300 ppm of added chemical oxygen demand (COD). For all experiments, PAA, COD, and ORP measurements were taken using specific portable meters and recorded. All experiments were conducted in triplicate.

Results: The addition of 0, 20, 40, 60, and 80 ppm PAA at the 0 ppm COD resulted 2, 74, 153, 248, and 338 ppm total COD values, respectively. With 100 ppm COD added resulted in values of 120, 121, 237, 321, and 414 ppm. With 300 ppm COD added, values were 264, 261, 319, 405, and 494 ppm. The results show that COD measurements while utilizing a PAA sanitizer resulted in values which indicated a lower water quality than the actual condition.

Significance: This study provides insights on how the measurement of the organic load in the model flume system was affected by the utilization of PAA. Using COD as an indicator for water cleanliness could negatively alter sanitation decisions when PAA is used as a sanitizer because of the misleading values that this sanitizer causes.

P1-43 Inactivation of *Escherichia coli* O157:H7 in Cabbage Seeds by Combined Treatments of Gaseous Chlorine Dioxide and Mild Wet Heat

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❖ ***Developing Scientist Entrant***

Introduction: Since foodborne pathogens such as *Escherichia coli* O157:H7 can exist in seeds of fresh vegetables, it is important to develop a method to inactivate foodborne pathogens in the seeds without decreasing the seed's viability.

Purpose: This study was done to develop a method to inactivate *E. coli* O157:H7 from cabbage seeds by the combined treatment of gaseous chlorine dioxide (ClO_2) and mild wet heat (60°C and 85% relative humidity [RH]).

Methods: Gaseous ClO_2 was spontaneously vaporized from a solution containing hydrochloric acid (HCl, 1 N) and sodium chlorite ($NaClO_2$, 100,000 µg/mL) in an airtight container (1.8 L). Firstly, the relationship between the amounts of HCl-$NaClO_2$ solution and the concentration of gaseous ClO_2 generated was determined. Next, cabbage seeds (5 g) were treated with gaseous ClO_2 (up to 3,000 ppm) under mild wet heat condition (60°C and 85% RH) for up to 120 min and the germination rate of the seeds was determined. Finally, cabbage seeds containing *E. coli* O157:H7 (*ca*. 7 log CFU/g) were exposed to gaseous ClO_2 gas (up to 3,000 ppm) at 60°C and 85% RH for up to min.

Results: The amount of HCl-$NaClO_2$ solution showed high correlation (R^2=0.9948) with the amount of gaseous ClO_2 in the container (y = 5687x). The germination rate (64.7 ± 6.4%) of cabbage seeds treated with 3,000 ppm of gaseous ClO_2 and mild wet heat for 120 min was not significantly reduced ($P > 0.05$) compared to that of control seeds (76.7 ± 8.3%). When the seeds were treated with 2,000 or 3,000 ppm of gaseous ClO_2 under mild wet heat condition, *E. coli* O157:H7 on the seeds was completely inactivated within 90 min.

Significance: The results of this study will provide useful information for the development of a method to inactivate foodborne pathogens from seeds using the combination of gaseous ClO_2 and mild wet heat treatments.

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P1-44 Antimicrobial Activities of Combined Treatments of Gaseous Chlorine Dioxide and Mild Wet Heat Against X*anthomonas campestris* and *Salmonella enterica*

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❖ ***Developing Scientist Entrant***

Introduction: Gaseous chlorine dioxide (ClO_2) has been known to have an antimicrobial activity, but the synergistic antimicrobial activities of combination of gaseous ClO_2 and mild wet heat on plant seeds have not been intensively studied yet.

Purpose: This study was done to develop a method to inactivate *Xanthomonas campestris* pv. *campestris* and *Salmonella enterica* on cabbage, chili pepper, and radish seeds using simultaneous treatments of gaseous ClO_2 and mild wet heat (70°C and 85% relative humidity [RH]) without decreasing seed's viability.

Methods: To determine the maximum treatment time which does not decrease seeds' viability, seeds were treated with 3,000 ppm of gaseous ClO_2 at 70°C and 85% RH for up to 60 min and their germination rates were measured. Seeds containing *X. campestris* or *S. enterica* were exposed to 3,000 ppm of gaseous ClO_2 under mild wet heat conditions (70°C and 85% RH) for up to 20 min. After treatments, the numbers of viable colonies of *X. campestris* or *S. enterica* were determined using direct plating method.

Results: The germination rates of seeds were not significantly ($P > 0.05$) changed after treatments with gaseous ClO_2 at 70°C and 85% RH for up to 20 min. The initial populations of *X. campestris* or *S. enterica* on seeds were *ca.* 6.3 or 6.4 log CFU/g, respectively. When treated with gaseous ClO_2 (*ca.* 3,000 ppm) and mild wet heat (70°C and 85% RH), *X. campestris* was completely inactivated within 20 min regardless types of seeds. For seeds inoculated with *S. enterica*, the numbers of the pathogen were decreased to below the detection limit (1.0 log CFU/g) for direct plating method within 20 min regardless types of seeds.

Significance: The results of this study may provide useful information for the method to decontaminate seeds using combination of gaseous ClO_2 and mild wet heat.

P1-45 Use of Antimicrobials and the Fate of *Salmonella* in Marinated Pork Loins

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Introduction: Foodborne disease outbreak has estimated the annual economic burden for human illness due to *Salmonella* infections in pork to be $1.9 billion. *Salmonella* is becoming an increasing concern for the pork industry despite several attempts to eliminate the pathogen in numerous critical steps. Out of 2,300 serotypes, *Salmonella* Enteritidis and *S.* Typhimurium account for almost half of all human infections in the U.S.

Purpose: The purpose of this study is to evaluate the effect of lytic bacteriophages (phage) and lactic acid (LA) in reducing the presence of *S.* Enteritidis, *S.* Montevideo, and *S*. Heidelberg on the surface and internal cores of marinated pork loins.

Methods: Locally purchased raw pork loins were sliced into 2"-2½" thick chops (n = 30/replication). Pork chops were inoculated with *Salmonella* strains' cocktail with a concentration of 10^7 CFU/mL for 30 min attachment. Inoculated chops were marinated for one hour. Marinated pork chops were randomly divided into one of the five treatments (Control, DI water, LA 2.5%, phage 5%, LA 2.5% + phage 5%) and later tenderized through a manual tenderizer. Surface swabs (50 cm^2) were taken before and after the meat tenderization. Sterile internal meat cores were homogenized and evaluated for pathogen translocation.

Results: The data indicate varied antimicrobial susceptibility patterns of *Salmonella* cocktail in marinated pork loins. Marinated loins treated with phage and LA + phage significantly ($P < 0.05$) reduced the surface and internal core pathogens. Similarly, no difference was observed on the surface pathogen counts for control, DI, and LA treated marinated loins and their subsequent internal cores.

Significance: *Salmonella-specific* Bacteriophage was able to reduce the number of surface pathogens on marinated pork loins (60 min) by more than 1.60 logs. However, the treatment itself was not enough to reduce the translocated pathogens from the surface to the internal cores.

P1-46 Control of *Listeria monocytogenes* in Model Wet Dog Foods by Using AAFCO-approved Inhibitor

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Introduction: There have been several recalls of pet foods due to contamination with *Listeria monocytogenes* and *Salmonella*. A validated cooking and proper refrigeration of finished products minimizes the *Salmonella* risks. However, *Listeria* is a common post-process contaminant that can easily grow in favorable food matrices even at refrigeration temperature. *Listeria* infections are uncommon in pets, but they are possible. People can get sick from handling contaminated pet foods. The use of inhibitors to control *Listeria* in ready-to-eat meats have been well documented. It is critical that the pet food ingredients have been accepted by FDA and adopted by the Association of American Feed Officials (AAFCO). Limited numbers of AAFCO-approved inhibitors are available commercially, but they have not been validated for *Listeria* control in a broad scope.

Purpose: Evaluate control of *Listeria* in two model meat-based wet dog foods formulated with AAFCO-approved inhibitor.

Methods: Two model dog foods formulated with turkey meat, grain varieties, dried fruits and vegetables, vitamin and mineral premix, and citric-acetic acid blend based inhibitor (0.6% w/w) were prepared by cooking, then diced or ground, and packaged in modified atmospheric pack (MAP) or non-MAP, respectively. Packaged samples were syringe inoculated with ca. 3-log CFU/g of a 5-strain cocktail of *L. monocytogenes* and incubated at 3.3°C. At every appropriate enumeration time-points (ten time-points), three inoculated samples along with two uninoculated control (pH, total-aerobic and lactic-acid bacteria counts) were evaluated. Three replications of the experiment were performed. Microbial data were log-transformed and reported as average.

Results: *Listeria* did not grow ($P > 0.05$) for 12 weeks in the diced-MAP model. In ground, non-MAP model, *Listeria* counts increased ($P < 0.05$) by 2 log CFU/g in 11 weeks. The aerobic and lactic-acid bacteria counts remained <100 CFU/g in both samples. Perhaps the difference in formulation (pH, moisture 5.74,68.1% vs. 6.05,71.4%, respectively) and gas composition inside the package affected growth. Yeast counts also increased overtime in ground, non-MAP model.

Significance: Processors can formulate pet foods with AAFCO -approved inhibitors to enhance food safety.

P1-47 In Plant Validation Study of Peracetic Acid Intervention on Whole Beef Carcasses Using *Escherichia coli* Surrogates

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Introduction: The efficacy of antimicrobials intervention during slaughter process shall be finally validated under in plant conditions. The levels of natural *Escherichia coli* on beef carcasses are very low in order to determine the real efficacy under commercial operation conditions. The use of *Escherichia coli* surrogates opens a great opportunity to validate beef carcass interventions in commercial facilities.

Purpose: To determine the antimicrobial efficacy of different levels of peracetic acid (PAA) on whole beef carcasses using proven *Escherichia coli* surrogates in a commercial beef processing plant environment.

Methods: On each repetition, 21 carcasses were railed off the processing line and sprayed on three different areas (100 cm^2) of the shank with an *E. coli* surrogate cocktail (BAA-1427, 1428, 1429, 1430 and 1431) targeting 6 log CFU/cm^2 of attachment. Samples were taken using 25 mL buffered peptone water (BPW) EZ-Reach™ swabs after 30 minutes for cell attachment, immediately after intervention, and 24 h after intervention. Treatments evaluated were PAA at 400, 600 and 800 ppm. Flow rate, pressure, concentration, and temperature were recorded for each treatment. TEMPO® system was used for *E. coli*

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enumeration. A total of three repetitions were conducted and a two-way ANOVA was performed using R (Version 4.0.3).

Results: For all tested concentrations, interventions significantly reduced ($P < 0.05$) *Escherichia coli* counts immediately after intervention and after 24 h. For 400, 600, and 800 ppm of PAA interventions, reductions were, on average, 4.62, 5.63, and 5.3 log CFU/cm^2 after intervention, respectively. There was no significant difference ($P > 0.05$) of attachment level between PAA concentrations.

Significance: The use of *E. coli* surrogate strains can become an alternative for obtaining more precise results in the effect of interventions on validation studies in commercial beef processing facilities, as well to represent more accurately the behavior of *E. coli* O157:H7 and *Salmonella*.

P1-48 Impact of Isolation Environment and Temperature on the Susceptibility of *Salmonella* to Biocides

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Introduction: Antimicrobial interventions are important mitigation strategies against *Salmonella*. However, factors that affect the susceptibility of *Salmonella* to biocides are not fully characterized.

Purpose: Evaluate the role of isolation environment and temperature on the susceptibility of *Salmonella* to biocides.

Methods: A 96-well panel of 12 biocides was designed to determine the minimum inhibitory concentrations (MIC) of these chemicals for *Salmonella*. The panel contained increasing concentrations of biocides including acetic acid (AA), citric acid (CA), lactic acid (LA), dodecyltrimethylammonium chloride (DC), benzalkonium chloride (BC), hexadecyltri-methylammonium chloride (HC), chlorhexidine (CH), trisodium phosphate (TP), acidified sodium chlorite (ASC), sodium hypochlorite (SH), sodium arsenate (ARA), sodium arsenite (ARI), silver (Ag) and Copper (Cu). Broth assay was incubated at 25°C or 37°C to evaluate the susceptibility of 55 and 62 *Salmonella* isolates from low-moisture foods (LMFs) and various meat sources, respectively.

Results: The concentration of biocide at which 50% of all isolates tested failed to grow was designated as the MIC_{50} (µg/mL) and used as a breakpoint. The MIC_{50} was 1640 for AA, 6304 for CA, 3776 for LA, 256 for DC, 20 for BC, 40 for HC, 2 for CH, 9428 for TP, 320 for ASC, 6304 for SH, 1664 for ARA, 112 for ARI, 4 for Ag, and 2048 for Cu. Isolates with MIC higher than the MIC_{50} by at least 4 folds were considered to be tolerant to the biocide. All LMF and meat isolates were equally susceptible to AA, CA, LA, DC, ASC, SH, TP and Cu. Higher percentage of meat isolates showed tolerance to BC, HC, CH, and Ag compared to LMF, while higher percentage of LMF isolates showed resistance to ARI and ARA. There was no observable effect of incubation temperature on the MIC of isolates tested.

Significance: Findings could improve the efficacy of antimicrobial interventions against *Salmonella*.

P1-49 Efficacy of Disinfectants Against Human Norovirus on Food Contact Surfaces

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Introduction: Human Norovirus (HuNoV) is particularly difficult to inactivate with commonly used disinfectants at concentrations appropriate for food contact surfaces. Recently two new surface disinfectants, Purell Surface Sanitizer (PSS) and Sink & Surface Cleaner Sanitizer (S&S), have come to market with 30 second label claims for HuNoV based on using murine norovirus (MNV) or feline calicivirus (FCV) surrogates.

Purpose: To characterize the viricidal efficacy of PSS (28.5% ethanol) and S&S (0.55 fl. oz/gal dilution, 0.06% dodecylbenzenesulfonic acid and 0.15% lactic acid), in comparison to 400 ppm QAC and 200 ppm hypochlorite, at 30 and 60 s using HuNoV GII.4 Sydney, and the cultivable HuNoV surrogate, Tulane Virus (TuV).

Methods: GII.4 Sydney positive stool suspension (20% in PBS), and semi-purified TuV cell culture lysates were used in viricidal surface assays using a modified ASTM E1053-11 protocol. HuNoV titer was evaluated using RNase treatment, followed by RNA extraction and RT-qPCR. TuV infectivity was determined by plaque assay using LLC-MK2 cells. Virus inactivation was calculated as the difference in titer between untreated and treated virus-inoculated Formica coupons by extrapolation to RT-qPCR standard curve (for HuNoV) or infectivity assay (for TuV).

Results: For GII.4 Sydney, PSS produced a 3.55 ± 0.72 and 4.03 ± 0.47 log reduction in genome equivalent copies (GEC), while S&S showed a 0.05 ± 0.14 and 0.23 ± 0.26 log reduction, after 30 and 60 s, respectively. In comparison 400 ppm QAC produced a 0.22 ± 0.05 and 0.13 ± 0.12 log reduction in GEC, while 200 ppm hypochlorite showed a 0.23 ± 0.06 and 0.31 ± 0.10 log reduction, after 30 and 60 s, respectively. Similar inactivation patterns were observed using infectivity assay with TuV, with PSS performing at a level matching the label claim, while S&S showed only minimal log reduction.

Significance: These data highlight the importance of using relevant surrogates and supplementing data with HuNoV studies to produce a more comprehensive picture of product efficacy in disinfection studies.

P1-50 Effect of Growth Conditions on the Relative Transcription of Plantaricin Genes and Antilisterial Capacity of *Lactiplantibacillus plantarum* Strains

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Introduction: Thirteen genes encoding the plantaricins, namely *plnNC8a*, *plnNC8b*, *plnK*, *plnJ*, *plnE*, *plnF, plnH, plnS, plnY, plnD, plnM, plnI* and *plnG* were detected through PCR reaction in two *Lpb. plantarum* strains. The presence of *pln423* and *plnG* was also detected in other five *Lpb. plantarum* strains.

Purpose: To assess the effect of parameters related to sourdough preparation, on the production of plantaricins and the transcriptomic response of plantaricin genes in 7 *Lpb. plantarum* strains.

Methods: Plantaricin production was assessed with the Well Diffusion Assay (WDA) against a mixture of 5 *Listeria monocytogenes* strains belonging to serotype 4b. Sourdough related parameters involved incubation temperature: 20, 30, 37°C; incubation time: 12 and 20 h; NaCl addition: 0 and 1.5%; initial pH value: 5.0 and 6.0; substrate: MRS broth, MRS broth containing wheat flour carbohydrates, wheat flour water extract supplemented with carbohydrates to the initial flour concentration; microbial strains: 7 *Lpb. plantarum* strains. The transcriptomic response of the plantaricin genes was studied with RT-qPCR.

Results: Plantaricin production ranged from 160 to 2560 AU/mL. The two bacterial strains, each harboring two plantaricin genes, exhibited high plantaricin production after growth in MRS broth, fortified with flour carbohydrates and combined with 30 and 37°C. On the other hand, the other five *Lpb. plantarum* strains, which harbored 13 plantaricin genes, exhibited the minimum plantaricin production. Analysis of variance revealed that only a marginal proportion of the variance observed could be explained by NaCl addition. Thus, these factors were excluded by RT-qPCR analysis. The latter revealed that depending on the strain, temperature and substrate had the most pronounced effect on relative gene transcription. The effect of different pH values was negligible, compared to the other parameters.

Significance: For the first time, the relative contribution of several plantaricins to the overall antilisterial capacity of *Lpb. plantarum* was assessed. This research has been co-financed by the European Union and Greek national funds, under the call RESEARCH-CREATE-INNOVATE (T1EDK-05339).

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P1-51 Evaluation of *Lactobacillus plantarum, Bifidobacterium longum* and *Saccharomyces boulardii* Attachment to Intestinal Mucosa and Inhibition of Pathogenic Microbes

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Introduction: Poultry performance and efficiency of feed utilization in poultry is highly influenced by gut health. Probiotics are viable microorganisms that beneficially affect the host animal by improving its gastrointestinal tract (GIT) microbial balance. However, environment favoring their colonization of the GIT and ability to inhibit growth of pathogenic microorganism is unclear.
Purpose: This study evaluated properties *of Lactobacillus plantarum, Bifidobacterium longum*, and *Saccharomyces boulardii* as potential probiotics in broiler production.
Methods: *L. plantarum, B. longum*, and *S. boulardii* potential to tolerate varying pH levels (pH 2 to 7) and bile concentrations (1 to 3%) in GIT of broilers was assessed. In vitro adhesion assay was also conducted to evaluate microbial attachment to intestinal epithelial tissue. QuantaTM FEG 250 Scanning Electron Microscopy was used to image bacteria epithelial attachment.
Results: *B. longum* counts were steady above 3 CFU/mL at pH 4-7 and from 0-5 hrs. Regardless of exposure time to various pH levels, *Lactobacillus plantarum* counts remained steady at 7.04-8.06 CFU/Ml. *S. boulardii* remained steady at 5.1-5.2 CFU/mL from time 0 h to 5 hrs. at varying pH levels (pH 3 -7). *L. plantarum* displayed significantly more survivability in bile than *B. longum* and *S. boulardii*. *B. longum* and *L. plantarum* showed remarkable ability to attach to the intestinal mucosa*. L. plantarum* and *B. longum* exhibited strong antimicrobial activity against*, E.coli O157:H7 Salmonella,* and *Campylobacter spp. S. boulardii* showed positive inhibition against *C. lari* and *C. jejuni*
Significance: *B. longum, L. plantarum,* and *S. boulardii* exhibited characteristics in vitro that suggest that they could be effective probiotics. Remarkable changes in the mucosal cell surface and cytoskeleton-associated with *B. longum* and *L. plantarum* attachment in vitro is evidence that these microbes inhibit the growth of pathogenic bacteria that cause challenges in the poultry industry.

P1-52 Isolation of *Listeria monocytogenes* Specific Bacteriophages and Application on Planktonic Cells and Biofilms Formed on Food Contact Surface

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❖ ***Developing Scientist Entrant***

Introduction: Bacteriophages are viruses that are ubiquitous in the environment and host bacteria. Among them, foodborne bacteria specific bacteriophages are considered a potential antimicrobial agent due to their lytic action against host cell. *Listeria monocytogenes* is a major *Listeria* species that occur listeriosis in human body.
Purpose: This study was aimed to isolate *L. monocytogenes* bacteriophages which were derived from an environment and evaluate their action as antimicrobial agent against planktonic cells and biofilms formed on food contact surfaces.
Methods: Isolate *Listeria*-specific bacteriophage from environment, confirm the host cells by host range test with efficiency of plating (EOP), measure adsorption rate and one-step growth curve, and observe morphology by transmission electron microscope. Isolated bacteriophages were mixed equal ratio to make it cocktail and incubated together with *L. monocytogenes* planktonic cells for lysis kinetics at MOI 0.1, 1, and 10 and different temperature (4, 15, and 30°C). Bacteriophage cocktail was also treated on *L. monocytogenes* biofilms formed on food contact surfaces (polyethylene [PE], polypropylene [PP], and stainless steel [SS]) to confirm biofilm reduction ability at MOI 1 and 10, and different temperature (4 and 15°C).
Results: Three bacteriophages were isolated from environment and confirmed two are myoviridae and one is siphoviridae. The treatment of mixed bacteriophages than the treatment of individual bacteriophages showed superior reduction ability against suspended bacteria and biofilms formed on the food contact surfaces. The mixed bacteriophage was able to inhibit the growth of planktonic cells for 24 hours and showed 2.05, 1.92, and 1.90 log CFU/cm^2 at 30°C, 0.86, 0.87, and 1.23 log CFU/cm^2 at 15°C, and 1.59, 1.50, 1.80 log CFU/cm^2 at 4°C reduction value of biofilm on PE, PP, and SS, respectively.
Significance: This study indicates that environment derived *L. monocytogenes*-specific bacteriophages are effective in controlling various types of *L. monocytogenes* (planktonic cells and biofilms) under refrigerated temperature to room temperature.

P1-53 Isolation and Characterization of Bacteriophages Which is Specific for *Salmonella* Thompson in South Korea

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* Thompson is a common pathogen of gastroenteritis with poultry and their bacteriophage is a naturally occurring bacterial virus that can infect certain host bacteria and considered a potential antimicrobial food intervention due to their ability to inactivate target bacteria in different environments.
Purpose: The aim of this study was to isolate, characterize, and evaluate the efficacy of phages against *S*. Thompson *in vitro*.
Methods: Two types of phages specific for *S*. Thompson were isolated from a natural environment, Han Cheon River in Ansung, South Korea. These phages were designated as CAU-STP-1 and CAU-STP-2. The host range was tested by spot test with 31 different bacterial strains. Survivability of bacteriophages were tested under pH (2-11) and temperature (20°C - 80°C). Bacteriophages adsorption rate and one-step growth curve were tested on original host strain of *S.* Thompson. Cocktail of bacteriophage was incubated together with host cell at MOI 10,000 and 30°C to conduct lysis ability.
Results: CAU-STP-1 and CAU-STP-2 bacteriophages showed the specificity against *S.* Thompson and were able to survive in wide range of pH. Both phages were resistant at 70°C for 30 min and more than 50% phages were readily adsorbed to host bacteria in 5 min. Based on step growth kinetics results, the latent period was 40 min, while the burst size was 100 PFU/cell for CAU-STP-1 and 19 PFU/cell for CAU-STP-2. Phages were able to lyse of *S*. Thompson cells at 10,000 multiplicity of infection values (MOI) in vitro.
Significance: According to lytic ability, 3.49 log CFU/mL and 2.08 log CFU/mL reduction was achieved for CAU-STP-1 and CAU-STP-2. These results suggest that bacteriophages have potential as a bactericidal agent for biocontrol of *S.* Thompson.

P1-54 Mutations Acquired on *Salmonella* Enteritidis upon Exposure to a Lytic Phage for 21 Days

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Introduction: Bacteriophage-based interventions to control *Salmonella* are getting global attention. Phage and bacteria are in constant interaction in the environment; and emergence of phage-resistant bacteria needs to be investigated, since resistance could limit the success of phage biocontrol.
Purpose: To determine and classify the emergence of mutations in *Salmonella* Enteritidis that coevolved with a lytic phage for 21 days in two culture

conditions.

Methods: *Salmonella* and a lytic phage were subjected to experimental evolution assays in two conditions, i) TSB and ii) minimal media (MM). Four replicates were conducted per assay, the mixture was transferred to fresh medium daily for 21 days. A total of 16 genomes of coevolved *Salmonella* were sequenced, these representing samples from day 1 and day 21 of the four replicates, along with controls. Filtered reads were quality trimmed and reads were further assembled with SPAdes. SNPs detection was conducted with Freebayes and GATK, finally phylogeny was conducted on the sequenced genomes.

Results: SNPs were classified based on the function of the gene that presented SNPs, being the most common found in LPS biosynthesis genes *rfbP, rfbD, rfbN,* in which effects observed represented frame shift and stop gained observed in TSB at day 21, and in MM at days 1 and 21. Membrane protein biosynthesis gene *oadA* presented SNPs with frame shift effects in both culture conditions at day 21. The majority of the SNPs were found in MM at day 21. Genomes were clustered in two clades on the phylogenetic analysis, based on the media used for coevolution.

Significance: This study shows the genetic changes observed in *Salmonella* upon coevolution with a lytic phage. SNPs founds represented potential phage receptors. This study is fundamental knowledge to understand phage resistance in *Salmonella.*

P1-55 Whole Genomic Characterization of Phage SB3 -induced *Salmonella* Bacteriophage-insensitive Mutants

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Developing Scientist Entrant

Introduction: Bacteriophages are emerging as natural antimicrobials to control the presence of *Salmonella* in foods and the food production environment. The consequences of bacteriophage induced resistance must be understood in order to increase the efficacy of bacteriophage-based antimicrobials.

Purpose: To evaluate the genetic mechanisms in Bacteriophage Insensitive Mutants (BIMs) of a *Salmonella* isolate, developed following incubation with bacteriophage SB3.

Methods: To generate BIMs, a series of 10-fold dilutions of the *Salmonella* siphophage SB3 was produced, and 100 microliters of each dilution was individually dropped on separate petri plates that contained a lawn of its host *Salmonella* Enteritidis. Following overnight incubation, colonies that formed in the clearing of each bacterial lawn were isolated and confirmed to be resistant to SB3. Whole genome sequencing of the wild type *S.* Enteritidis isolate and selected BIMs was conducted using MiSeq paired end sequencing, and sequencing reads were assembled and annotated using PATRIC. The draft genomes of three BIMS (A, B, and D) were compared to the wild type to identify mutations associated with bacteriophage resistance.

Results: Using GSAlign, single nucleotide variations (SNVs) and indels were identified in the BIMS. For example, BIM A had 21 SNVs and 2 deletions, BIM B had 22 SNVs, and 3 deletions, and BIM D had 44 SNVs. Two deletions common to mutants A and B were found in a homologous gene coding for a 68 aa hypothetical protein. One deletion (TT- > T) in mutant B was found in WbaP, which encodes an enzyme that transfers galactose from UDP-galactose to a polyprenyl carrier undecaprenyl-phosphate galactose during O-antigen biosynthesis in *Salmonella*, likely leading to a rough phenotype and increasing the permeability of the outer membrane to various antimicrobial compounds.

Significance: These results indicate that *Salmonella* BIMS likely exhibit reduced ability to survive in foods and also have reduced virulence.

P1-56 Characterization of the Potential of Lactic Acid Bacteria Isolated from Agroindustrial Waste in Costa Rica for the Production of Antimicrobial Compounds

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Undergraduate Student Award Entrant

Introduction: Agroindustrial waste is an important source of lactic acid bacteria (LAB) with biotechnological potential for biorrefinery and the production of antimicrobial compounds.

Purpose: Evaluation of the capacity of LAB isolated from Costa Rican agroindustrial waste to produce antimicrobial compounds.

Methods: Three *Lactobacillus casei* isolates from silage pineapple peel residuals in Costa Rica were used in the study. Growth curves were determined using a 96-well microplate that was filled with a constant volume (250 µL) of a suspension of each isolate (10^5) in MRS broth. Microplates were aerobically incubated at 35.0 ± 0.5°C for 24 ± 2 h in high humidity conditions and growth kinetics were determined by measuring the OD_{600} every 15 min. The cell-free supernatant of each isolate was obtained to determine the antagonistic activity against foodborne microorganisms (*Escherichia coli*, *Listeria innocua* and *Pseudomonas fluorescens*). A 96-well microplate was filled with a constant volume (50 µL) of Tryptic Soy Broth, 50 µL of the bacterial suspension (MacFarland 0.5), and variable volumes (50, 45, 40, 35, 30, 25, 20 and 15 µL) of filtered supernatant (pH adjusted to 7.0). Microplates were aerobically incubated as stated before and the absorbance at 620 nm was then measured. All determinations were performed in triplicate.

Results: The maximum growth rate of the isolates were between 0.191 and 0.266 h^{-1}. All the isolates were characterized by producing significant inhibition ($P < 0.05$) against all the surrogate bacterial strains and the effect was observed even with the most diluted sample (the exception was *L. casei* 6713 against *P. fluorescens*). Significant inhibition ($P < 0.05$) was observed against Gram positive and Gram negative microorganisms.

Significance: This result could be attributed to the production of bacteriocins considering that other metabolites (*i.e.,* lactic acid and acetic acid) were neutralized with NaOH. LAB strains isolated from agroindustrial waste could be an important source of antimicrobial compounds for food applications.

P1-57 Changes of Antimicrobial Activities of UV Irradiation Against *Staphylococcus aureus* on Plastic Surfaces as Affected by Intensity and Wavelength of UV Light

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Developing Scientist Entrant

Introduction: Ultraviolet (UV) irradiation has been known as an effective surface decontamination method, but influences of intensity and wavelength of UV light on its antimicrobial activities have not been intensively investigated.

Purpose: This study was done to determine the influence of intensity and wavelength of UV light on the antimicrobial activities of UV irradiation against *Staphylococcus aureus* on plastic surfaces.

Methods: *S. aureus* were spot-inoculated (*ca.* 7 log CFU/coupon) on plastic coupons (5 cm x 2 cm) followed by drying at 25 ± 1°C for 1 hour. The coupons were exposed to UV-B light (0.110, 0.180 and 0.320 mW/cm^2) and UV-C light (0.130, 0.180 and 0.320 mW/cm^2) generated from light-emitting diodes (LED) at 25°C and 43% relative humidity for 3 minutes and 10 seconds, respectively. After UV irradiation, the coupons were transferred to conical centrifuges tubes (50 mL) containing tryptic soy broth (30 mL) and glass beads (3 g), and vortexed at maximum speed for 1 minute to detach cells. The populations of *S. aureus* in suspensions were determined by a direct plating method.

Results: Antimicrobial activities of UV irradiation were increased as the wavelength was decreased under the same intensity. When the wavelength was fixed, the antimicrobial effects were increased as the intensity was increased. For example, when the coupons were exposed to 0.320 mW/cm^2 of UV-B and UV-C light, the populations of *S. aurues* on plastic surfaces were decreased by 4.3 and 5.2 log CFU/coupon, respectively. When the coupons were exposed

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to UV-C light for 0.130, 0.180 and 0.320 mW/cm^2, the populations of *S. aureus* were decreased by 3.6, 4.5 and 5.2 log CFU/coupon, respectively.

Significance: The results of this study may provide fundamental information in developing a method to inactivate foodborne pathogens on abiotic surfaces using UV irradiation.

P1-58 Inactivation of *Salmonella enterica* by UV-A, UV-B and UV-C Irradiation as Affected by Types of Abiotic Surfaces

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Developing Scientist Entrant

Introduction: Ultraviolet (UV) irradiation has received attention as a disinfection method on abiotic surfaces, but the influence of types of abiotic surfaces on the antimicrobial activities has not been intensively studied.

Purpose: This study was done to evaluate the antimicrobial activities of UV-A (369 nm), UV-B (303 nm) and UV-C (272 nm) irradiation on *Salmonella enterica* on glass, plastic, stainless steel, and wooden surfaces.

Methods: *S. enterica* were spot-inoculated (*ca.* 7 log CFU/coupon) on surfaces of coupons (glass, plastic, stainless steel, wood; 5 cm x 2 cm) and then dried at 25 ± 1°C for 1 hour. After drying, the coupons were irradiated (distance: 5 cm) by UV-A (369 nm, 0.800 mW/cm^2), UV-B (303 nm, 0.163 mW/cm^2), and UV-C (272 nm, 0.325 mW/cm^2) light generated from LED (100 mW) at 43% relative humidity and 25°C for up to 10, 10, and 5 min, respectively. To detach microorganisms from surfaces, coupons were transferred to conical tubes containing tryptic soy broth (30 mL) and glass beads (3 g) and vortexed at maximum speed for 1 min. The number of viable cells in the suspension was determined by a direct plating.

Results: UV-A irradiation did not significantly decrease the population of *S. enterica* ($P > 0.05$), but UV-B or UV-C irradiation reduced the number of *S. enterica* significantly ($P \leq 0.05$) on abiotic surfaces. It was observed that the lethality of UV-B and UV-C irradiation was affected by the types of surfaces. When exposed to UV-B irradiation, the number of *S. enterica* on glass, plastic, stainless steel, or wooden surface was reduced by >4, >4.3, >5.7, or 2.3 log CFU/coupon, respectively, within 10 min. For UV-C, the number was decreased by >5.6, >5.5, >5.5, or 2.3 log CFU/coupon, respectively, within 5 min.

Significance: These results may provide basic data for the development a method to inactivate foodborne pathogens on abiotic surfaces using UV irradiation.

P1-59 Comparison of Inactivation Efficacy of Plasma-activated Water Against Biofilms on Two Types of Lettuce

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Developing Scientist Entrant

Introduction: Bacterial biofilms are a major source of contamination in food processing environments and their removal has been a major challenge. Plasma-activated water (PAW) contains reactive oxygen and nitrogen species which can inactivate biofilms.

Purpose: The aim of this study was to establish a comparison of the inactivation of biofilms between romaine lettuce and iceberg lettuce by using PAW.

Methods: Lettuce samples of size 1" x 1" were dip inoculated with individual strains of *Listeria innocua* and *E. coli* DH5α to grow biofilms for 24 hours. PAW was prepared by activating deionized (DI) water with an atmospheric pressure air plasma jet for 15 minutes. The lettuce samples were incubated in PAW for 15 min. All the treatments were performed in triplicates and the inactivation efficacy of PAW against biofilms was determined by plate count and analyzed using ANOVA at $P < 0.05$.

Results: Reductions of *E. coli* biofilms by 1.7 ± 0.7 log CFU/in^2 with an initial concentration of 5.8 ± 0.3 log CFU/in^2 and 2.5 ± 1.1 log CFU/in^2 with an initial concentration of 6.5 ± 0.3 log CFU/in^2 were observed on romaine lettuce and iceberg lettuce, respectively. For *L. innocua* biofilm, PAW achieved a reduction of 2.0 ± 1.1 log CFU/in^2 on romaine lettuce when initial concentration was 5.9 ± 0.3 log CFU/in^2 while 2.5 ± 0.5 log CFU/in^2 reduction was observed in the case of iceberg lettuce when initial concentration was 6.6 ± 0.2 log CFU/in^2. Physical parameters of PAW were also measured including pH (2.3 ± 0.2), ORP (245 ± 13 mV), conductivity (185 ± 13 µS/cm), and temperature (33.9 ± 2.9°C).

Significance: PAW is a promising disinfectant for produce but the surface characteristic may affect inactivation efficacy.

P1-60 Investigation of Cross-Resistance Development between a Commercial Quaternary Ammonium Compound Sanitizer and Antibiotics in *Listeria monocytogenes* Isolated from Fresh Produce Environments

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Introduction: Listeria monocytogenes (Lm) contamination is of growing concern within the fresh produce industry. For these foods, the lack of a kill step demands emphasis on cleaning and sanitizing to mitigate the risk of cross-contamination from the processing environment.

Purpose: Assess potential for cross-resistance development between a commercial quaternary ammonium compound (cQAC) sanitizer and clinically relevant antibiotics.

Methods: Minimum inhibitory concentrations (MICs) of a cQAC against 11 Lm from fresh produce handling environments were assessed using microbroth dilution method in tryptic soy broth with 0.6% yeast extract. Strains were experimentally adapted to cQAC with 1 µg/mL increasing increments in concentrations (1-6 µg/mL). Adaptations to at least 1 µg/mL higher than the wild-type (w.t.) Lm MIC were stabilized by five passages at the respective concentration. Antimicrobial resistance (AMR) profiles were determined prior to and following strain adaptation using disk diffusion assay (CLSI) for 13 antibiotics (amikacin; ampicillin; cefoxitin; chloramphenicol; clindamycin; gentamicin; kanamycin; rifampin; co-trimoxazole; novobiocin; penicillin; streptomycin; ciprofloxacin). AMR of adapted and their respective w.t. strains were compared.

Results: MICs ranged from 3-4 µg/mL for all strains tested. Previously obtained genomic data showed 2/11 strains possessed QAC efflux pump-associated genes (qacC, ebrB), likely contributing to their increased MIC (4 µg/mL) to cQAC. There was no change in Lm susceptibility to amikacin, ampicillin, rifampin, co-trimoxazole, cefoxitin, or gentamicin, whereas 10/11 adapted isolates with initial intermediate resistance in w.t. to penicillin, kanamycin, ciprofloxacin, novobiocin and/or clindamycin were reclassified as resistant. A change in AMR from susceptible to intermediate was noted in 4/11 Lm for chloramphenicol and/or streptomycin.

Significance: The results highlight the potential for cross-resistance between cQAC and antibiotics; however, no cross-resistance between antibiotics typically used to treat listeriosis (e.g., amikacin, gentamicin) and cQAC provides confidence in the continued use of these antibiotics as listeriosis treatment options.

P1-61 Comparison of Planktonic Cells and Biofilms of Pressure-Stressed and Wild-Type Bacterial Pathogens of Food Industry Significance

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Introduction: While the vast majority of microbiological challenge studies are conducted using planktonic cells of bacterial pathogens, epidemiological studies indicate that as high as 80% of bacterial infections could be associated with bacterial biofilms.

Purpose: The current presentation provides an overview of recent microbial challenge studies performed in Public Health Microbiology laboratory for determining decontamination efficacy of common commercially-available sanitizers against planktonic and biofilm cells of foodborne bacteria. Pressure-stressed and wild-type phenotypes of the pathogens are additionally compared.

Methods: Presented studies are complete randomized block designs with two biologically independent repetitions as blocking factors, investigating effects of common commercially-available sanitizers against planktonic cells as well as one-week, two-week, and three-week mature biofilms of nontyphoidal *Salmonella* serovars, *Listeria monocytogenes*, Shiga toxin-producing *Escherichia coli*, and *Cronobacter sakazakii*.

Results: Vast majority of planktonic cells were reduced ($P < 0.05$) by >99.9% and/or to the detection limit after one-minute exposure to common industrial sanitizers such as sodium hypochlorite and quaternary ammonium compounds. Same treatments tested against one-, two-, and three-week mature bacterial biofilms were considerably less efficacious, leaving behind >2 log CFU/cm^2 of bacterial survivors. Pressure-stressed and wild-type phenotypes of the pathogens exhibited comparable ($P > 0.05$) biofilm formation and sensitivity to sanitizers. This indicates that a validated process against wild-type cells could almost certainly eliminate the pressure-stressed phenotypes as well.

Significance: Our results indicate that while commercial sanitizers are very efficacious for the elimination of planktonic cells, they have considerably lower efficacy for the elimination of bacterial biofilms. This major difference between the sensitivity of planktonic and sessile cells thus would need to be considered for developing successful SSOPs for products' food safety plans that are under the legislations of Hazard Analysis Critical Control Point and Food Safety Modernization Act.

P1-62 Occurrence and Distribution of Antibiotic-resistant *Staphylococcus aureus* in a Brazilian Pork Production Chain

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Introduction: The pork production chain is highly susceptible to contamination by staphylococci. Monitoring staphylococci contamination in the pork production chain may help understand the degree of hazard from this important group of bacteria.

Purpose: We characterized the distribution and the antibiotic resistance of staphylococci from a Brazilian pork production chain.

Methods: Samples (n = 1,114) from pig farms, pig lots and slaughterhouses, located in two Brazilian states (Minas Gerais and Paraná), were subjected to coagulase-positive *Staphylococcus* (CPS) enumeration. *S. aureus* isolates (n = 251) from this collection were further characterized for their resistance to oxacillin, cefoxitin, vancomycin and tetracycline through phenotypic and molecular assays.

Results: CPS counts from pig farms were higher when compared to other samples ($P < 0.05$). Other counts were relatively low, but present in all production stages. *S. aureus* isolates were commonly resistant oxacillin and cefoxitin (74.0%, 54/73), qualifying them as methicillin-resistant *S. aureus* (MRSA), but PCR assays indicated that few harbored the expected antimicrobial-resistance genes (*femB*, *mecA* and *mecC*). Vancomycin and tetracycline resistance were identified at lower frequencies (6.8% to 37.0%). Sensitivity (34.5% to 86.7%) and specificity (26.6% to 85.0%) of PCR for detecting antibiotic resistance genes varied based on the assessed antibiotic.

Significance: Antibiotic resistant staphylococci are widely distributed in the Brazilian pork production chain. MRSA can become a potential health and economic impediment for the Brazilian pork industry. Acknowledgments: CNPq, CAPES and FAPEMIG.

P1-63 Ciprofloxacin Resistance in *Salmonella enterica* Isolated from a Poultry Chain

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Introduction: Antimicrobial-resistant salmonellosis is a worldwide public health challenge, and poultry are a primary reservoir for the pathogen *Salmonella enterica*. The antibiotic ciprofloxacin is used to treat bacterial infections including salmonellosis. Widescale use of the veterinary analog, enrofloxacin, likely contributes to proliferation of ciprofloxacin-resistant strains of *S. enterica*.

Purpose: To characterize ciprofloxacin resistance and associated mechanisms for a panel of *S. enterica* isolated from poultry in Minas Gerais, Brazil.

Methods: The minimal inhibitory concentration (MIC) of ciprofloxacin was assessed for *S. enterica* isolates (n = 96) obtained from different steps of a poultry production chain (bird cages, slaughtering and chicken cuts). PCR was used to detect plasmid-borne quinolone resistance genes (*qnrB* and *qnrS*). Mutations in quinolone resistance-determining regions (QRDR) from *gyrA*, *gyrB* and *parC* were detected by high-resolution melting (HRM), based on comparison to a control sequence. Amplicons from selected isolates were sequenced to characterize the detected mutations.

Results: Most *S. enterica* (n = 94) presented resistance or intermediary resistance to ciprofloxacin (MIC ³ 0.125 µg/mL). *qnrB* was detected in all resistant isolates while *qnrS* was not detected. HRM temperature varied from 82.4 to 83.1°C for *gyrA*, 85.1 to 86.3°C for *gyrB* and 84.7 to 85.1°C for *parC*, and sequencing indicated no mutations for *gyrA* (n = 8 isolates), silent mutations for *gyrB* (n = 4 isolates) and a sense mutation (Thr57Ser) for *parC* (n = 3 isolates).

Significance: Ciprofloxacin resistance is highly prevalent for *S. enterica* isolates collected from poultry in Minas Gerais, likely explained by the presence of plasmid-encoded QnrB. These findings highlight the potential challenges for treating foodborne salmonellosis and the potential that poultry industry antibiotic use practices contribute to this challenge. Acknowledgments: CNPq, CAPES and FAPEMIG.

P1-64 Characterization of Antimicrobial Resistance in Indicator Bacteria (E. coli and Enterococcus spp.) from Surface Waters of Wyoming

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❖ ***Developing Scientist Entrant***

Introduction: Water bodies are important reservoirs for the emergence and dissemination of antibiotic resistant (AMR) bacteria and AMR genes (ARG), with exchange of ARGs potentially occurring in this environment among pathogenic and non-pathogenic bacteria. As this may direct the emergence of AMR genotypes and phenotypes in bacteria, including foodborne pathogens, extensive characterization of types and levels of resistance in bacteria from agricultural and recreational surface water is needed to help inform AMR mitigation strategies.

Purpose: To identify and characterize priority antimicrobial AMR phenotypes in fecal indicator bacteria (*E. coli* and *Enterococcus spp.*) used in assessment of water quality from surface waters of Wyoming.

Methods: Water samples (500 mL) were collected from seven different sites along the Laramie River. For presumptive enumeration and identification of *E. coli* and *Enterococcus* spp., respectively, U.S. EPA Method 1603 and 1600, were used. Confirmation was done via MALDI-TOF MS (Bruker MicroFlex, Biotyper RTC software Version 3.1). Antimicrobial susceptibility was determined using broth microdilution (Sensititre) and Gram Negative or Positive AST plate formats.

Results: A total of 543 isolates of *E. coli* and 287 isolates of *Enterococcus spp.* were processed for identification by MALDI-TOF MS, with (543/543) were confirmed to be generic *E.coli* and (280/287) identified as *Enterococcus spp.* AMR was often observed in *E. coli* isolate subsets, with 51% AMR isolates recorded (60/117, $P < 0.05$), and the majority of isolates showing resistance to tetracycline (52/117), ampicillin (23/117), chloramphenicol (20/117), sulfamethoxazole (14/117), amoxicillin (11/117), cefoxitin (11/117), ciprofloxacin (3/117), and ceftriaxone (2/117), while 16% (13/80) of *Enterococcus spp.* contained resistance to at least one antimicrobial, namely penicillin (10/80), erythromycin (9/80), vancomycin (6/80), and quinupristin/dalfopristin (1/80).

Significance: Our results revealed that priority AMR phenotypes were common among indicator bacteria from surface waters of Wyoming, indicating the need to control environmental inputs of AMR.

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P1-65 Use of Surrogate Bacteria for Cold-fill Processes Validation and Verification

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Introduction: In the dressings industry, most sauces are cold filled unless the quantity of low acid ingredients is greater than 10-20%, depending on particle size. These products are mostly shelf stable because of the fall in acid. There is currently no validation method at industrial scale for cold fill in the dressing and sauce industry. Validation of cold-fill processing currently consists in validating the reduction of pertinent pathogens of concern when stored at specific temperature conditions. These challenge studies are conducted with pathogens and spoilage organisms at a specific temperature by third party labs, which implies long and costly works and long product holding times. Alternative validation methodologies should be evaluated to pass through these inconveniences.

Purpose: The objective of the study was to evaluate the appropriateness of a surrogate candidate for use in validation/verification of cold-fill systems.

Methods: Two food matrices (ketchup, ranch dressing) were independently inoculated with a dry ready-to-use surrogate preparation derived from *Enterococcus faecium*, and three pathogenic organisms (*Salmonella*, *Listeria* and *E. coli* 0157:H7). Inoculated foods were distributed into sterile containers, stored at 25°C and enumerated at 0, 1, 2, 3 and 7 days. For each condition, 2 independent replicates were performed.

Results: *Listeria* is the most resistant pathogen in each matrix tested. In ranch dressing, surrogate and *Listeria* show respectively 1.3 and 1.7-log reduction after 3 days and are non-detectable after 7 days. In ketchup, surrogate shows 4.7-log reduction after 2 days, and is non-detectable after 3 days while *Listeria* is already non-detectable at day 1.

Significance: These first findings show promising results for using surrogate methodology to validate and verify microbial lethality of cold-fill matrices storage. Additional experiments must be performed to strengthen these results.

P1-66 Uptake and Redistribution of *Bacillus cereus* Spores in Kombucha Systems

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❖ ***Developing Scientist Entrant***

Introduction: Kombucha has a high acidity level and a high microbial population from the SCOBY that deters most foodborne pathogens from surviving in the product. However, there may be a chance for spore-formers to persist within the beverage, causing foodborne illness.

Purpose: This study will determine if *Bacillus cereus* spores incorporated into kombucha SCOBYs can survive and be passed onto the daughter SCOBY or finished product.

Methods: *B. cereus* spores (1,000) were added directly to each SCOBY (n = 3), sweetened tea (pre-fermentation), or the finished product in triplicate. Treatments were fermented at 25°C until pH ≤3.0 was achieved. Portions of the SCOBY or liquid were enriched in Tryptone Soy Polymyxin (TSP) broth, spread-plated on Mannitol Yolk Polymyxin (MYP) agar (incubated at 32°C for 18 hours). Plates were assessed for the presence of *B. cereus*. Results were analyzed by MANOVA in R with a Tukey's post hoc test.

Results: There were no significant differences ($P \leq 0.05$) in likelihood of spore recovery between the inoculation methods or in the presence of *B. cereus* in the daughter SCOBY or the broth. Raw data reveals few positives indicating spread is sporadic but possible. Equal survival is seen in the daughter SCOBY and the broth. There was no survival after long-term storage or secondary fermentation, indicating that implementation of a holding step may mitigate potential food safety threats.

Significance: These data show that *Bacillus cereus* contamination, while rare, may be possible, especially for homebrewers with limited sanitation control and lack of standardized production methodology. The hygienic handling of kombucha cultures and raw materials is crucial to prevent uptake of pathogenic organisms.

P1-67 Assessing the Microbial Variability and Chemical Composition in Kombucha during Repeated Brewing Cycles and Refrigerated Storage

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❖ ***Developing Scientist Entrant***

Introduction: Kombucha, a fermented beverage, is known for its health benefits. These may include toxin degradation, probiotic, antioxidant, and antimicrobial properties. Production requires SCOBYs that self-propagate for use in several sequential brewing cycles. Changes in microbial composition and resulting metabolites with repeated culture usage and prolonged storage however are unknown. Moreover, the number of repeated brewing cycles is not standardized. These may result in loss of functional properties or reduced consumer acceptability. There is a need to evaluate the effect of repeated brewing cycles and storage duration on the microbial community and their metabolites.

Purpose: To evaluate the reproducibility, stability, and functional components of microbial communities and chemical compositions in kombucha products over repeated brewing cycles and storage time.

Methods: Twelve SCOBYs were obtained from commercial suppliers (n = 9) and homebrewers (n = 3). Each SCOBY was brewed following a simplified standard recipe for 10 repeated cycles. Samples were collected at pH 3 and stored at 4°C for analysis at predetermined time points (2, 6, and 12 weeks). Samples were enumerated for total aerobic bacteria, LAB, AAB, and yeast on TSA, MRS, ABS, and APDA media respectively. HPLC was used for simultaneous detection of the major organic acids, sugars, and alcohol in the kombucha sample. Target analytes were identified by comparing retention times with corresponding analytical standards. Data was subject to ANOVA, MANCOVA, and mapping for visualization.

Results: The microbial population and chemical profile of kombucha were affected by both repeated brewing cycles and storage. There was an increase in LAB (5.3-6.7 log CFU/g) and AAB (5.8-6.5 7 log CFU/g) comparing the first and tenth brewing cycles. At the 12-week storage, these populations reduced within a range of 0.7-1.4 log CFU/g. Ethanol concentration and sucrose hydrolysis into component monosaccharides increased with increasing storage time.

Significance: These data suggest that the composition and functionality of kombucha changes over repeated brewing cycles and storage implying a need for standardization.

P1-68 Control of Spoilage Microorganisms in Cold Mix and Cold-filled Salad Dressings and Condiments

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Introduction: Lactic acid bacteria and yeast are well known as causative spoilage microorganisms in acidified and acid foods including salad dressing, condiments, soft drinks, and liquid syrup products. Spoilage by these microorganisms may involve slime, turbidity, off-flavors, and gas production in the product. Cold-filled and mixed products may be exposed to environmental spoilage as well as microbial loads from ingredients. A challenge study is conducted to evaluate the control of these organisms by formulation.

Purpose: The purpose of this study was to determine the outgrowth of heterofermentative lactic acid bacteria and yeast in six different salad dressings (pH<4.0) and five different condiments (pH<4.0) during storage at 30°C for up to 9 months.

Methods: Six salad dressings (5 fruit-based and one barbeque) and five condiments (mustard, vinaigrette, mayonnaise-based) were separately inoculated with acid-adapted cocktail cultures of heterofermentative lactic acid bacteria and yeast to achieve an initial inoculum level of 3-4 log CFU/g. One

lot of each product was formulated to represent the worst-case pH. The dressings and condiments were held at 30°C for 9 months and were analyzed in triplicate per time point using plate count methods to evaluate the growth of the inoculated target organisms. One replicate of the uninoculated sample was evaluated for lactic acid bacteria, yeast, and mold at each pull time. Failure was defined as the growth of spoilage organisms. Growth was considered as 1 log or greater increase in counts compared to inoculum levels.

Results: The inoculated heterofermentative lactic acid bacteria counts decreased to <10 CFU/g when stored at 30°C by week 3 and were maintained at this level over the 9 months of storage. The inoculated yeast counts decreased to <10 CFU/g when stored at 30°C by week 1 and were maintained at this level over the 9 months of storage.

Significance: The results demonstrated the stability of all the evaluated dressings and condiments against spoilage-causing organisms. The formulations can withstand normal production variability and prevent economic loss.

P1-69 Determination of 5-Log Reduction of Acid Tolerant Pathogens in Cold-filled Sauces

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Introduction: Acidified foods are defined as low-acid food products to which acid or acid food ingredients have been added. The final pH value for acidified foods must be ≤ 4.6. FDA requests scientific support for cold-fill products to demonstrate the reduction of non-sporeforming pathogenic bacteria.

Purpose: To determine the 5-log reduction of acid-tolerant *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes* in Ketchup-based sauces with different levels of acetic acid stored at 18°C, and in a soy-based sauce stored at 10°C and 18°C.

Methods: Four Ketchup-based sauces (Version 1-4) and one soy-based sauce, were separately inoculated with acid-adapted cocktails of *Salmonella* spp., *E. coli* O157:H7 and *L. monocytogenes* to achieve a level of 6-7 log CFU/g. The Ketchup-based sauce versions 1, 2, 3, 4 had acidity levels of 1.02%, 1.19%, 1.4%, and 1.51%, respectively, and pH levels of 3.59-3.60.

Results: Average counts of *Salmonella* reduced by >5 log CFU/g by day 1 in all Ketchup-based sauces. *L. monocytogenes* demonstrated an average 5-log CFU/g reduction by day 1 or 2 depending on the version. *E. coli* O157 demonstrated >5-log CFU/g average reduction by day 7 in version 1, day 5 in version 2 and 3 and by day 3 in version 4. In the soy-based sauce, >5-log CFU/g average reduction of *Salmonella* was observed by day 1 and 2 at 18 and 10°C, respectively. *L. monocytogenes* demonstrated >5-log CFU/g average reduction in 2 days at both temperatures. *E. coli* O157 H7 demonstrated >5-log CFU/g average reduction by day 2 and 10 at 18 and 10°C, respectively.

Significance: The level of acidity had an impact on the 5-log CFU/g reduction times of *E. coli* O157 H7 in the Ketchup-based sauces. In the soy-based sauce, storage temperature affected the 5-log CFU/g reduction times of *Salmonella* and *E. coli* O157:H7 and did not impact *L. monocytogenes*.

P1-70 Development of *D*- and *Z*-Values for Shiga Toxin-producing *Escherichia coli* in Cheesemilk to Reduce Pathogen Risks in Cheese Made with Unpasteurized Milk

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❖ ***Developing Scientist Entrant***

Introduction: Several US cheese varieties can be legally produced using raw milk, but certain varieties have been shown to allow survival of Shiga toxin-producing *Escherichia coli* (STEC) beyond the 60-day aging requirements. Thermization (i.e., sub-pasteurization) has been proposed to reduce the risk of STEC in these cheeses. However, the temperature-times needed to enhance safety have not been well characterized.

Purpose: To determine and validate *D*- and *z*-values of STEC in cheesemilk at thermization temperatures 60.0°C, 62.8°C, and 65.6°C.

Methods: Non-homogenized, pasteurized whole milk was inoculated with 8-log CFU/mL STEC (7-strain mixture; O157:H7 plus 6 non-O157 strains). One-mL samples were vacuum-sealed in moisture-impermeable pouches and heated to test temperatures via waterbath submersion. Duplicate samples were removed from heating at appropriate times and immediately cooled in an ice bath. Surviving STEC were enumerated by plating on Sorbitol MacConkey agar overlaid with Tryptic Soy agar to aid in the recovery of heat-injured cells. Duplicate trials were conducted and survival data used to calculate thermal inactivation rates. Predicted times to 3-log reduction were validated in triplicate trials for each test temperature using 100 mL cheesemilk heated with constant stirring in a water bath, with 2 mL pipetted into chilled tubes and immediately cooled at beginning, middle, and end of heating before enumeration.

Results: *D*-values of 60.0 ± 7.4, 16.9 ± 1.3, and 7.2 ± 0.7 s at 60.0, 62.8, and 65.6°C, respectively, were observed, corresponding to 3-log reductions in 3:00, 0:51, and 0:21 (min:sec), and an overall *z*-value of 6.1°C (R^2 = 0.98). Results from the validation study confirmed STEC reduction to a less-than-detectable limit of <0.48 log CFU/mL in 94, 12-25, and 5-10 seconds for test temperatures of 60.0, 62.8, and 65.6°C, representing fail-safety of the generated *D*-values.

Significance: These data can be used to develop a flexible thermal process for artisanal raw milk cheese to reduce STEC populations to levels where they are not infectious to consumers.

P1-71 Shifts of Microbiota during Cheese Production: Impact on Quality and Safety

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❖ ***Developing Scientist Entrant***

Introduction: Understanding the microbiome of cheese is significant in the dairy industry because the microbiota contributes to the physicochemical, quality, and safety of cheese.

Purpose: The purpose of this review is to understand the microbiota of different types of cheese and delineate the influence of the microbiota on cheese quality and safety.

Methods: Microbiome data during the processing of six different types of cheese were collected; Mexican artisanal cheese, Italian artisanal cheese, Mozzarella cheese, Brazilian artisanal cheese, Cheddar cheese, and Edam cheese. The samples of each cheese were collected from milk, curd, whey, and cheese during aging. With the collected data, the microbial diversity of each cheese throughout the cheese production was observed and specific microbes of each step of the cheese making process were examined. Foodborne pathogens related to outbreaks in cheese were discussed to prevent potential threats.

Results: The microbiome of the six different types of cheese appeared highly diverse then became significantly less diverse after starting culture was added. The cheese using natural starter cultures (Mexican artisanal cheese, Italian artisanal cheese, traditional mozzarella cheese, and Brazilian artisanal) resulted in less standardized microbiota than cheese using commercial starter cultures (Cheddar cheese and Edam cheese). The microbiota of raw milk cheese (Italian artisanal cheese) contained undesirable bacteria such as *Pseudomonas*, *Serratia*, and *Staphylococcus* that cause spoilage due to proteolytic and lipolytic enzyme activity. However, during the aging step of the cheese-making procedure, the abundance of the spoilage bacteria decreased and starter culture bacteria (*Lactococcus lactis* and *Leuconostoc mesenteroides*) were predominant. An *E. coli* O157:H7 outbreak associated with a Gouda cheese raised a concern about the effectiveness of the antimicrobial activity of lactic acid bacteria.

Significance: The result obtained from the microbiome of different types of cheese processed with different starter cultures and pasteurized or unpasteurized milk will contribute to the dairy industry to produce cheese that meets both quality and safety.

P1-72 Characterization of the Microbiome Present in Established Biofilms Collected from Dairy Environments

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Introduction: The presence of foodborne pathogens in food industry is often associated with the formation of biofilms. The microbiota present in these biofilms may determine the fate of the pathogens as a result of symbiotic but also antagonistic interactions.

Purpose: This study aimed to characterize the microbiome of the dairy environments where established biofilms could be found.

Methods: Fifty-four environmental samples were collected in two artisanal dairies, characterized by the production of a raw cow's milk cheese, using scrapers and swabs focusing on previously hygienized surfaces and covering approximately 400 cm^2 each. Biofilm disruption was promoted using cation exchange resin during a high-speed shaking. The samples were then centrifuged and the supernatant filter sterilized. The DNA in the cell pellets was extracted and used for enumeration of the bacterial load by qPCR. The DNA was also used for analyzing the microbiome using a 16S rRNA gene amplicon sequencing approach followed by further bioinformatic downstream analysis. Biofilm detection was based on presence of at least 2 of the 3 main matrix components: Proteins, detected by precipitation, followed by SDS-PAGE and silver staining. Exopolysaccharides using a phenol-sulfuric acid method. eDNA, detection made by spectrophotometry after precipitation.

Results: Overall, 23 samples were found to have a bacterial load of >1 BCE/cm^2. The remaining 31 samples had <1 BCE/cm^2. In these 23 samples it was possible to detect the three matrix components in 11, only 2 components in other 11 and for one sample only 1 matrix component was detected. When analyzed the microbiome of the samples, the most prevalent genera detected were *Staphylococcus*, *Psychrobacter*, *Brevidobacterium*, *Halomonas* and *Corynebacterium*. Among the environments sampled, the ones associated with ventilation systems showed high microbial diversity.

Significance: This study highlights the importance of knowing the composition of the microbiota in dairy facilities as it contributes to clarify the types of niches available for foodborne pathogens.

P1-73 Safety, Technological and Functional Characterization of Lactic Acid Bacteria Isolated from Sheep Milk and Dairy Products

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Introduction: Autochthonous LAB isolated from milk and dairy products to be used as technological or protective cultures are often first evaluated apart from their technological properties, also for the absence of potential virulence factors and the lack of antibiotic resistance traits.

Purpose: To phenotypically assess the safety, technological and *in vitro* functional potential of 189 LAB strains isolated from 5 sheep milk and 4 dairy products.

Methods: The ability to acidify milk was tested in skimmed milk agar, and the antimicrobial activity against mixture of *Listeria monocytogenes*, *Salmonella* and *Escherichia coli* O157:H7 strains. Proteolytic and lipolytic activity, along with their resistance to ampicillin, bacitracin, chloramphenicol, erythromycin, vancomycin, tetracycline, novobiocin and streptomycin were assessed with well-diffusion assay. LAB isolates were screened for their tolerance to low pH (1, 2 and 3/ 3h) and bile bovine (0.5, 1 and 2% w/v/ 4h) as well as their ability to hydrolyze bile bovine. Finally, their ability to produce exopolysaccharides, gelatinase and biogenic amines and the cholesterol-lowering potential of LAB strains were investigated.

Results: Among the LAB tested, 62 strains showed rapid milk acidification and 64 strains presented weak proteolytic activity. Production of proteinaceous antimicrobial compounds against *L. monocytogenes* and *E. coli* O157:H7 were detected in 14 and 4 strains, respectively. All strains presented resistance to at least one antibiotic, while indicating highest sensitivity to ampicillin. None of the strains survived exposure to pH 1, 25.9% of the strains survived exposure to pH 2 and 88.8% to pH 3. Population reduction was greater than 0.5 log units for 6, 10 and 15 strains after exposure to 0.5, 1 and 2 % (w/v) bile bovine, respectively. None of the others previously mentioned properties were detected.

Significance: These data highlight the potential of using isolated LAB as starters provided that further research concerning the evaluation of their health risks takes place. This research has been co-financed by the European Union and Greek national funds, under the call RESEARCH-CREATE-INNOVATE (T1EDK-05339).

P1-74 Pasteurization of Ice Cream Evaluated Using a Chemiluminescent Assay Measuring Alkaline Phosphatase Activity

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Introduction: Several assays that measure alkaline phosphatase (ALP) activity are available to measure pasteurization of dairy products. The first colorimetric assays are less sensitive than chemiluminescent assays that are available. A chemiluminescent assay approved by National Conference on Interstate Milk Shipments (NCIMS) for fluid dairy products was evaluated for use with ice cream.

Purpose: This study used a sensitive chemiluminescent assay to measure ALP in ice cream to determine pasteurization effectiveness.

Methods: A chemiluminescent assay approved by NCIMS to determine pasteurization of fluid milk products was evaluated for use with ice cream. The assay uses a handheld luminometer to measure ALP activity. Several types of ice cream were evaluated including homestyle vanilla, vanilla bean and strawberry ice cream spiked with eight levels of raw commingled milk from 0.002% to 0.5% and ALP activities were measured in triplicate. Melted ice cream was diluted 1:1 in pure water prior to assay. The luminometer was calibrated using pasteurized ice cream as the blank and pasteurized ice cream spiked with 350 mU/L alkaline phosphatase as the positive control. A flocked sample handle provided in the kit was dipped into the diluted ice cream for 3 s and then the sample handle was placed in an assay sampler which contained the reagents required for the luminescent reaction. After a 2 min incubation period, the amount of light generated was detected using the luminometer and reported in mU/L.

Results: For all ice cream varieties, the results were linear from 0.002% to 0.5% raw milk with all correlation coefficients > 0.99. Limit of detection of raw milk ranged from 0.002% to 0.004% depending on ice cream variety.

Significance: A chemiluminescent assay approved for evaluation of pasteurization of fluid milk products was evaluated for use with ice cream. Acceptable performance of the assay was demonstrated for three types of ice cream.

P1-75 Rapid Quantification of *Enterobacteria* in Raw Milk Using Real-Time PCR Methods

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Introduction: We developed a new, highly sensitive, real-time PCR kit for quantification of *Enterobacteriaceae* in raw milk and milk products with a multiplex kit with a lypholized user-friendly format. The kit is the **food**proof® *Enterobacteriaceae* plus *Salmonella* Detection LyoKit.

Purpose: Raw milk can be contaminated with *Enterobacteriaceae* and these organisms are often used as microbial hygiene and quality indicators during milk production. Challenges for *Enterobacteriaceae* quantification is ensuring reagents are free of any contaminating foreign DNA that can result in a high false-positive signal. By developing a new method for decontaminating the reagents, we can significantly reduce this contamination while preserving quali-

ty, stability, and efficiency at the level more sensitive than traditional microbiology.

Methods: DNA extraction and amplification was based on the **food**proof® StarPrep Three Kit and **food**proof® *Enterobacteriaceae* plus *Salmonella* Detection LyoKit. Reagent D was used during extraction to bind extracellular DNA to discriminate live cells from dead cells. We spiked matrices (e.g., Growing up milk and BPW) with 106 cells/mL of *Cronobacter* to compare Cq values of untreated and reagent D treated samples. Also, we spiked raw milk with live *Escherichia coli* (DSM 3008310) to test for sensitivity and recovery.

Results: We developed a process to produce contamination-free PCR reagents. Untreated PCR mix had significantly more positive signals from extracellular DNA templates contaminating the reagents compared to treated and reduced detection of up to > 6 log (approx. ΔCq 20) dead cells. The Limit of Detection (LOD 95) for *Enterobacteriaceae* in raw milk samples was 0.8 genome copies (GE) per reaction.

Significance: We develop a rapid and convenient method for detection and quantification of *Enterobacteriaceae* and *Salmonella* in raw milk and milk products by producing and confirming DNA free PCR reagents. Finally, we quantified live vs dead cells in samples with a method more sensitive than traditional microbiology.

P1-76 Demonstration of Hygiena High Sensitivity SuperSnap ATP Surface Monitoring as an Excellent Proxy Alternative Method for Remediation of True Allergens from Surfaces during Cleaning Verification

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Introduction: Use of swabs to collect and quickly verify allergen levels from surfaces has been expanding becoming more relevant recently. Using true specific allergen lateral flows is being complimented using high sensitivity ATP swabs as proxy measurements for surface cleaning efficiency.

Purpose: To demonstrate dilution levels capable of being detected and concomitant true allergen, ATP and protein levels in a survey of complex food matrix dilutions dried onto surface. This allows users to run verifications with lateral flows but also regular cleaning verification using high sensitivity ATP swabs.

Methods: Forty-one food matrices across 10 different allergen proteins used to generate dose response curves by producing a 10% suspension which was then diluted from -1 down to -7 in a decreasing decimal dilution series. These dilutions were then dried onto stainless steel surface and swabbed (*n* = 3) using Hygiena SuperSnap and allergen specific ELISA tests. The allergens covered were peanut, hazelnut, sesame, pistachio, almond, crustacea, soya, egg, cashew, milk and gluten.

Results: ATP levels were detected in 100% of food tested, true allergens in 83% and non-specific protein in 97%. The frequency of lowest detectable level in each dilution using each analyte was as follows: (ATP)(Allergen)(Protein) – 10% dilution – 4, 3, 5 for 1% dilution – 5, 2, 32 for 0.1% dilution – 10, 4, 3 for 0.01% dilution – 17, 7, 0 for 0.001% dilution 5, 14, 0 for 0.0001% - 0, 4, 0 for 0.00001% dilution – 0, 0, 0. The frequency histogram shows peaks for ATP and true allergens at (-4) 0.01% food to (-6) 0.0001% dilution, with a distribution behind this level. The protein content shows a distribution from (-1) 10% to (-3) 0.1% food dilutions.

Significance: The use of ATP measurements as a complimentary measurand with true allergens can be advocated due to the level of dilution from complex food matrices achievable.

P1-77 Recovery of Gluten Residue from Environmental Swabs Following Specific Storage Times and Temperatures

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❖ ***Developing Scientist Entrant***

Introduction: Environmental swabs are used in food facilities to validate cleaning procedures and ensure foods are free from unintentional allergens. Swabs can be sent to third-party laboratories for evaluation, but the effect of swab storage time and temperature on the reliability of results is unknown.

Purpose: Two commercial ELISA kits were utilized to determine allergen recovery from swabs spiked with known amounts of gluten which were stored at different temperatures for a predetermined number of days.

Methods: Neogen Environmental Swabs were spiked with 10, 25, 50, and 100 ppm gluten and stored at room temperature (RT), 37, 4, and -20°C for 0, 1, 3, 5, 7, 10, and 14 days. Following this, swabs were analyzed using the Neogen Veratox® for Gliadin R5 kit and the Morinaga Wheat/Gluten (Gliadin) ELISA kit II. The percent recovery of gluten for all data points was calculated.

Results: Swabs spiked with 10 ppm gluten were below the limit of quantification (BLQ) with the Veratox kit, from day 3-14 with one or more of the replicates. However, the Morinaga kit was able to detect 10 ppm gluten across all days of storage. Swabs stored at RT and 37 °C and tested with the Veratox kit presented a 20-30% decrease in recovery with time while swabs stored at 4 and -20 °C did not show a significant decrease in recovery with time. With the Morinaga kit, a less than 10% decrease in recovery was observed from day 0 to 14 for each concentration across all four temperatures.

Significance: Swabs should be stored and transported at refrigerated or lower temperatures to ensure optimum recovery and detection of gluten residue. These results can be useful for both the food industry and testing laboratories when shipping and analyzing swabs for the presence of gluten.

P1-78 Effectiveness of a Dry-Cleaning Strategy for Removal of Milk and Egg Powder from a Continuous Mixer/Auger System

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Introduction: Shared processing equipment in dry manufacturing facilities can pose allergen cross-contact risks if appropriate dry-cleaning strategies are not implemented.

Purpose: This study evaluated the effectiveness of a push-through cleaning method using flour to purge egg or milk powder from a continuous mixer/auger system.

Methods: Nonfat dry milk (NFDM; 500 g) or whole egg powder (WEP; 500 g) was conveyed through a continuous lab-scale mixer/auger system comprised of a hopper, tee and screw conveyor. All-purpose flour (9,000 g) was then added to the hopper to push-through residual allergen remaining in the system (2-10 g). Flour samples (~88 g) collected at one-minute increments as they exited the unit were homogenized and analyzed in triplicate for milk or egg concentrations by ELISA (Neogen Veratox for Total Milk or Egg). Push-through trials were completed in triplicate for each allergen. Visual inspection and qualitative allergen-specific lateral flow devices (LFDs; Neogen Reveal 3D tests for milk or egg) were also utilized for residual allergen determination.

Results: Egg concentrations in flour decreased as a function of the amount of push-through. The final flour samples obtained after 9,000 g of push-through contained <LOQ, 11 ppm (18% CV) and 4.5 ppm (3% CV) egg in the triplicate trials. Preliminary trials with NFDM indicate that less than 9,000 g of push-through flour was required to achieve flour samples having <2.5 ppm milk. This suggests that NFDM was easier to flush from the system than WEP. However, when the system was disassembled, NFDM or WEP residue was visibly present or detectable by LFDs on the auger and other inner surfaces of the unit after the push-through trials were completed.

Significance: Push-through with flour was successful in reducing residual NFDM and WEP from the mixer/auger unit and aided in reducing allergen cross-contact. However, full unit disassembly was required to ensure complete allergen removal.

P1-79 Occurrence of Aflatoxins in Edible Vegetable Seeds and Oil Samples Available in Retail Markets and Estimation of Dietary Intake in Consumers

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Introduction: The contamination of food and food products with aflatoxins (AFs) is a global food safety concern. AFs are recognized as dangerous and toxic natural compounds. The subclass of AFs, e.g., Aflatoxin B_1 (AFB_1), is recognized as the most toxic and carcinogenic. The fungi like *Aspergillus flavus*, *Aspergillus nomius*, and *Aspergillus parasiticus* are the primary producer of aflatoxins.

Purpose: In Pakistan, no previous reports have recorded the occurrence of AFs in edible oils. However, a high incidence of AFs in feed samples (cereals products) was reported and recently in animal feed. Therefore, the study has designed to investigate AFB_1 and total AFs in edible vegetable oil samples, compare the levels with European Union recommended limits, and estimate dietary intake evaluation in the local population.

Methods: A total of 744 samples of vegetable seeds and oil (soybean, sunflower, canola, olive, corn, and mustard) were collected for the presence of AFB_1 and total aflatoxins (AFs). The samples were analyzed using HPLC with fluorescence detector.

Results: The maximum mean of AFB_1 and total AFs in non-branded soybean seeds was 21.01 ± 4.70 and 36.37 ± 6.10 µg/kg, respectively. Furthermore, all samples of edible seeds have concentrations of AFB_1 greater than the proposed limit of European Union (EU, 2 µg/kg) and 12 (7.40%) samples of branded seeds and 14 (7.40%) samples of non-branded seeds were found in the range ≥ 50 µg/kg. About 78 (43.3%) samples of branded edible oil and 103 (48.3%) sample of non-branded edible oil were observed to be positive with AFs, and the elevated average of AFB_1 (14.29 ± 2.51 µg/kg) and total AFs (25.61 ± 7.50) µg/kg were found in non-branded soybean oil samples. Furthermore, 16 (8.88%) and 6 (3.33%) samples of branded vegetable oil have levels of total AFs in a range (21 - 50 µg/kg) and ≥ 50 µg/kg, respectively. The highest dietary intake was found in non-branded sunflower oil sample (0.90 µg/kg/day) in female individuals (16-22 age group).

Significance:

The findings have indicated significant difference of AFs levels between branded and non-branded vegetable oil samples ($t = 22.274$ and $p = 0.000$) at $\alpha = 0.05$ and significant difference of AFs levels in vegetable seeds and oil samples ($t = -17.75$, $p = 0.000$) at $\alpha = 0.05$. All samples of oil have levels of AFB_1 higher than European Union limit i.e. 2 µg/kg.

P1-80 Effects of High Hydrostatic Pressure on Allergenicity and Fish Protein of Mackerel

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Introduction: High hydrostatic pressure (HHP) is a non-thermal technology that can be applied to achieve food safety via non-heating pasteurization for food preservation. However, there is little information in the literature about the effect of HHP on the allergenicity of marine fish such as mackerel.

Purpose: The objective of this study is to investigate the effects of HPP treatment on the allergenic properties and protein content of mackerel meat.

Methods: The allergenicity with monoclonal antibody against fish allergen and protein content of mackerel meat extract by HHP treatment (200 MPa and 600 MPa for 1 to 15 min, respectively) were studied. We treated the mackerel meats at 200 and 600 MPa for 1 for 0, 1, 3, 5, 10, 15 min at 20℃, used SDS–PAGE to separate the proteins, measured protein content by Lowry method, and determined fish allergens by ELISA with monoclonal antibody.

Results: After 200 MPa for 1 to 15 min, the allergenicity of mackerel extracts was not significantly different ($P > 0.05$) as compared to control (untreated sample). In contrast, the samples treated with 600 MPa for 1 to 15 min showed significantly ($P < 0.05$) lower allergenicity than control or 200 MPa samples. After HHP at 200 MPa for 1 to 15 min, there was no significant difference in SDS-PAGE profile between control and 200 MPa samples. However, the protein contents in SDS-PAGE bands of 600 MPa samples were drastically diminished and seemed to decrease with increased pressure and time.

Significance: This result showed that 200 MPa of HHP did not change the allergenicity and protein content of mackerel meat, whereas those were significantly decreased after 600 MPa treatment. In summary, 600 MPa of HHP can reduce allergenicity in mackerel meat.

P1-81 Continued Monitoring of PFAS in U.S. Food Supply

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Introduction: Per- and polyfluoroalkyl substances (PFAS) are a group of man-made chemicals, known to bio-accumulate, persist in the environment, and cause adverse health effects in humans. Water used for feed crops or live animals may be contaminated from industrial sources of PFAS and could enter the food supply via contaminated meat. In a government-wide effort, the Food Safety and Inspection Service (FSIS) is working with Federal partners at the Food and Drug Administration (FDA), Environmental Protection Agency (EPA), and Agricultural Research Service (ARS), to monitor the food supply for PFAS contamination.

Purpose: FSIS will continue monitoring of the U.S. beef supply and to extend the monitoring to other major food species as part of the National Residue Program (NRP), as outlined in the FSIS FY2021 annual sampling plan.

Methods: FSIS developed and validated a method that screens, confirms, and quantifies PFAS compounds in beef muscle and plasma, using UPLC-MS-MS quantitation. The method includes 16 PFAS analytes and has an analytical range of 0.50 – 125 ng/g. The method has been extended to include three new species – swine, poultry, and *Siluriformes* – and implemented across all three FSIS Field Service labs through a novel equipment efficiency strategy.

Results: Throughout 2020, FSIS used this method on beef muscle samples collected at slaughter to monitor for PFAS and has tested over 2,100 samples to date. After the extension to other species, FSIS will test over 1,000 samples throughout 2021. Few positive samples (4 beef samples) with low-level concentrations (all under 1 ppb) have been found. Results from this monitoring program are presented.

Significance: FSIS continues working with Federal partners to test for PFAS compounds in the U.S. food supply. Using this proactive and exploratory program helps FSIS identify and evaluate a potential public health concern in the meat supply arising from these environmental contaminants that have garnered significant public attention.

P1-82 Application of a Universal ELISA Method to Detect Aflatoxin B1 in Diverse Commodities with Optimized Extraction Procedures

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Introduction: Aflatoxins are toxic metabolites known to cause cancer, immune disorders, and even death, and therefore, it's of paramount importance to accurately estimate the level of aflatoxin contamination in agricultural commodities, which can exhibit high matrix effects.

Purpose: To optimize extraction of aflatoxin B1 for over twenty commodities for quantification using the Aflatoxin B1 Low Matrix ELISA.

Methods: Bulk commodities, such as beans, rice, flours, corn starch, cereal milk, dextrose, glucose, puffed food, jams, fructose syrup, maltose, oil, oatmeal, glucose, white sugar, xylitol, tapioca starch and yogurt, were obtained from the local market and were finely ground. Each sample was confirmed negative for aflatoxins, then spiked with aflatoxin B1 at known concentrations and extracted using different solvents for detection by ELISA, which is a competitive immunoassay. Optimized extraction procedures were validated, and % recoveries were calculated along with % CV. Sample extracts were diluted and the dilution factor was considered to estimate the amount of aflatoxin in the sample.

Results: Ground samples were spiked at 5 ng/g and/or 20 ng/g followed by extraction using 70% methanol or 90% methanol or 50% acetonitrile or 80% acetonitrile. Extraction procedures were developed and validated using Helica™ Aflatoxin B1 Low Matrix ELISA assay. Overall recovery was in the range 84 -

122%. At least three independent extractions for each of the commodity were performed, which resulted in % CV in the range of 1-11%, demonstrating the repeatability of the method.

Significance: The aflatoxin B1 low matrix ELISA is a universal assay fit to ensure food safety because it can accurately and reliably quantify aflatoxin B1 from such disparate sample types, which were successfully tested and validated using the optimized extraction procedures.

P1-83 Comparison of Two Commercial ELISA Kits on Their Efficacy of Detecting Fish Proteins from Nine Different Fish Species Using Two Extraction Buffers

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❖ ***Developing Scientist Entrant***

Introduction: Fish is an important source of healthy nutrients and its consumption is increasing; however, minimizing cross-contact of fish residue on shared equipment is critical for those with fish allergy. Several commercial fish ELISA kits detect parvalbumin which has been identified as a major fish allergen. However, one limitation of these methods is the ability to detect a diversity of commercially important and commonly consumed fish species.

Purpose: The objective was to evaluate the ability of two commercial fish ELISA kits to detect and quantify nine commonly consumed fish species.

Methods: Nine commonly consumed fish species (cod, pollock, herring, salmon, tuna, skate, tilapia, grouper, and halibut) representing a broad range of taxonomies were selected. Proteins were extracted from fish fillets using two extraction buffers (Phosphate Buffered Saline (PBS, pH 7.4) and PBS with sodium sulfite (PBS-S, pH 7.4)) in triplicate. SDS-PAGE, Western-blotting, ELISA, and 2D-Quant methods were used for protein characterization and quantification. Commercial fish ELISA Kit A (LOQ: 4-100 ppm fish (cod)) and Kit B (LOQ: 1-27 ppm fish protein) were evaluated.

Results: Western blotting indicated that antibodies from the two kits strongly recognized a protein (~12 kDa) in cod, pollock, herring, and salmon, but showed weak or no recognition with the remaining fish species. Consistent ELISA results were observed with Kit A for only cod and pollock, although percentage recovery was overestimated with both buffers (~240-650%). In comparison, Kit B showed a better detection ability. The percentage recovery from Kit B ranged from ~70-150% for four fish species with both buffers. However, both kits failed to detect skate which was in agreement with the Western-blotting results.

Significance: These results indicate the limitations of two commercial ELISAs to detect a range of commonly consumed fish species. Therefore, it is important to develop a reliable method capable of detecting fish across commercially important fish species.

P1-84 Performance Verification of an ELISA-based Assay and a Rapid Lateral Flow Immunoassay for Specific Quantification and Detection of Almond Protein in Food Matrices, Clean-in-Place (CIP) Rinse Water and Environmental Samples

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Introduction: Almond is a tree nut and a highly used food ingredient that can cause food allergy. Products containing almonds may be processed utilizing shared equipment, increasing the risk of cross-contact contamination. Cleaning verification and food testing are important elements for assessing allergen control management. Thus, effective tools for food allergen analysis are required.

Purpose: To evaluate the performance of two specific protein immunoassays for qualitative and quantitative detection of almond proteins in food, CIP and environmental samples.

Methods: An ELISA method and a rapid lateral flow (LF) immunoassay were evaluated for cross-reactivity and capability for quantitative and qualitative detection of almond proteins from different sources. Foods (n = 22) were first screened and spiked in duplicate with almond to determine the protein recovery and detection after sample extraction. Cross-reactivity was assessed in 38 different commodities. Recovery was also assessed in CIP rinse water (n = 3) and swabs (n = 3). For LF method the probability of detection was determined in water and food samples and for ELISA accuracy and linearity were also evaluated.

Results: Almond protein standard was used to construct a 4-parameter logistic curve which enabled quantification of almond protein in the range of 1-27 ppm with a precision of <10% CV. The ELISA method showed linearity in samples containing raw and processed almonds. Cross-reactivity was not detected in most of the evaluated food commodities except Brazil nuts which showed a slight cross-reaction with ELISA. The probability of detection by LF was 2 ppm of almond protein in food. Recovery of almond proteins by ELISA was above 80%, aligned to AOAC SMPR requirements, and detection of almond proteins at 2 ppm was achieved by LF in all spiked foods.

Significance: Almond protein-specific ELISA and LF are reliable analytical methods for specific quantification and detection, respectively, of almond proteins in a variety of matrices in the food industry.

P1-85 Performance Verification of an ELISA-based Assay Fish Allergen in Asian Matrices

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Introduction: Fish is included in human consumption around the world and contained many vitamins and minerals for healthy diet. In addition to milk, eggs, peanuts, tree nuts, soy, wheat and seafood, fish is counted among the most frequent triggers of IgE-mediated food allergies. Fish allergy prevalence could range from up to 7%. And is more frequent in Asia.

Purpose: There are many ingredients in each Asian food and condiments. As there are changes of recipes in manufacturing plant, there is a possibility of fish allergen found in matrices that do not contain fish. Fish allergy consumers could only depend on food allergen labels to determine. In this study, several Asian food and condiments are bought from supermarket to analyze for Fish allergen.

Methods: The following are bought from a local supermarket. Food such as vanilla mix, tomato pizza sauce, plain flour (that do not fish allergen), Oyster sauce, Korean hot chili sauce that labelled "fish allergen present," rice cakes, luncheon meat, sambal chili sauce that do not declare fish allergen are bought. We tested all the matrices using Fish ELISA test kit. The Non – detectable matrices are fortified to spike with 4 ppm of fish that is of LOQ level of the kit and 12 ppm which is 3 times of LOQ level. Matrices which are positive are prepared in serial dilutions to confirm is of true positive.

Results: The recovery of fish spike fortification is between 80-100%. The RSD is <10%. Oyster sauce, Korean Chili sauce and sambal chili sauce is found to be positive. Luncheon meat and rice cakes are found to be negative.

Significance: The AgraQuant® Fish Allergen plus offers a rapid and reliable tool for testing fish allergen in simple food and complex Asian food matrices.

P1-86 Performance Verification of an ELISA-based Assay Fish Allergen on Different Environmental Surfaces and Clean-in-Place (CIP) Rinse Water

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Introduction: Fish is included in human consumption around the world and contained many vitamins and minerals for healthy diet. In addition to milk, eggs, peanuts, tree nuts, soy, wheat and seafood, fish is counted among the most frequent triggers of IgE-mediated food allergies. Fish allergy prevalence could range from up to 7%. And is more frequent in Asia.

Purpose: There are many fish ingredients in each Asian food and condiments. As there are changes of recipes in manufacturing plant, there is a possibility of fish allergen found in matrixes that do not contain fish. The causes are mainly due to insufficient cleaning and testing of allergens between change of recipes. In this study, we determine the recovery in spiked rinses and recovery on swabs on different surfaces, steel, Teflon coated tray and plastic.

Methods: The rinse is fortified with 4 ppm of fish spike that is of LOQ level of the kit and 12 ppm which is 3 times of LOQ level. Each of the three surfaces was sectioned into 18 cm x 25 cm. Spike solutions were applied using micropipette to each of the 3 surfaces and left to dry for 2 hours at room temperature. The AgraQuant® Allergen swabbing kit is used to swab on the surface and AgraQuant® fish allergen is used to tested on the recovery of fish allergen.

Results: The recovery of fish spike fortification is between 80-100%. The RSD is <10%. Recovery of the 3 surface is between 80-90% % and RSD <10%.

Significance: The AgraQuant® Fish Allergen and AgraQuant® Allergen swabbing kit offers a rapid and reliable tool for testing fish allergen presence in environment such as rinse and surface.

P1-87 Performance Verification of an ELISA-based Assay Milk in Chocolate Matrices

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Introduction: Cow's milk allergy is defined as an immune-mediated response to proteins in cow's milk that occurs consistently with ingestion. It is one of the most common food allergies in early life with an estimated prevalence in developed countries ranging from 0.5% to 3% at age 1 year. Even though most children eventually outgrow their allergy to milk, milk allergy is also among the most common food allergies in adults.

Purpose: One of the most common ingredients in chocolate is milk. During manufacture of even dark chocolate, milk might be an ingredient as well. There is also a possibility of milk contamination in the dark chocolate even though it is not listed as an ingredient. In this study, dark chocolates with different milk declaration are bought from supermarket and analyzed.

Methods: Four different brands of dark chocolate, the first that is declared milk-free, the second that do not declare, the third that stated "traces of milk " and the fourth that listed milk as the ingredient. All these are analyzed with AgraQuant® Milk Allergen to determine the milk presence. The non-detectable milk dark chocolate is fortified with milk spike at at LOQ level of 0.4 ppm and 3x LOQ at 1.2 ppm level to determine the recovery.

Results: The results for milk-free dark chocolate is <LOD level, and the recovery of milk spike fortification is between 80-100%. RSD is <5%. Results for the rest of the dark chocolate yield positive results. The dark chocolate that listed milk as the ingredient yielded results over the quantification range of The AgraQuant® Milk Allergen.

Significance: The AgraQuant® Milk Allergen offers a rapid and reliable tool for testing milk allergens in complex matrices like dark chocolate.

P1-88 Evaluation of Allergens in a Survey of Frozen Meals and Meals Ready-to-Eat (MREs)

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Introduction: Multiplex immunoassay, an efficient way to determine multiple allergens simultaneously, was evaluated and the results compared with singlet ELISA for complex matrices such as frozen meals and meals, ready to eat (MREs).

Purpose: To determine the number of undeclared allergens as well as allergens declared but not verified by immunoassay in frozen meals and MREs from a market survey.

Methods: A total of 113 frozen meal types and 24 MREs were purchased in duplicate. The entire edible portion of each frozen-meal or the main course of each MRE was homogenized. The homogenized samples were extracted with PBS-0.05% Tween. A 7-plex array was used to detect soy, egg, cashew, hazelnut, milk, peanut, and shrimp allergens. ELISAs capable of determining individual allergens (singlet ELISA) were also used to determine allergens in food samples. The results were analyzed statistically using the Bland-Altman approach.

Results: Both the 7-plex and the singlet ELISAs accurately detected cashew, peanut and shrimp allergens relative to product label declarations. However, for soy, egg, and milk, both assays failed to detect labeled antigens in MREs and frozen food. Undeclared allergens were detected for egg in 1.8% and for soy in 7.1% of frozen meals. Labeled allergens were not detected in 0.9% of milk, 4.4% of egg, and 15% of soy allergens in frozen meals. Both the 7-plex array and singlet ELISAs performed poorly on the analyses of MREs ranging from no correlation for egg ($r = 0$) to marginal correlation ($r = 0.5976$) for milk antigens. The singlet ELISA returned higher concentrations to all allergens in comparison to 7-plex ELISA.

Significance: Both 7-plex and single allergen ELISAs detected undeclared allergens and failed to detect declared allergens. More accurate methods are needed to assay allergens in processed foods such as frozen meals and MREs.

P1-89 USDA-FSIS Validation of Sodium Chloride Replacement in Biltong Marinade to Achieve >5-Log Reduction of *Salmonella*

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❖ ***Developing Scientist Entrant***

Introduction: Biltong is a dried beef product manufactured by salt, spice, and vinegar marination followed by moderate temperature drying and therefore requires validated process approval by USDA-FSIS.

Purpose: This study aimed to evaluate the use of alternative salts, potassium chloride (KCl) and calcium chloride ($CaCl_2$), in biltong marinade which the USDA-FSIS does not accept unless an adequate log reduction of the pathogen of interest is demonstrated (2- or 5-log reduction depending on the process).

Methods: Beef pieces (1.9 cm x 5.1 cm x 7.6 cm) were inoculated with a five-serovar mixture of *Salmonella*, vacuum-tumbled in a traditional biltong marinade comprised of spices (coriander, black pepper), 4% 100 grain vinegar, and 2.2% salt (NaCl, KCl, or $CaCl_2$) and then dried in a humidity controlled oven for 8-10 days at 23.9°C and 55% relative humidity. Microbial enumeration of *Salmonella* was conducted post-inoculation, post-marination, and after 2, 4, 6, 8, and 10 days of drying. Sodium, calcium, and potassium ion concentrations were measured using ion-specific electrode meters. Trials were performed in duplicate replication with triplicate samples per time period and analyzed by RM-ANOVA.

Results: Biltong produced with $CaCl_2$, NaCl, or KCl achieved a > 5-log reduction of *Salmonella* after 6, 7, and 8 days, respectively, with water activity < 0.85. Biltong processes made with NaCl or $CaCl_2$ were not significantly different ($P < 0.05$) but both were significantly different from biltong made with KCl ($P > 0.05$). Regardless of the salt used in the marinade, potassium ion levels were moderately elevated in all samples and was attributed to potassium found in beef.

Significance: KCl or $CaCl_2$ used in place of NaCl in biltong marinade was able to achieve >5-log reduction of *Salmonella* to satisfy USDA-FSIS process conditions. The data provided herein with these alternative salts will enable manufacturers to produce a safe, healthy, and low-sodium RTE dried beef product for consumers.

P1-90 Performance Evaluation of the Hygiena™ BAX® System for *E. coli* O157:H7 in Tree Nuts

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Introduction: *E. coli* O157:H7 contamination is typically associated with ground beef and leafy greens. However, a multistate outbreak in hazelnuts reported within the last decade highlighted the ability of this organism to adapt and colonize new and diverse surfaces.

Purpose: In this study, the performance of two real-time PCR assays were compared to the U. S. FDA BAM reference method for the detection of *E. coli*

O157:H7 in experimentally contaminated tree nuts.

Methods: In two separate matrix studies, almonds and pecans were inoculated with *E. coli* O157:H7 at two target levels: a low level of 1 MPN/25 g and a high-level of 10 MPN/25 g. Prior to enrichment, samples were equilibrated at room temperature for two weeks. Unpaired samples were then prepared for either the test method (n = 30) or the FDA BAM method (n = 30) using different preenrichment protocols (BPW or mBPWp + ACV, respectively). Test method samples were analyzed with 2 PCR assays and all samples were confirmed according to the procedures in the FDA BAM Chapter 4A.

Results: For almonds, *E. coli* O157:H7 was detected in 13/20 low-spiked and 5/5 high spiked samples. For pecans, a 1:50 dilution was required before lysis which resulted in positives for 17/20 low-spiked and 5/5 high-spiked samples. Both PCR assays were identical, and all presumptive results matched culture with 100% sensitivity and 100% specificity. For the method comparisons, the difference in the probability of detection (dPOD) indicated no significant differences between the test and reference method.

Significance: Overall, the results between the BAX® System method and the FDA BAM reference method were indistinguishable, allowing tree nut processors to utilize a rapid and reliable PCR method for screening *E. coli* O157:H7.

P1-91 Effect of Flow Rate on *Salmonella* Removal in a Simple Model Peanut Butter Push-Through System

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Introduction: *Salmonella* contamination in nut butter processing equipment remains a concern to public health. Purging has been examined for *Salmonella* removal, but the effect of flow rate remains unknown.

Purpose: To examine the effect of flow rate on *Salmonella* reduction during peanut butter push-through cleaning of a simple model system.

Methods: A section of stainless steel piping, ID 9.7 mm, was contaminated with *Salmonella* inoculated creamy peanut butter. The inoculated piping was attached to a stainless steel assembly and connected to a peristaltic pump with silicone tubing. Uninoculated peanut butter was pre-heated to ~60°C and pushed through at 220.8 mL/min or 878.4 mL/min. At set time points (n = 6), ~5 g samples were collected from the exit. Samples were enumerated on TSAYE and XLD. At time 0 and 60 seconds, contaminated piping was wiped to a visual clean using a Kimwipe and the inside of the piping was swabbed and plated for enumeration. Experiments were conducted in triplicate.

Results: Initial inoculation was 7.98 ± 0.92 and 7.79 ± 1.10 log CFU/g on TSAYE and XLD, respectively. After 60 s at 220.8 mL/min there was 5.82 ± 0.92 and 5.99 ± 1.52 log CFU/g of reduction in *Salmonella* population on TSAYE and XLD, respectively. After 60 s at 878.4 mL/min there was 4.94 ± 0.77 and 5.01 ± 1.18 log CFU/g of reduction in *Salmonella* population on TSAYE and XLD, respectively. No significant difference in log reduction (P > 0.05) was observed at 220.8mL/min than at 878.4 mL/min after 60 s. *Salmonella* was not eliminated at the end of the 60 seconds push through at either speed.

Significance: Significant increase in flow rate did not lead to improvement in push-through efficacy. Though *Salmonella* was greatly reduced during 60 seconds of peanut butter push-through, it was not totally removed from the system.

P1-92 Efficacy of UV-C Treatment to Inactivate *Salmonella* on Seeds, Treenuts and Their Flours

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Introduction: *Salmonella* is a known concern in low moisture foods; however, lack of sufficient validation data limits the applicability of alternative intervention strategies such as UV light to mitigate the risk.

Purpose: To determine the efficacy of UV-C treatment for eliminating *Salmonella* on seeds, treenuts and their flours at different water activity levels.

Methods: A 10 g of seeds (inshell sunflower and pumpkin and their kernels), treenuts (Almond and Hazelnut) and their respective flours were wet seed inoculated with nalidixic acid-adapted *Salmonella* spp., (3-strain) to a target concentration of ~ 10^7 CFU/g. The samples were equilibrated to a target water activity of 0.5, 0.7 and 0.9 ± 0.05 in an environmental chamber at 21°C. The equilibrated samples were arranged either as a monolayer or 1 cm thick (to improve efficacy of treatment) then subjected to UV-C treatment at 10 to 25 mW/cm^2 for up to 60 min. The log survivors were enumerated by plating on both selective (XLDN) and non-selective (TSAN) media. The data will be analyzed by ANOVA using SPSS.

Results: A reduction of 1.56 and 1.72 log CFU/g was observed on inshell sunflower and pumpkin seeds after 60 min treatment at 12.5 mW/cm^2 and 0.5 water activity. No significant difference in the reduction was observed between inshell sunflower/pumpkin seeds and their kernels. Similarly, a reduction of up to 2.5 log on tested treenuts and <1 log on flour samples was observed at 12.5 mW/cm^2. Data from the ongoing studies indicate a further increase in log reduction by increasing the UV-C light density and sample water activity.

Significance: UV-C treatment showed promise to reduce the *Salmonella* population on tested low water activity foods and sample water activity seems to impact treatment efficacy.

P1-93 Consumption of Raw Flour in the U.S.: Results from the 2019 FDA Food Safety and Nutrition Survey

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Introduction: In the past decade, several outbreaks of Shiga toxin-producing *E. coli* (STEC) associated with flour and products containing flour have raised concerns that the consumption of raw flour represents a significant public health risk as a vehicle of foodborne pathogens. The extent to which consumers know and understand that they should not consume raw flour is unclear.

Purpose: This study examines reported U.S. consumer behavior and perceptions about consuming raw flour.

Methods: In the Fall of 2019, FDA collected data on raw flour knowledge, perceptions, and self-reported behaviors via the Food Safety and Nutrition Survey (FSANS), a national probability survey of U.S. adults (18+). The FSANS used addressed-based sampling, "mail-push-to-web" procedures. Frequencies, cross-tabulation, and ANOVAs were used to analyze the data (n = 2,171).

Results: Thirty-five percent of respondents reported having consumed something with uncooked flour in it in the last 12 months. Responses differed significantly by demographics with females (41%), Whites (42%), college graduates (42%), those with higher incomes (42%), and those in the 18 to 29 age category (47%) consuming uncooked flour more than other categories. On average, only 13% of respondents indicated that uncooked flour is likely to contain germs that can make people sick, with significant differences noted by demographic categories. Thirty-two percent of respondents rated raw homemade cookie dough as likely to have germs that can make people sick, with significant demographic differences.

Significance: These data suggest that U.S. consumers are largely unaware that raw flour is risky to consume, and a sizeable number are consuming products that contain raw flour.

P1-94 Fate of *Salmonella* and Shiga Toxin-producing *E. coli* (STEC) on Soft Wheat Kernels during Tempering

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❖ ***Developing Scientist Entrant***

Introduction: Outbreaks of *Salmonella* and Shiga toxin-producing *E. coli* (STEC) linked to wheat flour led to increased interest in characterizing the fate of these pathogens on wheat grain during processing. Tempering, where water is added to wheat kernels to increase the moisture content before milling, has the potential to facilitate microbial spread. While the amount of water added to the kernels is small, the intention is that water coats the kernel

and is slowly absorbed, effectively increasing the a_w from ~0.5 to ~0.7. Published data for generic *E. coli* indicates that the tempering process can lead to increased bacterial numbers.

Purpose: This study aimed to quantify changes in pathogen numbers on inoculated wheat during tempering.

Methods: Soft wheat kernels were inoculated with four *Salmonella* strains and six STEC strains at 6 log CFU/g. The lab-scale tempering process was conducted using 50-g aliquots following AACC method 26-95 to achieve a final moisture content of 15% after 16 hours. A_w was measured, and pathogens were enumerated by plating at eight time points over 16 hours. Uninoculated wheat was also tempered, and total plate count and coliform counts were measured at each time point.

Results: The a_w increased from 0.51 ± 0.01 to 0.81 ± 0.04 during the first half-hour after the addition of water, changes in *Salmonella* cell density ranged from -0.13 to +0.10 log CFU/g while changes in STEC cell density ranged from +0.06 to +0.27 log CFU/g. Over the 16 hours of tempering, the a_w increased by 0.25 ± 0.13, and the cell density for *Salmonella* ranged from -0.24 to 0 log CFU/g, changes in STEC cell density ranged from -0.51 to +0.14 log CFU/g.

Significance: The data show minimal changes in *Salmonella* or STEC numbers during tempering. Future research will determine if bacterial cells localize to the kernel crease during tempering.

P1-95 Assessment of Consumer Flour Thermal Treatments on the Reduction of Salmonella

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✦ ***Undergraduate Student Award Entrant***

Introduction: Increased public awareness of risks associated with raw flour and products containing raw flour, such as raw cookie dough, has resulted in online consumer resources offering home-scale solutions for reducing pathogens in such foods. However, there is limited evidence validating the efficacy of these treatments.

Purpose: The purpose of this study was to determine the efficacy of home-scale raw flour heat treatments on the reduction of *Salmonella* in a variety of flour types.

Methods: An online search for home-scale instructions for flour heat treatment informed the experimental design. All-purpose, whole-wheat, and gluten-free (rice-based) varieties of flour were inoculated with *Salmonella* Enteritidis PT 30 (~8.65 log CFU/g), and conditioned to a water activity (a_w) of ~0.45 for ≥ 2 days. Samples (three replications with triplicate 4-8 g subsamples) were spread into a uniform layer ~0.5 cm thick, heat-treated in a convection oven at 177°C up to 10 min, then transferred to sterile bags, cooled, serially diluted, and plated on differential media. Temperature profiles and a_w also were measured.

Results: After a 10 min treatment, *Salmonella* in all-purpose, whole wheat, and gluten free flours resulted in log reductions (mean ± standard deviation) of 3.28 ± 0.52, 4.09 ± 0.46, and 4.13 ± 0.67, respectively. There were significantly less *Salmonella* reductions in all-purpose than in whole-wheat and gluten-free flour. Similar a_w trends were observed for all products, with values < 0.1 by 7.5 min. Samples did not achieve greater than an average 5-log reduction after a 10 min treatment ($P < 0.05$).

Significance: Awareness of microbial hazards associated with low-moisture products is increasing; however, none of the home-scale solutions evaluated were scientifically supported. While treated flour resulted in less *Salmonella*, it is currently unknown whether this is a sufficient or best practice for consumers.

P1-96 A Meta-Analysis of the Effect of Water Activity on the Thermal Inactivation of Different Microorganisms in Low Moisture Foods

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❖ ***Developing Scientist Entrant***

Introduction: Water activity (a_w) and temperature are major factors in determining microbial inactivation in low moisture foods (LMF), and a number of studies have calculated cell death kinetics in different foods. Rather than continuing to conduct individual studies for each product and time/temperature combination, identification of generalizable trends and key variables would improve our understanding of LMF safety. However, development of such a model may be confounded by variation in microbial target, matrix composition, inoculation strategies, and processing conditions.

Purpose: The purpose of this study is to estimate microbial inactivation kinetics in LMF by performing a meta-analysis of previously published studies.

Methods: Electronic databases and selection criteria were used to identify 27 studies which contained 782 mean *D*-values for different foodborne microorganisms under varying a_w and temperature conditions. Linear mixed effect models were developed to assess the effect of microbial target, matrix structure and composition, and inoculation strategies on cell death kinetics. Additionally, a global response surface model was developed for each organism, accounting for temperature and a_w.

Results: Data were partitioned by microbial target and matrix structure. Interclass correlation statistics (I^2) and conditional R^2 values of the linear mixed effects models by organism were: *E. coli* (R^2-0.91, I^2-83.18), fungi (R^2-0.88, I^2-85.86), *L. monocytogenes* (R^2-0.84, I^2-75.8), *Salmonella* (R^2-0.69, I^2-46.98). Model results by matrix structure were: solids (R^2-0.96, I^2-85.63), solution (R^2-0.92, I^2-88.53), and powder (R^2-0.87, I^2-83.18). Inoculation strategies and sample composition did not have a significant on cell death kinetics ($P > 0.05$). Response surface models were developed to estimate cell death kinetics as function of a_w and temperature and varied by organisms from R^2 0.88 (*E. coli)* to 0.23 (fungi).

Significance: The findings from this study can be used to identify generalizable trends and key variables for microbial inactivation in LMF. This may reduce redundancy in future research on isothermal inactivation in LMF.

P1-97 [v] Impact of Water Activity Alteration during Heating and Extended Storage on Thermal Resistance of *Salmonella* in Almond Meal

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Introduction: Raw almond products are often kept for one year at various temperatures. *Salmonella* can survive in almonds during this extended storage. Thus, it is important to understand the thermal resistance of *Salmonella* during thermal processing of almond meal before or after extended storage.

Purpose: To assess the impacts of the alteration of a_w during heating on thermal tolerance of *Salmonella* in almond meal before and post one-year storage.

Methods: Almond meal was inoculated with a lawn-grown three-strain *Salmonella* cocktail at 10^{8-9} log CFU/g, and equilibrated to a_w 0.25 or a_w 0.45 at 22°C. The inoculated almond meal was subjected to isothermal treatments parallelly using two different thermal inactivation test cells, thermal death time (TDT, where a_w subjected to change during heating) or thermal water activity (TWA, where a_w maintained relatively stable during heating) cells, before or after one-year of storage.

Results: *Salmonella* maintained a stable population in the almond meal for one year at 4°C regardless of a_w. *Salmonella* count in a_w 0.25 and a_w 0.45 almond meal declined by 0.77 or 1.53 log CFU/g in one-year storage at 22°C. The thermal resistance of *Salmonella* in almond meal was inversely related to the equilibrated a_w of samples. The death time for one log reduction (*D*-value) of *Salmonella* at 85-95°C in a_w 0.25 almond meal obtained using TWA cells were 2.0-2.4 times of those determined using TDT cells. Data indicated that the alteration of a_w during heating is an important factor affecting *Salmonella* thermal resistance in almond meal. The D_{85}-values of *Salmonella* in almond meal maintained or slightly increased after 1-year storage regardless of a_w and storage temperature.

Significance: Data provide new insight into the thermal resistance of *Salmonella* in low-moisture foods and useful information for the food industry in

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designing their thermal pasteurization processing of almond meal. (USDA-NIFA-2020-68012-31822)

P1-98 Effect of Relative Humidity on the Survival Kinetics of *Salmonella* in Different Treenut Flours

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Introduction: *Salmonella* is known to persist in low water activity foods however its persistence in treenut flours as effected by flour composition and storage conditions is not well understood.
Purpose: To study the effect of relative humidity on the growth kinetics of *Salmonella* spp., in various treenut flours during an extended storage
Methods: Treenut flours (almond, chestnut, and hazelnut) of varying protein and fat contents were seed inoculated with *Salmonella* spp. (3-strain) to achieve a 10^{8-9} CFU/g. The inoculated samples (100 g each) were packed into sterile polyethylene bags and stored in RH controlled chambers at 25, 50 and 75% relative humidity and 23°C for up to 6 months. At each sampling time (0, 1, 7, 14, 28, 45, 60, 90 and 180 days) the samples were analyzed in triplicates for the levels of *Salmonella* on both selective (XLD) and non-selective (TSA) media. Uninoculated controls were also analyzed for changes in water activity. All the experiments were conducted in duplicates. Statistical analysis of the data was performed by ANOVA using SPSS and the data was tested to check the predictive power of different models.
Results: Type of treenut flour showed significant effect on the growth of *Salmonella.* Increasing the RH from 25 to 75% increased the log survival except for chestnut flour at 75% RH. No significant difference ($P > 0.05$) in the reductions was observed when the samples were stored at 50 and 75% RH. A reduction of 2.27 (hazelnut), 1.68 (almond), and 0.5 (chestnut) log were observed after 45 days storage at 25% RH. Initial water activities of almond, chestnut and hazelnut flours were found to be 0.65, 0.47, and 0.67 ± 0.05, respectively, and they all subsequently equilibrated to around 0.3, 0.45, and 0.7 ± 0.05 when stored at 25, 50 and 75% RH.
Significance: The findings help to better understand growth kinetics of *Salmonella* in treenut flours and develop suitable storage conditions to mitigate the risk.

P1-99 *Salmonella* and *Escherichia coli* Populations in Wheat Kernels are Reduced Following Tempering in Lactic Acid and Novel Lactic Acid Solutions

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Introduction: Outbreaks of *Escherichia coli* and *Salmonella* spp. in retail flour have prompted the milling industry to investigate methods of microbial control. Antimicrobials may be introduced is the tempering process, when water is used to soften wheat kernels in advance of milling.
Purpose: Quantify the reductions of microorganisms on wheat by application of antimicrobials in tempering water in a bench-top model.
Methods: Wheat (200 g/sample) was dried to a target moisture (to accommodate moisture addition at inoculation) and irradiated at 12.5 kGy to destroy background microflora. Wheat was inoculated with a cocktail of surrogate *E. coli* (ATCC no. BAA-1427, BAA-1428, BAA-1429, BAA-1430, and BAA-1431) or a cocktail of *Salmonella* (*S. enterica* subsp. Enteritidis [ATCC 13076 and 31194], Typhimurium [ATCC 13311 and 14028], and Heidelberg [ATCC 8326]) to obtain a starting population of ca. 6 log CFU/g. Tempering solutions were prepared containing (a) 5% lactic acid (PURAC FCC 88), (b) novel lactic acid solution, or (c) water. To temper, solutions were applied to wheat in bags at quantities to reach desired moisture content (ca. 11%). Bags were intermittently shaken and permitted to temper for ca. 14 h. Wheat was sampled post-temper by serially diluting samples in Butterfield's buffer and plating on sorbitol MacConkey agar (SMAC; 35°C for 48 h) to detect *E. coli* and xylose-lysine-tergitol 4 agar (XLT-4; 35°C for 24 h) to detect *Salmonella* spp.
Results: Both 5% lactic acid and novel lactic acid solution significantly ($P < 0.05$) reduced populations of *Salmonella* and *E. coli* in post-tempered wheat compared to water control. In *E. coli* challenged kernels, reductions in treated samples were 0.96-1.48 log CFU/g lower than water-tempered samples in lactic acid and novel lactic acid solution, respectively.
Significance: Use of antimicrobial interventions in tempering water can reduce populations of pathogens and potentially impact pathogen prevalence in brans and flours downstream.

P1-100 Thermal Resistance of *Bacillus* spp. in Naturally Contaminated Mesquite Flour with Two Water Activities

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Introduction: A number of foodborne pathogens, including *Salmonella* spp. and *Bacillus cereus*, have been found in low-moisture foods such as flours. Mesquite flour with inherent high sugar content has been recalled due to contamination with *Bacillus cereus*. Therefore, treatments of mesquite flour are desirable to reduce the populations of *Bacillus* to levels that do not cause foodborne illness.
Purpose: The purposes of the present study were to evaluate the thermal resistance of *Bacillus* spp. in naturally contaminated mesquite flour and to study the influence of water activity (a_w) on thermal inactivation.
Methods: Mesquite flours with water activities of 0.34 and 0.71 were treated in ovens at various temperatures (100-140°C) and times (up to 2 h). Total mesophilic bacteria and *Bacillus* spp. in the treated flour samples were enumerated using tryptic soy agar and Brilliance Bacillus Cereus Agar media, respectively.
Results: Results revealed that naturally contaminated *Bacillus* spp. and other mesophilic bacteria in mesquite flour with unadjusted water activity (a_w = 0.34) were highly resistant to heat. To reduce the initial populations (4.75 log CFU/g) of *Bacillus* spp. to non-detectable levels (<1.18 log CFU/g), thermal treatments of 120°C for 2 h were required. $D_{100°C}$-values for total mesophilic bacteria were 5.6-fold higher than those of *Bacillus* spp. With increasing treatment temperature, the *D*-value between total mesophilic bacteria and *Bacillus* spp. became smaller. When the a_w of flour was adjusted from 0.34 to 0.71, the *D*-values for *Bacillus* spp. decreased significantly. Treatment at 100°C for 1 h reduced *Bacillus* spp. populations to nondetectable levels.
Significance: Our results demonstrated that *Bacillus* spp. of naturally present *Bacillus* in the flour were highly resistant to heat, while increasing water activity increased heat sensitivity of the bacteria. The information reported here will help develop strategies to minimize contamination of this low moisture, high sugar food with *Bacillus cereus*.

P1-101 Radio Frequency Pasteurization Against *Salmonella* and *Listeria monocytogenes* in Cocoa Powder

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Introduction: Radio frequency (RF) pasteurization is identified as a potential technology to pasteurize low-moisture food due to its rapid and relatively uniform heating of bulk food powders.
Purpose: This study developed a thermal microbial validation method for RF pasteurization for cocoa powder at a_w 0.45 against *L. monocytogenes* and *Salmonella* using surrogates, *Listeria innocua* and *Enterococcus faecium* NRRL B-2354.
Methods: Heat resistance parameters (*D*- and *z*-values) of *Salmonella, L. monocytogenes* and their surrogate *Enterococcus faecium* NRRL B-2354 in cocoa powder were determined. For process validation, one-gram inoculated pack of cocoa powder inoculated with either surrogates were subjected to RF heating at 75 and 90°C. An insulated device was developed to improve energy efficiency and achieve greater microbial reduction.
Results: The thermal inactivation of *E. faecium* and *L. innocua* was not significantly different from *Salmonella* and *L. monocytogenes.* From the measured temperature profiles and microbial thermal resistance parameters, a 7.6 min RF heating plus 48 min insulated holding attained a 5-log reduction of *E. faecium* without a negative impact on color of cocoa powder. *L. innocua* was less resistance to RF heating in a_w 0.45 cocoa powder; a 6.2 min RF heating plus

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18 min insulated holding delivered a 5.04-log CFU/g reduction.

Significance: This study provides a comprehensive study on achieving maximal microbial reduction using insulated holding following RF heating.

P1-102 Determination of Thermal Inactivation Parameters of *Salmonella* and *Listeria monocytogenes* in Brownie Batter

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* and *Listeria monocytogenes* can survive under the dry environment of flour during prolonged storage and could multiply when flour is hydrated to prepare batter or dough. Therefore, determining the thermal inactivation parameters of *Salmonella* and *L. monocytogenes* to examine the effectiveness of thermal treatment is vital to ensure brownie's microbiological safety.

Purpose: To determine thermal inactivation parameters for controlling *Salmonella* and *Listeria monocytogenes* in brownie batter.

Methods: This research consisted of two individual studies for determining thermal inactivation parameters of *Salmonella* and *L. monocytogenes* in brownie batter and were designed as completely randomized with three replications. The flour was individually inoculated with 5-serovar *Salmonella* cocktail or 3-strains of *L. monocytogenes* cocktail, and dried back to original pre-inoculation a_w. Inoculated flour was weighed and mixed with granulated sugar, powdered sugar, cocoa powder, salt, vegetable oil, water, liquid whole egg and vanilla in a kitchen mixer for 1 min. The batter (~10 g) was transferred into five thermal-death-time disks, sealed and placed in hot-water baths set at 64, 68, 72, and 76°C. The samples were held for 0 to 96 min in hot-water baths and quickly transferred to cold-water baths at pre-determined time intervals. The samples were enumerated using injury-recovery media, and *D*- and *z*-values were calculated.

Results: *D*-values of *Salmonella* were 53.4, 27.2, 10.7, and 4.6 min at 64, 68, 72, and 76°C, respectively, and 37.5, 16.9, 9.1, and 7.3 min at 64, 68, 72, and 76°C, respectively, for *L. monocytogenes*. The *z*-values of *Salmonella* and *L. monocytogenes* in brownie batter were 11.1 and 16.4°C, respectively.

Significance: *D*- and z-values from this study will provide necessary information about the heat resistance of *Salmonella* and *L. monocytogenes* in the brownie batter at the start of the baking process.

P1-103 Thermal Inactivation of *Enterococcus faecium* NRRL B-2354, *Escherichia coli*, and *Salmonella* in Peanut Butter Cookies at Various Moisture Levels

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Introduction: *Salmonella* and *Escherichia coli* may be present in raw ingredients such as flour. *E. faecium* has been used as surrogate microorganism in low-moisture food validation studies.

Purpose: This study investigated the thermal death time characteristics of *E. coli, Salmonella* and *E. faecium* in peanut butter cookies at three moisture levels.

Methods: Wheat flour was inoculated with freeze-dried *E. faecium,* a cocktail of *E. coli*, or *Salmonella* associated with low moisture foods at approximately 7 log CFU/g, and acclimated at 25°C for 24 h. Inoculated flour was combined with peanut butter, sugar, starch, eggs, flavoring and coloring agents and water to achieve target moistures. Samples were dispensed into pouches, vacuum sealed, exposed to 65 to 85°C and pulled at predetermined intervals. Surviving organisms were counted, averaged, then transformed to log CFU/g.

Results: The dough moisture levels were 8.0% (a_w 0.421), 12.8% (a_w 0.694), and 17.4% (a_w 0.793). *E. faecium* was the most heat resistant, followed by *Salmonella* and *E. coli*. *E. faecium* was 1.5 to 3.0 times more heat resistant compared to *Salmonella* under the same moisture and temperature condition. The *E. faecium* to *Salmonella* ratio was larger at higher moisture levels. *Salmonella D*-values at 70, 75, and 80°C for 17.4% cookie moisture were 2.98, 0.85, 0.38 min, respectively, while the *D*-values at 75, 80 and 85°C for 12.8% moisture were 3.5, 1.96, and 0.62 min, respectively, and for 8.0% moisture were 4.97, 2.42, and 1.24 min, respectively.

Significance: Thermal inactivation data showed that *E. faecium* would be a suitable surrogate for in-plant validation studies of peanut butter cookies. *Salmonella* showed higher resistance at lower moisture level during baking. These data can be used as a scientific basis for thermal validation of similar soft cookies with similar moisture.

P1-104 Correlation of Intracellular Moisture and Thermal Inactivation Kinetics of Desiccated *Salmonella* at Acidic pH Conditions

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Introduction: Previous research has indicated that thermal inactivation kinetics of desiccated *Salmonella* are affected by pH conditions, and that *Salmonella* cells retain significant intracellular moisture after desiccation. Inflection points were observed in the drying and inactivation curves for desiccated *Salmonella* in acidic conditions, after which drying/inactivation rate decreases. Since both curves exhibit this behavior, it may be that the moisture loss is related to the change in inactivation rate.

Purpose: The objective of this study was to investigate the correlation of intracellular moisture content and thermal inactivation kinetics of desiccated *Salmonella* at acidic conditions.

Methods: Data previously gathered for the inactivation rate (n = 3) and drying curve (n = 3) of desiccated *Salmonella* Anatum 6802 and control samples was analyzed. Desiccated *Salmonella* and control samples consisted of 0.1 mL concentrated *Salmonella* inoculum in buffer and 0.1 mL buffered peptone water, respectively pipetted onto cellulose filters and dried 24 h at ~25°C/30% relative humidity. The second derivative of mass change over time was used to locate the drying inflection point for both inoculated and control samples. ANCOVA was used to compare inactivation rates of desiccated *Salmonella* at various pH conditions, as well as before and after the inflection point at pH4.

Results: Inoculated samples required significantly greater ($P < 0.05$) treatment time than control to reach an inflection point after which drying rate decreased (5.54 ± 0.03 and 3.30 ± 0.38 min, respectively), indicating desiccated cells retain more intracellular moisture than control. The inactivation rate of desiccated *Salmonella* at pH4 from 0-8 min of treatment was significantly greater ($P < 0.05$) than both the rate from 8-32 minutes of thermal treatment and *Salmonella* at pH7 (decimal reduction times of 4.49 ± 0.54, 26.85 ± 8.26, and 31.8 ± 3.30 min, respectively).

Significance: Drying and inactivation curves both exhibiting similar inflection points indicates that the loss of intracellular moisture may be correlated to the change in thermal resistance of desiccated *Salmonella* at pH 4.

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P1-105 Antimicrobial Efficacy of Gaseous Chlorine Dioxide for Inactivation of *Salmonella* and *Enterococcufaecium* NRRL B-2354 on Dried Basil Leaves

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❖ ***Developing Scientist Entrant***

Introduction: The repeated association of low moisture foods with foodborne outbreaks requires a need for novel processing technologies. Chlorine dioxide (ClO_2) gaseous technology is a promising non-thermal treatment that has been employed to reduce the microbial load in various food products and is approved under U.S. regulation 21CFR173.300.

Purpose: The research aims to (i) investigate the efficacy of ClO_2 gas on the inactivation of *Salmonella* on dried basil leaves and (ii) evaluate the suitability of *E. faecium* as a surrogate for *Salmonella*.

Methods: Dried basil leaves (2.0 ± 0.1 g) inoculated with a 5-strain *Salmonella* cocktail or *E. faecium* were packed in a mesh tea bag, heat-sealed, and placed inside the treatment chamber. The samples were exposed to different levels of ClO_2 gas concentration (5.0, 10.0, and 15.0 mg/L) and relative humidity (RH; 60, 70, 80%) for various exposure times (0-5 h) to develop a microbial inactivation model. The treated samples were diluted with 18 mL of neutralizing buffer (NB) and homogenized for 1 min. The dispersions were serially diluted with NB plated onto Tryptic Soy Agar modified to enumerate *Salmonella* and *E. faecium.*

Results: The gas concentration and RH showed a significant effect ($P < 0.05$) on the resistance of *Salmonella* and *E. faecium*. As the gas concentration and RH increased, the *D*-values of both microorganisms decreased. For example, at 5.0 mg/L, as the RH increased from 70 to 80%, the *D*-value of *Salmonella* and *E. faecium* decreased from 59.17 to 38.61 min and 90.91 to 48.08 min, respectively. *E. faecium* showed greater resistance to the treatments compared to *Salmonella* at all conditions tested in this study.

Significance: The research will provide food processors with a starting point for implementing gaseous technologies to improving the safety of low moisture foods. Additionally, *E. faecium* can be used as a surrogate for *Salmonella* to conduct in-plant validations of similar treatments of basil leaves.

P1-106 Inactivation of *Salmonella* and *Enterococcus faecium* NRRL B-2354 in Black Peppercorn and Cumin Seeds Using Gaseous Chlorine Dioxide Technology

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❖ ***Developing Scientist Entrant***

Introduction: The antimicrobial gaseous chlorine dioxide technology is a promising waterless and non-thermal process for inactivation of food pathogens in low moisture foods. Chlorine dioxide has been approved by FDA for improving the safety of food products with minimum chemical residuals.

Purpose: The objectives of this study were to 1) evaluate the effect of gas concentration, relative humidity, and exposure time on the efficacy for *Salmonella* inactivation during gaseous chlorine dioxide treatment for black peppercorn and cumin seeds, and 2) evaluate the suitability of *Enterococcus faecium* NRRL B-2354 as a surrogate for *Salmonella*.

Methods: Black peppercorn and cumin seeds were first inoculated with a 5-strain *Salmonella* cocktail or *E. faecium*. Inoculated samples (3.0 ± 0.1 g) were placed on a petri dish and transferred to a custom-made gas treatment chamber. The chlorine dioxide treatments were conducted at three gas concentrations (5.0, 10.0, and 15.0 mg/L) and three relative humidity (RH) levels (60, 70, 80%) for up to 5 h of exposure time. The sampling was conducted at every hour during the exposure and collected samples were enumerated to obtain the microbial inactivation curve during each treatment.

Results: More than 5-log reduction of *Salmonella* was achieved on both spices with 300 min of ClO_2 gas treatment at 15 mg/L and 80% RH. The ClO_2 gas inactivation kinetics were determined at each gas concentration and RH using the log-linear model. The reduction of *E. faecium* was found to be consistently lower than *Salmonella* in all treatment conditions.

Significance: The study provides technical information to the spice industry for implementing this novel antimicrobial gaseous technology to improve the safety of their products. *E. faecium* was found to be a suitable surrogate for *Salmonella* for the process validation of chlorine dioxide treatments similar to those tested in the present study.

P1-107 Survival of *Salmonella* and *Enterococcus faecium* in Spices ss Influenced by Water Activity during Storage

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❖ ***Developing Scientist Entrant***

Introduction: Spices as minor low-moisture ready-to-eat food ingredients are able to cause large-scale outbreaks and recalls due to the potential foodborne pathogen contamination. *Salmonella* has been widely implicated by the outbreaks in spices and it can survive for long periods in spices.

Purpose: Spices have natural bioactive compounds to inhibit microbial growth. This study aims to investigate the microbial stability in different spices as influenced by a_w during storage, and to evaluate the feasibility of *Enterococcus faecium* as a surrogate for *Salmonella* in foods with antimicrobial activity.

Methods: The ground cinnamon, chili powder, and black pepper were inoculated with a three-strain *Salmonella* cocktail (*S*. Enteritidis PT 30, *S*. Agona 447967 and *S*. Tennessee K4643) or *E*. faecium, and the homogeneous samples were conditioned by a humidity-controllable chamber for 3 days to two a_w levels (0.3 and 0.5). Then, samples were divided into small packs, sealed in water barrier bags, and stored at room temperature. Sample bags were taken for survival tests weekly/monthly.

Results: Bacteria are generally stable in spices during the first month of storage and they are more resistant at a lower a_w. *E. faecium* is more stable than *Salmonella* during storage. For example, around 2 log reductions of *E. faecium* were obtained in chili at a_w of 0.5 after 20 months, while no *Salmonella* could be observed after 16 months (more than 5-log reduction). Spices also show the different antimicrobial activities to inhibit microbial growth during storage. More microbial reduction is observed in chili powder, followed by ground cinnamon, and bacteria are relatively stable in black pepper powder during storage.

Significance: This work aims to profile the microbial risks for spices during storage and provides information that a_w influences the microbial resistance in spices during storage. It also evaluated the feasibility of *E. faecium* as a surrogate for *Salmonella* in low-moisture foods with antimicrobial activity, such as spices.

P1-108 Detection of *Salmonella* in Garlic Powder Using the Hygiena™ BAX® System

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Introduction: A risk profile initiated by the FDA to survey the prevalence of *Salmonella* in 11 imported spices, estimated that between 1.7-18% are contaminated. With more than half of spices imported in the United States for retail, sensitive screening methods must be available to assess the food safety risk.

Purpose: Spices such as garlic, white pepper and black pepper have all been previously recalled due to the presence of *Salmonella*. Garlic, specifically, is one of the most commonly used ingredients as a flavor enhancement for foods. However, the bioactive compounds that create its pungent taste also contribute towards its antimicrobial properties. The objective of this study was to therefore evaluate the performance of a PCR method for the detection of

Salmonella in garlic powder.

Methods: Using a paired study design, *Salmonella* Typhimurium was artificially inoculated into 25-g test portions of garlic powder at 2 levels: a low level expected to yield fractional recovery and a high level expected to yield all positives. Samples were held at 25°C for 72 hours to equilibrate and then enriched according to the procedure in the FDA BAM Chapter 5. Aliquots were removed for PCR testing, and all samples regardless of presumptive result were confirmed by culture.

Results: Real-time and standard PCR assays returned positive results for 7/20 low-spiked samples (0.42 MPN/25 g) and 4/5 high spiked samples (1.6 MPN/25 g). Paired samples confirmed by the reference method returned identical results to presumptive PCR results. Statistical analysis conducting according to the probability of detection (POD) determined that there is no significant difference between PCR and culture.

Significance: With no false positives or false negatives, the BAX® System PCR assays are a suitable screening method to accurately detect *Salmonella* using the FDA BAM reference enrichment.

P1-109 Determining the Suitability of *Enterococcus faecium* NRRL B-2354 as a Potential Surrogate for *Salmonella enterica* in Fine Ground Black Pepper

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Introduction: Black pepper has been reported to be increasingly associated with *Salmonella* contamination. For validating the thermal process in the industry, a suitable surrogate needs to be identified.

Purpose: 1) To identify a suitable surrogate for *Salmonella* for industrial process validations. 2) To investigate the effect of a_w on the thermal inactivation kinetics of *Salmonella* and *E. faecium.*

Methods: The fine ground black pepper sample was inoculated with a 5-serotypes of *Salmonella* cocktail or *E. faecium* and equilibrated to target water activities (0.40, 0.55, 0.70) in a humidity chamber. Stability and homogeneity tests were conducted to determine the effectiveness of the inoculation method. Isothermal treatments were conducted at different temperatures (65-80°C) for five different exposure times ranging from 18 s - 250 min. The survivor data of *Salmonella* and *E. faecium* were fitted to log-linear and Weibull model.

Results: *Salmonella* and *E. faecium* population stabilized within 5 days of equilibration. At higher water activity, the rate of inactivation significantly ($P < 0.05$) increased for both microorganisms indicating shorter treatment times are enough to achieve target bacterial inactivation. Weibull model ($R^2 = 0.85$-0.99) fits better than log-linear ($R^2 = 0.80$-0.97) and was used to determine the thermal resistance since survival curves were non-linear. Among the two pathogens tested, *E. faecium* was found to be significantly ($P < 0.05$) more heat resistant than *Salmonella* corresponding to its higher δ-values indicating its suitability as a conservative surrogate. For a_w of 0.55, the δ-values at 70, 75 and 80°C were 0.99 ± 0.65, 0.47 ± 0.32 and 0.85 ± 0.49 min for *Salmonella*; and 19.64 ± 3.01, 7.44 ± 1.83 and 0.98 ± 0.29 min for *E. faecium,* respectively.

Significance: This output of the present study will enable spice industries to develop suitable thermal pasteurization methods for microbial inactivation of fine ground black pepper. In addition, *E. faecium* can be used a non-pathogenic surrogate for *Salmonella* in the validation studies of thermal processing.

P1-110 Using Whole Genome Sequencing to Characterize the Genetic Diversity of Salmonella enterica Isolated from Raw Inshell Pistachios

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❖ ***Developing Scientist Entrant***

Introduction: A total of seven *Salmonella* serovars with nine PFGE fingerprints was isolated from 68 of 3,996 inshell pistachio samples over three California harvests (2010, 2011, and 2012). A limited number of PFGE patterns suggested a narrow and persistent contamination source.

Purpose: To characterize the genetic diversity using whole genome sequencing (WGS) of the five *Salmonella* serovars isolated from the pistachios.

Methods: DNA was isolated from 121 *Salmonella* isolates and paired-end libraries were constructed with the Nextera XT, Nextera DNA Flex (Illumina Inc., San Diego, CA) or by High-Throughput processing (UC Davis Genome Center). Pooled libraries were sequenced with MiSeq, HiSeq, or NovaSeq Illumina platform. Sequences were trimmed and assembled de novo using Trimmomatic software v. 0.36 and SPAdes v. 3.14.1, respectively. Genomes were aligned and core genome single nucleotide trees were built using default parameters of Parsnp v.1.2. The Center for Food Safety and Applied Nutrition Single Nucleotide Polymorphism (SNP) Pipeline v2.2.0 was used to compare differences among survey isolates and other isolates selected by geography, isolation source, and year.

Results: A phylogenetic tree generated by Parsnp clustered *Salmonella* isolates by serotypes Agona, Enteritidis, Montevideo, Senftenberg, and Worthington. Within each serotype, isolates grouped in ≤2 clusters. Within each cluster, isolates differed by 0 to 14 SNPs. Comparison of WGS sequences at the National Center for Biotechnology Information identified additional *Salmonella* Montevideo or *Salmonella* Senftenberg isolated from pistachios from 2005 through 2018 that differed from the survey isolates by ≤10 SNPs. Survey isolates fell within two of 18 clusters of *Salmonella* Montevideo; one of the clusters was exclusive to pistachio isolates while the other included isolates from a broad range of food and environmental sources in California.

Significance: Preliminary WGS data analysis confirms a limited *Salmonella* diversity isolated from pistachios. This information could serve as the basis for identifying potential contamination sources for US pistachios.

P1-111 Efficacy of Gaseous Chlorine Dioxide Against *Salmonella enterica* and *Enterococcus faecium* NRRL B-2354 on Chia Seeds

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❖ ***Developing Scientist Entrant***

Introduction: Consumption of chia seeds especially in raw form is increasing due to its health benefits. Often chia seeds are soaked in water overnight prior to consumption, any *Salmonella* contamination may grow to a dangerous level due to favorable nutrition-rich environment.

Purpose: The objective of this study is to investigate the efficacy of chlorine dioxide (ClO_2) gas in inactivating *Salmonella enterica* in chia seeds and to evaluate *Enterococcus faecium* NRRL B-2354 as a potential surrogate.

Methods: Chia seeds were inoculated with either a cocktail of five *Salmonella enterica* serovars or *E. faecium* and equilibrated at a target a_w of 0.53. The Minidox-M (Clordisys) system was used to generate ClO_2 gas, monitor and maintain the target gas concentration and RH throughout the treatment. The bactericidal effect of ClO_2 was studied at various combinations of gas concentrations (5, 10, and 15 mg/L), exposure times (1-5 h) and process relative humidity (60, 70, and 80%).

Results: Inactivation of *Salmonella* and *E. faecium* was significantly ($P < 0.05$) affected by the RH and exposure time. The higher the RH and exposure time, the greater the log reduction for both *Salmonella* and *E. faecium*. At 80% RH and 15 mg/L ClO_2 concentration and 5 h exposure time, *Salmonella* and *E. faecium* were reduced by 1.83 and 1.76 logs, respectively. However, the concentration did not have significant effect on microbial inactivation at any of the RH condition tested. No visual quality changes were observed. Higher fat content and lower a_w in chia seeds might have attributed to a protective effect on microbial inactivation.

Significance: This is the first study of its kind to investigate the effect of ClO_2 against *Salmonella* in chia seeds. The outcome of the present study enlightens the challenges associated with the microbial inactivation of chia seeds using ClO_2 treatment. *E. faecium* is a suitable surrogate for *Salmonella* for ClO_2

Poster

treatment of chia seeds.

P1-112 Natural Antimicrobials Suitable for Combating Desiccation-resistant *Salmonella enterica* in Milk Powder

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Introduction: *Salmonella enterica* can survive well in low–water activity foods, and therefore, causes frequent salmonellosis outbreaks in these products. Methods are needed to overcome such desiccation-resistant *Salmonella* to improve the safety of low–water activity foods.

Purpose: (a) Screening natural food additives for ability to compromise the desiccation–resistance in *S. enterica*, and (b) assess the ability of a membrane-active additive to inactivate *S. enterica* during milk spray–drying and freeze–drying.

Methods: Ten food additives (Carvacrol, thymol, trans-cinnamaldehyde, eugenol, vanillin, citric acid, lactic acid, benzoic acid, diacetyl, and catechin hydrate) were screened for ability to cause leakage of potassium ions from desiccation–adapted *S. enterica* cells (air dried in a biosafety cabinet for 24 h at 22-25°C under ca. 40% relative humidity) using a potassium-binding probe. Based on the screening results, carvacrol and thymol caused significant potassium leakage and so, were assessed at 200 and 100 ppm, respectively, for their capability to sensitize *S. enterica* strains to mild heat treatment at 55°C for 15 min. Furthermore, carvacrol at levels of 200 to 5,000 ppm was added to liquid milk samples inoculated with *S. enterica* cells to assess the pathogen inactivation during spray– or freeze–drying. *Salmonella* survivors were enumerated in milk powder samples immediately following the drying processing and during storage of the milk powder at room temperature. Data were analyzed in GraphPad software.

Results: The membrane–active carvacrol and thymol caused potassium leakage ($P < 0.05$) from desiccation–adapted *S. enterica* strains. Carvacrol (200 ppm) and thymol (100 ppm), combined with heat treatment, reduced desiccation–adapted *Salmonella* by 3.1 ± 0.21–5.5 log CFU/mL compared to sole heat treatment (2.4 ± 0.53–3.2 ± 0.11 log CFU/mL reduction). Addition of carvacrol at 500 or 5,000 ppm during spray– or freeze–drying lowered *Salmonella* populations in milk powder by 1.3 ± 0.1 to more than 4.5 log CFU/g, respectively.

Significance: Carvacrol is a potent food additive capable of combating desiccation–resistant *Salmonella* and thus may improve safety of low–water activity foods.

P1-113 Microbial Risks Associated with Soaking and Subsequent Drying of Walnut Kernels

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Introduction: Soaking nuts has increased in popularity, but the food safety implications of these practices have not been adequately assessed.

Purpose: To evaluate the behavior of foodborne pathogens on walnut kernels during soaking and subsequent low-temperature drying using at-home practices common in cooking and nutrition blogs.

Methods: Individual five-strain cocktails of rifampicin-resistant *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* prepared from cultures grown on agar plates and diluted in water, were inoculated onto untreated walnut kernels and then dried for 7 days under ambient conditions. Walnut kernels were combined with sterile water at a 1:4 ratio (w/v), soaked at 15, 18, 23°C for up to 24 h, and dried at 66°C for up to 24 h (*Salmonella* only). Pathogen populations were enumerated during soaking and drying by plating onto corresponding selective agars; moisture content of the walnuts was measured using standard methods.

Results: Initial populations of native microbiota were 3.14 log CFU/g on uninoculated walnuts; increases of 1.20, 1.87, and 2.58 log CFU/g were observed at 15, 18, and 23°C, respectively. Initial *E. coli*, *L. monocytogenes*, and *Salmonella* populations were 1.34, 1.41, and 2.55 log CFU/g, respectively, after inoculation and drying. No significant ($P > 0.05$) increase in populations was observed for any of the pathogens after 24 h at 15 and 18°C or after 12 h at 23°C; increases of 3.0, 2.0, and 1.7 log CFU/g were observed for *E. coli*, *L. monocytogenes*, and *Salmonella*, respectively, after 24 h of soaking at 23°C. Moisture levels were ~4% (initial), ~30% (24 h of soaking), and ~4 and <1% (6 and 24 h of drying, respectively). Populations of *Salmonella* declined by 1.04 log CFU/g over 12 h of drying; further significant ($P > 0.05$) decreases were not observed at 24 h.

Significance: Soaking walnuts at ≤18°C or for ≤12 h should prevent growth of pathogens.

P1-114 *Enterobacteriaceae* and Coliform Contamination Patterns in Peanuts Produced and Sold in the Senegalese Peanut Basin

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❖ ***Developing Scientist Entrant***

Introduction: Peanuts and peanut products are significant revenue sources for smallholder farmers in Senegal, however, microbial contamination during production and storage can greatly affect market access for producers on a global scale. Recently, peanut products have emerged as possible sources of foodborne illness, encouraging discussion of global standards for exported peanuts.

Purpose: This is the first study analyzing bacterial contamination of peanuts from Senegal and to elucidate the relationship between producer understanding of bacteria with contamination levels.

Methods: A survey was implemented to assess current production practices, storage methods, and producers' knowledge of microbial contamination. A single member of each household orally completed the survey with a trained enumerator and the results were correlated to the microbiological results obtained from collected peanut samples using a linear regression analysis and an analysis of variance model. Samples were collected from stored peanuts at each household; peanuts were shelled and total *Enterobacteriaceae*, coliform, and yeast and mold populations were enumerated on Petrifilm.

Results: Of the 198 samples, 13.0% and 13.6% were greater than the detection limit (5.0 log CFU/g) for *Enterobacteriaceae* and coliforms, respectively; 21.2% of samples were above the detection limit (4.6 log CFU/g) for yeast and mold. Only 22.7% and 18.7% of producers knew of bacteria or aflatoxins, respectively, prior to this study. There were no significant differences ($P \geq 0.05$) between households who took preventative measures against microbial contamination. Additionally, only four households reported washing their kitchen utensils before using them to eat, while 60.1% reported always washing their hands before eating. Neither storage container type used nor if the containers were elevated off the ground was useful in predicting bacterial contamination ($R^2 < 0.02$).

Significance: These results provide preliminary data to inform future studies to evaluate pathogen prevalence and identify impactful preventative measures to minimize microbial contamination of peanuts produced in Senegal.

P1-115 Treatment of Wheat Kernels during Simulated Tempering to Control Foodborne Pathogens

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Introduction: Recent *Salmonella* and enterohemorrhagic *Escherichia coli* (STEC) outbreaks linked to wheat flour consumption have revealed its potential risk. Tempering is the addition of water (2-4%) to wheat kernels before milling that is as a potential point for antimicrobial interventions. *Listeria monocytogenes* has not been involved in outbreaks, but because flour is a raw agricultural commodity its risk is also recognized. We previously reported that kernels treated with 4% of a 0.5 M pelargonic acid emulsion (PAE) reduced viability of *Salmonella* and STEC >4 log CFU/g.

Purpose: This project was undertaken to 1) determine the effect of PAE treatment on *L. monocytogenes* on wheat kernels and 2) optimize the PAE treatment in combination with other ingredients.

Methods: Separate five-strain cocktails of *Salmonella*, STEC O121 and *L. monocytogenes* strains were grown in rich complex media, diluted, and inoculated onto wheat kernels (8 log CFU/g). After drying, kernels were mixed at 4% (v/w) with antimicrobial treatments and incubated for 17 h at different temperatures. Kernels mixed with buffer and plated on specific differential media to determine viability. Statistical differences among means was determined by Student's *t*-test.

Results: Hard wheat kernels mixed with 4% of 0.5 M PAE at 23°C for 1, 6 and 17 h reduced the viable count of *L. monocytogenes* by > 4.0 log CFU/g ($P < 0.05$). *Listeria* viability reduction on soft wheat was approximately 1.0 log CFU/g less than on hard wheat. A combination of 0.25 M PAE and 0.5% lactic acid (LA) inactivated *Salmonella* by approximately 1.8 log CFU/g at 23°C. Additional reduction of PAE concentration (0.1 M) with 0.8% LA killed 2.0 log CFU/g *Salmonella*. Similar reductions were observed with STEC O121 with latter combinations.

Significance: The findings from this study identified antimicrobial treatments to mitigate pathogen contamination of wheat kernels used for flour production.

P1-116 Evaluation of Charged Chalk as a Seeding Medium of Low Moisture Powders

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❖ ***Developing Scientist Entrant***

Introduction: Low-moisture food powders such as cake mix have been increasingly implicated in outbreaks of foodborne illness, notably as novel vehicles for shigatoxigenic *E. coli* (STEC). Currently, there is a lack of literature describing how the initial contamination and subsequent survival of STEC in food powders can be accurately modeled in experimental settings.

Purpose: This study evaluated the use of chalk powder as a seeding medium for inoculating selected strains of *E. coli* into cake mix powder as compared to a traditional liquid inoculum.

Methods: *E. coli* serotypes O26, O121, and O157:H7 were transformed with green fluorescent protein and resistance to ampicillin (100 µg/mL) and streptomycin (100 µg/mL). Chalk sticks (3.75 ± 0.15 g) were soaked in 7-log CFU/mL suspensions of each *E. coli* strain for 12 h at 4°C, then dried pulverized to create a powder. The powders and liquid bacterial suspensions were added to individual portions of commercial cake mix, which were made into batter per the manufacturer's instructions and stored at 22°C for 5 days. Cells were quantified on days 0, 3, and 5 by plating on tryptic soy agar containing ampicillin and streptomycin (100 µg/mL) (LOD=2.70 log CFU/g).

Results: No significant difference in *E. coli* survival was observed among bacterial strains nor between inoculation methods on each sampling day ($P > 0.05$), indicating that a dry seeding medium like chalk may be used in lieu of a liquid medium. *E. coli* survival was found to be significantly greater ($P < 0.05$) for all three strains and both inoculation methods on days 3 (7.56 log CFU/g) and 5 (9.60 log CFU/g) as compared to day 0 (4.99 log CFU/g), demonstrating that these bacteria were able to proliferate in the cake batter.

Significance: These results suggest that a charged chalk powder is suitable for use as a seeding medium of *E. coli* in low-moisture food powders, and that *E. coli* can survive dehydration and grow in reconstituted batters.

P1-117 Difference in Growth Rates for Native and Antibiotic-resistant Strains of *E. coli* O26, O121, and O157:H7

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Poster

Introduction: Commercial cake mixes contain flour, which is a raw agricultural product whose robust microflora may interfere with microbiological analysis. Antibiotic-resistant growth media can be used to improve detection, but previous works have suggested that resistant bacterial strains may possess growth characteristics different from their native strains.

Purpose: This study evaluated the growth of three strains of *E. coli* which have been associated with low-moisture food outbreaks: O26, O121, and O157:H7. Growth of these native strains (NR) was compared to transformants with resistance to ampicillin (+Amp), and both ampicillin and streptomycin (Amp+Strep).

Methods: *E. coli* strains O26, O121, and O157 were transformed via electroporation using the pGFPuv plasmid which encodes resistance to ampicillin (100 µg/mL) and GFP gene. Strains were additionally made resistant to streptomycin (100 µg/mL) through incremental exposure. These native and resistant strains were inoculated into tryptic soy broth ($n = 3$) and grown for 24 h at 37°C. Growth was monitored by microplate detection.

Results: *E. coli* O26+Amp grew more quickly (0.225 log CFU/h) than its native and Amp+Strep resistant strain (0.179 and 0.127 log CFU/h, respectively). Much less variation in growth rate was observed between *E. coli* O121 NR, +Amp, and Amp+Strep (0.174, 0.184, and 0.163 log CFU/h, respectively) and almost no variation was observed among the strains of *E. coli* O157:H7. There was little change in the duration of the lag phase across all strains (ca. 7.3 h).

Significance: These results demonstrate that conferring antibiotic resistance to certain strains of *E. coli* can alter their growth behavior. These findings advance our understanding of these strains, which are significant in the context of low-moisture foods, and may aid in the development of risk assessment tools.

P1-118 Effects of the Early and Repeated Administration of the Lactic Acid Bacterium Lactobacillus kefiranofaciens DN1 and the Yeast Kluyveromyces marxianus KU140723-05 on the Inhibition of Salmonella Enteritidis Colonization in Young Layers

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Introduction: *Salmonella* Enteritidis (SE) can be contracted via the consumption of poultry and poultry-derived products contaminated with SE. Today's restrictions on the prophylactic usage of antibiotics in feed have led to reductions in meat production and a concomitant increase in bacterial infections in the poultry. Therefore, alternative antibiotics are needed to reduce the pathogen load in the guts of poultry.

Purpose: We evaluated two probiotic strains with *Lactobacillus kefiranofaciens* DN1 (LKF_DN1) and *Kluyveromyces marxianus* KU140723-05 (KMA5) for the prospective applicability to reduce the colonization of SE in the gut of young layers.

Methods: A total of 90 one-day-old male layers were used and randomly divided into five groups; negative control (no probiotic and SE), positive control, lactic acid bacteria (LKF_DN1), yeast (KMA5), and a commercial probiotics product (IDF-7). The probiotics were administered into the experimental chicks every five days up to three times Day (D)1, D5, and D11. Each group consists of three subgroups (SG1; probiotics or their culture media administered only one time (A1) at D1, SG2; two repeated administration (A2) at D1 and D6, SG3; three repeated administration (A3) at D1, D6, and D11. Eighteen

chicks from each group were sacrificed at D13 to collect the ceca for viable cell counting of the SE strain resistant to rifampicin (SERR). Groups, except NC, were co-administered with SERR. The number of SERR in the cecal digesta obtained from the intestinal segments of the experimental chickens were determined at the end of experimental period (D13).

Results: The administration of probiotics decreased the number of viable SERR in chicks by up to 2.00 log when compared to PC chicks. Additionally, repeated administration of the probiotics was significantly reduced the number of SERR cells colonized in the cecum, compared with repeated administration (D1 and/or D6).

Significance: Probiotics are effective to enhance host health, particularly during the rearing of chickens.

P1-119 Homespun *Salmonella* Biofilms: Invisible Chicken Guests in the Kitchen

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Undergraduate Student Award Entrant

Introduction: *Salmonella* spp. cause around 93.8 million of illnesses each year, being chicken one of the most associated food products. *Salmonella* spp. have the ability to form biofilms on food and abiotic surfaces.

Purpose: The aim of this study was to collect information of chicken handling at home among the population of the central region Mexico (CRM), and evaluate the biofilm formation (BF) of *Salmonella* spp. in common risky scenarios during chicken handling.

Methods: An online survey on chicken handling practices during purchase, storage, and preparation at home was performed among the population of CRM; from it risky scenarios were selected. Three *S. enterica* isolates were inoculated individually on stainless steel, plastic, and glass in presence of sterile raw breast (RB), cooked breast (CB) and skin (SC) chicken extracts and incubated at 25°C for up to 72 h; surfaces in presence of RB were incubated at 9 and 14° for up to 15 days. The BF was evaluated by biopolymers quantification (BQ) and quantification of the pathogen embedded in the biofilm (QP).

Results: A total of 688 surveys were analyzed, finding that the use of the same surface for raw and cooked chicken at least one time (47.7%) and improper storage (38.6%) are the most frequently unsafe practices. Regarding BF, the highest BQ (0.63 ± 0.08 to 1.24 ± 0.40 OD_{600nm}) and QP (6.0 ± 0.83 to 7.24 ± 1.24 log CFU/surface) were observed on plastic surfaces stored at 14°C, while the lowest BQ (0.13 ± 0.03 to 0.41 ± 0.08 D_{600nm}) and QP (4.28 ± 0.71 to 6.67 ± .07 log CFU/surface) were in glass at 9°C. It was not found a correlation between BQ and QP at any temperature.

Significance: *Salmonella* spp. proved to have the ability to form biofilms in all scenarios, being higher in plastic, which is the most common material used in kitchens in CRM. This handling practice can increase the risk of cross-contamination scenarios.

P1-120 SMART Design of a Multi-Receptor Phage Cocktail to Tackle *Salmonella* in Poultry

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Developing Scientist Entrant

Introduction: *Salmonella* is the most prevalent foodborne bacterial pathogen around the world causing gastroenteritis in humans. This has been usually attributed to the consumption of contaminated poultry products. Bacteriophages (phages) are viruses that specifically infect and kill bacteria. For this reason, their use as antimicrobials has been proposed to enhance food safety in different food products including poultry.

Purpose: The purpose of this study is to design a cocktail comprised of broad-host range lytic phages targeting multiple receptors to mitigate the risk of *Salmonella* contamination of poultry products.

Methods: Six phages isolated from environmental samples were selected based on their host range, bacterial receptor recognition, and ease of propagation for cocktail composition. A total of 22 *Salmonella* serovars were challenged with different cocktail concentrations for 24 hours in liquid culture at 25°C to evaluate growth inhibition. Furthermore, *Salmonella* Enteritidis was used to study growth inhibition at 25°C and 15°C for 48 and 96 hours, respectively, and the development of resistance.

Results: Application of phage cocktail in vitro at MOI of 10^3 completely inhibited the growth of ten different *Salmonella* serovars, while delaying the growth of six serovars for more than five hours and less than five hours of four serovars. Bacteria challenge experiment using *S.* Enteritidis treated with different phage concentrations (MOIs 10^{-1}-10^3) showed complete growth inhibition at 25°C and 15°C for 48 and 96 hours, respectively. No cross-resistance to all phages in the cocktail was observed. Biocontrol experiments on chicken carcass are in progress.

Significance: These results suggest that using phage candidates that bind to different receptors to formulate the phage cocktail is a promising strategy to ensure mitigating *Salmonella* contamination risk during poultry processing.

P1-121 Inhibition of *Clostridium perfringens* during Cooling of Model Uncured Poultry Products Using Combinations of Lactate, Diacetate, and Propionate

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Introduction: The 2017 USDA Appendix B guidelines are intended to inhibit sporeforming bacteria during cooling, regardless of product formulation. However, Phase 1 cooling from 48.9°C to 27°C in 1 hour is thermodynamically difficult in achieve in large diameter uncured products; therefore, these products may require antimicrobial addition to extend cooling.

Purpose: To determine inhibition of *Clostridium perfringens* in uncured poultry using combinations of organic acid antimicrobials (sodium lactate, diacetate, and propionate) during extended Phase 1 cooling.

Methods: Twenty-seven model uncured turkey formulations (pH 6.4, 1.4% salt, 75% moisture) were tested representing 3 levels each of propionate or diacetate (0, 0.25, and 0.5%) or lactate (0, 1.25, and 2.5%) using a multilevel factorial design. Formulations were inoculated with *C. perfringens* spores (3 log CFU/g), vacuum-packaged, and cooked to 70°C for two minutes. Samples were cooled from 48.9°C to 27°C (3, 4, or 5 hours), from 27°C to 12.8°C in 5 hours, and 12.8°C to 4.4°C in 5 hours (13-15 hours total cooling). Triplicate samples were enumerated for *C. perfringens* at pre-cook, post-cook, and end of each cooling phase.

Results: *C. perfringens* populations increased 4.0, 2.9, and 1.8 log in control (no antimicrobial), 0.25 and 0.5% diacetate treatments, respectively, when Phase 1 cooling was extended to 3 hours. Similarly, *C. perfringens* increased >1 log with extended cooling in products formulated with low levels of propionate, alone or in combination with diacetate. In contrast, *C. perfringens* increased <1 log when Phase 1 cooling was extended to 5 hours with 2.5% lactate alone or 1.25% lactate+0.5% diacetate, but only for 4 hours with 1.25% lactate+0.5% propionate+0.25% diacetate.

Significance: Phase 1 cooling can be extended to 5 hours depending on the combination of organic acid salts. Data from this study can be used to identify minimum concentrations of organic acids needed in cultured sugar-vinegar blends to extend cooling of uncured meat and poultry products.

P1-122 Novel Multi-Strain Probiotics Reduces *Pasteurella multocida* Induced Fowl Cholera Mortality in Broilers: A Randomized Control Study

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Introduction: *Pasteurella multocida* causes fowl cholera, a highly contagious poultry disease of global concern, causing significant ecological and economic challenges to the poultry industry each year. With the reduction of antibiotics usage in animal production, the need for the application of naturally safe alternatives including probiotics for the improvement of animal growth performance as well as control infectious diseases is imperative.

Purpose: This study evaluated the effects of novel multi-strain probiotics consisting of *Lactobacillus plantarum*, *L. fermentum*, *Pediococcus acidilactici*, *Enterococcus faecium* and *Saccharomyces cerevisiae* on growth performance, intestinal microbiota, haemato-biochemical parameters and anti-inflammatory properties on broilers experimentally induced with *P. multocida*.

Methods: Birds were fed with basal diet supplemented with probiotics (10^8 CFU/kg) and then orally challenged with 10^8 CFU/mL of *P. multocida*. Parameters for growth performance, feed efficiency and gut microbiota were assessed as well as the expression of anti-inflammatory properties using real time polymerase chain reaction (RT-PCR).

Results: Probiotics supplementation significantly ($P < 0.05$) improved growth performance and feed efficiency as well as reducing ($P < 0.05$) the population of intestinal *P. multocida*, enterobacteria, and mortality. Haemato-biochemical parameters including total cholesterol, white blood cells (WBC), proteins, glucose, PCV and lymphocytes improved ($P < 0.05$) among probiotic fed birds when compared with the controls. Transcriptional profiles of anti-inflammatory genes including hypoxia inducible factor 1 alpha (HIF1A), tumor necrosis factor- (TNF) stimulated gene-6 (TSG-6) and prostaglandin E receptor 2 (PTGER2) in the intestinal mucosa were upregulated ($P < 0.05$) in probiotics fed birds.

Significance: The dietary inclusion of the novel multi-strain probiotics improves growth performance, feed efficiency and intestinal health while attenuating inflammatory reaction, clinical signs and mortality associated with *P. multocida* infection in broilers.

P1-123 Bio-Mapping of Pathogens and Indicator Organisms throughout the Poultry Processing Chain Using Hygiena's Microsnap™ and BAX® System Salquant™, and Biomérieux Tempo® Methods

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Introduction: Physical and chemical interventions are heavily used in the poultry industry to reduce *Salmonella* and *Campylobacter* presence on final poultry products driven by the USDA-FSIS performance standards. When only prevalence testing is performed, intervention efficacy is difficult to evaluate, creating the need for quantification-based baseline evaluations.

Purpose: The purpose of this study was to develop an indicator organism and pathogen baseline, with and without chemical interventions, by bio-mapping the processing chain from flock-to-final product of a large USDA-inspected poultry processor.

Methods: Five poultry samples were collected at each poultry sampling location (9), with and without interventions, across 5 days ($n = 450$; Boot swabs, Live-receiving, Rehang, Post-evisceration, Post-chill, Skin-On-Thighs, Skinless-Thigh, Wing-Parts, Ground-Wings). All samples were prepared utilizing a single enrichment source for indicator organisms (Total-Viable-Count and *Enterobacteriaceae*) tested with Hygiena MicroSnap™ and TEMPO®, *Salmonella* enumeration and prevalence tested with BAX® System Real-Time *Salmonella* and SalQuant™, and bioMérieux TEMPO® for *Campylobacter* enumeration. All bacterial counts were converted to log CFU/carcass with comparisons using an ANOVA in JMP® (Version 14.3.0. SAS Institute Inc., Cary, NC, 1989-2019) with significance at $P \leq 0.05$.

Results: There was no intervention effect on the sample population ($P = 0.167$) indicating that physical interventions were just as effective at reducing indicator organisms and pathogens compared to chemical interventions. Only indicator organism counts decreased from live-production boot swabs samples to initial live-receiving samples, however, *Campylobacter* and *Salmonella* counts increased by 2.3 and 0.5 log CFU/carcass, respectively. Post-live receiving, both pathogens and indicator organism counts continued to decrease, regardless of intervention, until further processing where ground wing samples rebounded for prevalence and quantification.

Significance: This study provides evidence that physical interventions are just as effective at reducing indicator organisms and pathogen counts to safe levels. These conclusions can further promote reducing chemical usage while still producing a safe and wholesome product.

P1-124 Salmonella Quantification (SalQuant™) with the Hygiena™ BAX® System for Ground Turkey

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Introduction: Poultry is a well-known reservoir of *Salmonella* and processors have been seeking for analytical methods to quickly determine the levels of *Salmonella* in the environment, flocks, and in-plant products in order to mitigate the risk of foodborne illnesses.

Purpose: Artificial inoculation studies were conducted using specific concentrations of *Salmonella* known to have a negative impact on public health to develop and verify a limits approach and quantification (SalQuant™) in ground turkey using the BAX® System Real-Time PCR assay.

Methods: In two separate experiments, ground turkey was divided into 325-g test portions and inoculated with *Salmonella* Heidelberg at 1 CFU/g (LOD1) and 10 CFU/g (LOD10) for a limits approach or *Salmonella* Typhimurium at 5 levels (1, 10, 1,000, and 10,000 CFU/mL) for quantification. Enrichments were prepared 1:1 in pre-warmed MP media and then a 30 mL aliquot was removed and combined with additional MP media with antibiotics to create 75 samples for limits and 16 samples for quantification. Samples were incubated using shortened enrichment times and tested by real-time PCR. Data for limits was assessed using qualitative results until all 75 samples were positive. Data for quantification was assessed using linear regression to compare the CT value and log CFU/g.

Results: Using the limits approach, 100% positivity was achieved for LOD1 at 8 hours and LOD10 at 6 hours. All results were confirmed as positive following USDA MLG culture procedures. For quantification, the 8-hour enrichment produced the best linear fit equation with an *R*-squared of 0.88 and Log RMSE of 0.53. Compared to MPN, there were no differences in estimations.

Significance: These results demonstrate the ability of the BAX® System Real-Time PCR assay to be used for limits testing to accurately detect 1 CFU/g and 10 CFU/g enriched for 8 and 6 hours, respectively. In addition, complete quantification from 1-10,0000 CFU/g can be achieved utilizing the same sample at 8 hours.

P1-125 Evaluation of Ozonated Water as a Microbial Decontamination Strategy for Chicken Parts

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Introduction: Poultry meat remains one of the food categories responsible for the most outbreak-associated foodborne illness cases. Prevalence of foodborne pathogens, such as *Salmonella*, can be higher in poultry parts than in whole carcasses. Ozone treatment has potential as an antimicrobial intervention for poultry parts in post-chill immersion tanks.

Purpose: To evaluate the effectiveness of ozonated water for reducing *Salmonella* and microbial indicators in chicken parts.

Methods: Chicken wings (average weight 51.3 ± 12.4 g) were immersed in ozonated water generated by a Tetraclean® aqueous ozone system at three concentrations (2.5, 5.0, and 10.0 ppm) for different exposure times (15, 30, and 45 s). Aerobic plate counts (APC) were determined on Petrifilm® plates for rinsates (100 mL/wing). Treatments were repeated on wings inoculated with a five-strain cocktail of poultry-borne *Salmonella* spp. (10^6 CFU/mL per wing). *Salmonella* counts (SC) were determined on XLD plates. Chicken wings treated with water were used as controls. Experiments were performed in triplicate (three wings/per treatment/per replicate).

Results: Increasing treatment time by 30 s reduced APC of treated wings by 0.5 and 1.0 log CFU/mL rinsate at 5 ppm and 10 ppm. Increasing ozone concentration from 2.5 ppm to 10 ppm reduced APC by 1.3 and 0.9 log CFU/mL rinsate at 30 s and 45 s. SC ranged from 5.96 ± 0.10 to 6.20 ± 0.02 log CFU/mL rinsate, with average reductions of 0.5 ± 0.06 log CFU/mL rinsate compared to the inoculated control. Reductions in SC were not significant ($P > 0.05$) and no time or concentration effect was observed.

Significance: Ozonated water treatment at the concentrations tested can reduce *Salmonella* by 0.5 log CFU/mL rinsate in chicken wings. Studies are underway to optimize process parameters to increase reductions, as well as to determine the effect of these treatments on other pathogens, such as *Campylobacter*, and on other chicken parts.

P1-126 Efficacy of Multiple Sequential Interventions at Different Concentrations of Peracetic Acid on *Salmonella* and *Campylobacter* on Chicken Wings

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Introduction: Poultry processors use peroxyacetic acid (PAA) at several steps during processing to reduce the risk of *Salmonella* and *Campylobacter*. However, these sequential steps using the same antimicrobial may not achieve additive reductions in the microorganisms.

Purpose: The objective of the research was to evaluate PAA treatments individually and sequentially on chicken wings to reduce *Salmonella* and *Campylobacter* populations.

Methods: Fresh chicken wings (0.45 kg) inoculated with a cocktail of nalidixic acid-resistant *Salmonella* Typhimurium (200 ppm) and gentamicin-resistant *Campylobacter coli* (200 ppm) were immersed in PAA solutions 100 ppm for 60 min (main chiller) alone or followed by immersion in PAA solutions of 750 and 1,000 ppm for 15 s (post-chill treatment) and/or 250, 500, 750 or 1,000 ppm for 15 s alone or in combinations. There was a total of 13 treatments, an inoculated and an uninoculated control. Treated wings were rinsed in BPW containing sodium thiosulfate (100 mL; 0.1 %; 1 min), serially diluted in phosphate buffered saline (0.1%) containing nalidixic acid (PBS^N; 200 ppm), and plated on APC Petrifilm™ for *Salmonella* in duplicate; diluted in PBS and plated on Campy-cefex agar supplemented with gentamicin (200 ppm) in duplicate for *Campylobacter* enumeration. The experiment was performed in triplicate for each treatment combination on separate days.

Results: Immersion of inoculated chicken wings in 100 ppm PAA for 60 min resulted in 2.03 log CFU/mL reduction ($P \leq 0.05$) in *Salmonella* population. Immersion of inoculated chicken wings in 250, 500, 750 and 1,000 ppm PAA for 15 s resulted in 1.25, 1.57, 1.91 and 1.98 log CFU/mL reduction ($P \leq 0.05$) in *Salmonella* population, respectively. Reductions in *Salmonella* population on chicken wings by immersion in 100 ppm PAA for 60 followed by additional PAA solutions representing post-chill and parts treatments were lower ($P \leq 0.05$) than the additive reductions achieved by individual treatments. Similar results were observed for *Campylobacter*.

Significance: Sequential treatments with the same antimicrobial (PAA) did not achieve additive reductions in *Salmonella* and *Campylobacter* populations on chicken wings.

P1-127 Shelf-life Extension of Raw Chicken Drumsticks by Injection Application of Vinegar- and Ferment-based Antimicrobial Solutions

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Introduction: Raw chicken shelf life can be extended by myriad factors, including antimicrobial application, reducing food waste and supporting supply chain stability. Verdad® N8 and Verdad® Opti Powder N450 are vinegar-based antimicrobials ("V-1" and "V-2", respectively), and Verdad® Opti Powder N60 ("F-1") is a ferment-based antimicrobial.

Purpose: Confirm the efficacy of vinegar- and ferment-based antimicrobials to extend the shelf life of raw chicken drumsticks.

Methods: Raw chicken drumsticks were injected with 1% V-1, 0.75% V-2, 0.90% F-1, or water (control) at a target 15% pump. Post-injection, samples were vacuum-packaged and stored (4.4°C) until enumeration (0, 2, 7, 9, 14, 21, 24, and 30 days). Upon enumeration, drumsticks were diluted in 100 mL neutralizing buffer and hand shaken for 30 s. Appropriate serial dilutions were made in Butterfield's buffer, and samples were plated on aerobic plate count (APC) 3M Petrifilm™ (48 h at 35°C). Three drumsticks were analyzed per sampling date.

Results: When aerobic plate count populations exceeded 6 log CFU/g, treatments were deemed "spoiled." Control drumsticks spoiled by ca. day 8; meanwhile, V-1 samples spoiled at ca. day 22, V-2 samples at ca. day 27, and F-1 samples at ca. day 25. By sampling day 7, control populations (5.34 log CFU/g) were greater ($P \leq 0.05$) than all treatments (3.02, 3.03, and 2.1 log CFU/g for V-1, V-2, and F-1, respectively). Control samples were not plated on day 30 due to spoilage organism proliferation.

Significance: When utilizing a spoilage threshold of 6 log CFU/g aerobic plate count, application of Verdad® N8, Verdad® Opti Powder N450, and Verdad® Opti Powder N60 by injection extended the shelf life of raw chicken drumsticks 14-19 days compared to control.

P1-128 Impact of Set-up Temperatures and Pump Rates for Survival of *Salmonella* and the Surrogate *Enterococcus faecium* in Moisture-Enhanced, Reconstructed Chicken Patties after Double Pan-Broiling

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❖ ***Developing Scientist Entrant***

Introduction: Reconstructed chicken products can be contaminated with foodborne pathogens in the process of moisture enhancement (MH). *Enterococcus faecium* has been recognized as a surrogate for *Salmonella* however they are not validated in cooking of chicken products.

Purpose: This study was designed to compare kinetic parameters of *Salmonella* and *E. faecium* in MH-reconstructed chicken patties with different pump rates during double pan-broiling with various set-up temperatures.

Methods: Fresh 1.5-kg chicken breast was course grounded, inoculated with *S.* Typhimurium and Tennessee, or *E. faecium*, followed by adding NaCl (2.0%) + Na-tripolyphosphate (0.5%) solutions to achieve pump rates of 1%, 5% or 11.1%. Samples were manually manufactured into patties with the thickness of 2.0 cm and diameter of 10.5 cm. Patties were packaged with polyvinyl chloride films in the foam-tray stored at 4°C for 42 h before double pan-broiling on a griller set at 200, 300, or 425°F for 0, 30, to 420 s. Counts of pathogens were analyzed on XLT-4 and bile esculin agars with tryptic soy agar layers. Microbial data and kinetic parameters ($n = 9$, USDA-Integrated-Predictive-Modeling-Program/USDA-Global-Fit software) were analyzed by the Mixed Model Procedure (SAS).

Results: Double pan-broiling reduced >5 log CFU/g ($P < 0.05$) of *Salmonella* after 360 (200°F), 180-225 (300°F), and 150-165 s (425°F), and of *E. faecium* after 270 s (300°F), and 180 s (425°F) across all samples. *D*-values (Weibull-model) of *Salmonella* and *E. faecium* in 1% MH samples cooked at 200-425°F (102.7-248.2 and 115.5-271.0-s) were lower ($P < 0.05$) than 11.1% samples (119.8-263.7 and 122.5-298.3-s). *Salmonella* were more susceptible ($P < 0.05$) to heat than *E. faecium*. "Shoulder-time" (Buchanan-Two-Phase-model) of *Salmonella* cooking at 200-425°F increased ($P < 0.05$) from 82.3-229.0 to 116.6-246.2-s as pump rate increased from 1 to 11.1%, whereas this phenomenon was not shown for *E. faecium*.

Significance: *E. faecium* can be used as a surrogate for *Salmonella* in thermal inactivation validation studies of chicken products.

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P1-129 Application of Peroxyacetic Acid in Combination with an Acidifier Post-Defeathering for Reduction of Campylobacter from Broiler Chicken

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❖ ***Developing Scientist Entrant***

Introduction: *Campylobacter* is commonly found in association with poultry meat. Various antimicrobial interventions during poultry processing are currently being tested to minimize or eliminate *Campylobacter* on poultry meat.

Purpose: The purpose of this study was to assess the effect of a peroxyacetic acid (PAA) and acidifier combination applied post-defeathering for reduction of *Campylobacter* from poultry carcasses and parts in a commercial broiler chicken processing facility.

Methods: In a commercial broiler processing facility with two lines, line 1 served as a control while on line 2 broiler carcasses were dipped for 5 s in PAA (475–525 ppm) solution, maintained at 1.4 to 1.6 pH with an acidifier (Amplon), immediately post-defeathering. For each of eight repetitions, three carcasses or pooled wings from each line were sampled post-defeathering, post-dip (line 2 only), post-chill, and post-deboning (wings) for the counts and prevalence of *Campylobacter*. Data were analyzed by sampling location and processing line using the GLM or Chi-square procedure in SAS.

Results: There was no differences in *Campylobacter* counts (log CFU/mL) or prevalence at each location between lines 1 and 2 ($P > 0.05$). However, *Campylobacter* counts and prevalence significantly decreased during the processes between defeathering and carcass chilling ($P = 0.0026$, $P < 0.0001$, respectively). The *Campylobacter* counts post-defeathering, post-dip, post-chill, and post-deboning were 2.86, 3.00, 0.70, and 1.20 (detection level = 5 CFU/mL). Similarly, *Campylobacter* prevalence following enrichment post-defeathering, post-dip, post-chill, and post-deboning was 48/48, 24/24, 16/48, and 23/48 (detection level = 50 CFU/mL).

Significance: These results indicate that dipping in acidified PAA following carcass defeathering did not have significant downstream impacts on post-chill broiler chicken carcass or post-deboning wing *Campylobacter* levels or prevalence. Carcass treatment following defeathering may not be beneficial for *Campylobacter* final product safety. However, commercial practices occurring between defeathering and post-chill are effective in reducing *Campylobacter* levels and prevalence.

P1-130 Efficacy of On-Site Generated Peroxyacetic Acid (PAA) in Reducing *Salmonella* and *Campylobacter* Populations on Chicken Wings

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Introduction: Peroxyacetic acid (PAA) is widely used in poultry processing as an antimicrobial and is transported as a concentrated PAA solution. On-site generation of PAA using non-hazardous ingredients will be helpful to minimize transportation and storage.

Purpose: The objective of this research was to evaluate the efficacy of on-site generated PAA in reducing *Salmonella* and *Campylobacter* populations and its effect on product color.

Methods: A 2 (PAA types; SaniDate [PAA] or OxyFusion [O-PAA) x 2 (Concentrations; 50 and 100 ppm) x 2 (pH; 8.5 or 10) x 2 (exposure times; 10 s or 60 min (to replicate broiler carcass chilling)) experimental design was used. Three independent replications ($n = 3$) were performed using fresh chicken wings (0.45 kg; 8 pieces/treatment for each replication). Chicken wings were inoculated with a cocktail of nalidixic acid-resistant *Salmonella* Typhimurium and gentamicin-resistant *Campylobacter coli* and were subjected to different treatments. Treated chicken wings were rinsed in BPW containing sodium thiosulfate (100 mL; 0.1%; 1 min), serially diluted in peptone water (0.1%) containing nalidixic acid (PW^N; 200 ppm) and plated on APC Petrifilm™ for *Salmonella*; diluted in PW and plated on Campy-cefex agar supplemented with gentamicin (200 ppm) for *Campylobacter* enumeration.

Results: Immersion of chicken wings in 100 ppm PAA for 60 min, irrespective of pH resulted in greater reductions ($P \leq 0.05$) of *Salmonella* by 1.68 and 1.42 log CFU/mL for PAA, 1.82 and 1.83 log CFU/mL for O-PAA, respectively. Similarly, *Campylobacter* reductions were 1.59 and 1.36 log CFU/mL for PAA, 1.63 and 1.71 log CFU/mL for O-PAA. The antimicrobial efficiency of PAA was not affected by pH ($P > 0.05$) and type of PAA solution ($P > 0.05$). Treatments did not affect ($P > 0.05$) the color of the chicken wings.

Significance: On-site generated PAA may provide poultry processors an effective alternative to concentrated PAA solutions, minimizing transportation and storage hazards.

P1-131 Efficacy of Peracetic Acid Gel Against *Salmonella* Typhimurium Inoculated on Chicken Skin

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❖ ***Developing Scientist Entrant***

Introduction: Peracetic acid (PAA) is a common antimicrobial used in poultry slaughter processing plants used in aqueous form to improve *Salmonella* and *Campylobacter* food safety. As a sustained-release mechanism, the antimicrobial efficacy of PAA incorporated in edible gels was investigated to reduce PAA usage while maintaining food safety.

Purpose: The purpose of the study was to evaluate PAA gel's antimicrobial efficacy on the survival of *Salmonella* Typhimurium (ST) inoculated on chicken skin.

Methods: The PAA gel was prepared by combining locust bean gum (0.25 g) and guar gum (0.25 g) in a 0.0105% peracetic acid (PAA) solution (100 mL). Chicken skin coupons (1 cm diameter) ($n = 36$/trial) were cut along the feather line and inoculated with nalidixic acid resistance (35 µm/mL) *ST* (50 µL) on coupons (target inoculation 103 CFU/mL) and placed at 4°C for 30 min for microbial attachment. Inoculated coupons were subjected to the following treatments for 0, 5, and 15 min: 1. Control – No treatment; 2. PAA 0.0105% (50 µL); 3. Control gel without PAA (50 µL); 4. PAA (0.0105%) gel (50 µL). At each sampling time, three coupons per treatment were rinsed in neutralizing buffered peptone water (0.52 % Na2S2O3) (nBPW), placed in a sterile whirl-pak bag, and rinsed with 2 mL BPW for 1 min, rinsates were spread plated on XLT4 + nalidixic acid (35 µm/mL), and the plates were incubated at 37°C for 24 h. Viable colonies were reported as log CFU/mL of rinsate. A total of 108 samples (3 samples/trt x 4trt x 3 sampling times x 3 trials) were analyzed over three independent trials and data was analyzed using GLM procedure with Tukey HSD ($P < 0.05$).

Results: PAA gel did not perform significantly different ($P < 0.05$) than the control.

Significance: We were able to successfully make PAA gels which can be used in poultry processing, but its effective concentrations and contact times should be determined.

P1-132 Pathogens Turn Hypervirulent during Colonization of Food: *Salmonella* Enteritidis in Egg as an Example

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❖ ***Developing Scientist Entrant***

Introduction: Outbreak data demonstrated that *Salmonella* infectious dose varied drastically. Studies suggested that food vehicles involved in those outbreaks may play a significant role in such variation, but direct evidence is lacking.

Purpose: To demonstrate the contribution of different growth matrices, including egg yolk, to the virulence of *Salmonella* Enteritidis.

Methods: To measure changes in the expression of its virulence genes in response to growth matrices, *Salmonella* was transformed with reporter plasmids containing an antibiotic-resistant marker and a luciferase operon (producing luminescence) the promoter of which was replaced with regulatory region of *Salmonella* virulence gene *sopB* or *sseA*. Luminescence was monitored during *Salmonella* growth in yolk or TSB and results from three repeats were compared using two-way ANOVA. Female C57BL/6 mice were fed with *Salmonella* grown in TSB, *Salmonella* grown in yolk, or *Salmonella* grown in TSB followed by sterile yolk. Within each treatment group, each cage of 5 mice was challenged with one of the 10-fold dilutions of *Salmonella* (10^7 CFU-10^2 CFU). Controls included sterile TSB, sterile yolk and sterile TSB followed by sterile yolk. The experiment was repeated twice and LD_{50} was calculated using GraphPad Prism.

Results: At late exponential phase, *Salmonella* virulence genes *sopB* and *sseA* were profusely expressed. Expression of both genes were significantly higher when *Salmonella* was grown in the yolk compared to those in TSB (P = 0.0019 and 0.0306, respectively). In the mouse study, *Salmonella* LD_{50} was the lowest when the pathogen was grown in yolk. In this case the LD_{50} was 280.7 CFU, compared to 1,086 CFU in TSB and 4,616 CFU in TSB followed by sterile yolk.

Significance: The study provided both transcriptomic and *in vivo* evidence that a food matrix (egg yolk) could turn a foodborne bacterium (*Salmonella*) into a hypervirulent pathogen. This knowledge is crucial for preventing and monitoring salmonellosis outbreaks.

P1-133 *Salmonella* Quantification of Various Pork Matrices Utilizing Hygiena's BAX® System SalQuant™

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❖ ***Developing Scientist Entrant***

Introduction: In 2019, the United States Department of Agriculture Food Safety Inspection Service (USDA-FSIS) announced the final rule to the Modernization of Swine Slaughter Inspection that requires pork producers to conduct additional microbial testing for *Salmonella*. Quantification of *Salmonella* utilizing BAX® System SalQuant™ can provide the pork industry with a reliable and efficient system to monitor contamination throughout pork production.

Purpose: Multiple studies were performed to develop rapid and predictable enumeration method for *Salmonella* in pork products: ground pork, pork trim, MicroTally™ Manual-Sampling-Device (MSD) for pork trim, pork carcass swabs, and pork head trim rinsates utilizing SalQuant™.

Methods: Primary enrichments were created for each matrix, apart from Head Trim Rinse, using varying levels of Buffered Peptone Water (BPW). Each matrix utilized a secondary enrichment which included 30 mL of the primary enrichment homogenate and 30 mL of quant solution, except Pork Trim and MicroTally™, tested directly from primary enrichment. Sixteen samples per matrix were spiked with 0.00-4.00 log CFU/mL(g) of *Salmonella* Typhimurium with three biological replicates per level. Samples were incubated at 42°C and five technical replicates per biological replicate were tested on the BAX® System at various timepoints. Pre-enrichment levels of *Salmonella* in pork products were estimated by creating linear-fit equations utilizing BAX® Cycle Threshold (CT) value. Matrices were compared to MPN with no statistical difference between populations ($P \leq 0.05$).

Results: The results of this study demonstrate the ability of BAX® System SalQuant™ to accurately detect and enumerate pre-enrichment levels of *Salmonella* across a wide enumerable range for pork matrices. All linear-fit equation estimations with shortened enrichment times met statistical parameters with R^2 ranging from 0.79 – 0.93 and Log RMSE from 0.39 to 0.67.

Significance: Utilization of a rapid PCR-based enumerative method provides the pork industry with an enumeration tool for data-driven decisions throughout the pork supply chain to mitigate the risk of foodborne illness.

P1-134 Detection and Prevalence of Salmonella in Swine Lymph Nodes at Harvest

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Introduction: Research demonstrates *Salmonella*-positive lymphoid tissues present a potential hazard to the safety of ground, or other final pork products, and the United States Department of Agriculture (USDA) will require increased *Salmonella* testing standards, thus prompting the need for a better understanding of *Salmonella* prevalence within different swine tissues.

Purpose: The purpose of this study was to gather *Salmonella* prevalence data from somatic and splanchnic lymph nodes in swine.

Methods: Lymph nodes (n=197) were aseptically collected from splanchnic (mesenteric and tracheobronchial) and somatic (inguinal and subiliac) locations from 50 different swine carcasses at harvest. Lymph nodes were trimmed of surrounding fat and fascia, sterilized via submersion in a boiling water bath, pulverized with a rubber mallet, and incubated in tryptic soy broth (TSB) for 6 hours at 42°C. Sample enrichments were subject to immunomagnetic separation (IMS) using anti-*Salmonella* beads, spread plated onto xylose lysine desoxycholate (XLD) and brilliant green sulfa (BGS) agar, and incubated for 18-24 hours at 35°C. *Salmonella* positive samples were identified via latex agglutination and confirmed using PCR.

Results: The overall *Salmonella* prevalence rate was 21.8%, with 43 positive lymph node samples from 31 different animals. Lymph node prevalence varied based on location (mesenteric, 34%; inguinal, 18.4%; subiliac, 18.4%; tracheobronchial, 16.3%) but, these differences were not statistically different (P=0.1403).

Significance: This research demonstrates that splanchnic and somatic lymph nodes are a source of *Salmonella* in swine carcasses. Testing lymph node tissues for *Salmonella* is not routine; therefore, these data enhance understanding of *Salmonella* in swine and may inform future intervention or testing techniques.

P1-135 Validation of Post-Harvest Antimicrobial Interventions to Control Shiga Toxin-producing Escherichia coli (STEC) on Market Hog Carcass Surfaces

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❖ ***Developing Scientist Entrant***

Introduction: Even though swine-associated STEC outbreaks have been reported less frequently than cattle, the likelihood that swine represent an important source of STEC infections in human beings cannot be underestimated.

Purpose: This study compared 8 carcass antimicrobial intervention technologies applied using commercial equipment and parameters to quantify their ability to reduce STEC contamination on market hog carcasses.

Methods: Hogs were harvested to provide skin-on carcass sides, and 8 sides (per replication) were inoculated with a STEC cocktail (ca. 5 log CFU/cm^2) including *E. coli* O157:H7 and non-O157 strains *E. coli* O145:NM, O121:H19, O111:H-, O103:H2, O45:H2, and O26:H11.Each side was treated in a commercial CHAD cabinet using a spray [low-volume: 3% lactic acid (*l*LA), 400 ppm peracetic acid (*l*PAA), acidified 400 ppm peracetic acid (*l*aPAA)] or wash [high volume: ambient water (*h*AW), 400 ppm PAA, 400 or 600 ppm bromous acid (*h*DBDMH), 71°C water (*h*HW)] treatment within a randomized complete block study design. Post-treatment and post-chilling STEC reductions were compared. Color changes were determined on lean, adipose, and skin after treatment application and after 18 hours of carcass chilling (2°C).

Results: The *h*HW, *h*PAA, and *h*DBDMH$_{600}$ deluge washes achieved the greatest post-chilling STEC reductions (3.8, 3.4, and 3.2 log CFU/cm^2, respectively), and were significantly ($P \leq 0.05$) more effective than the other intervention technologies, including the 1.7-log reduction achieved by the *ambient water* control. The carcass washes were less effective at reducing STEC populations attached to interior carcass cavity, with post-chilling STEC populations reduced

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by 0.9-2.2 log cycles, while the *h*AW control wash achieved a 0.6-log reduction. None of the treatments negatively impacted carcass color quality.

Significance: All market hog carcass interventions reduced STEC populations, thus equipping pork processors with information to support decision-making and flexibility in choice when selecting an intervention that is appropriate for their specific facility and operation.

P1-136 Creation and Characterization of a Film with a Color pH Indicator Coating to Determine the Spoilage of Beef, Using Bio-Based Materials

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❖ ***Developing Scientist Entrant***

Introduction: Intelligent packaging utilizes chemical sensors to identify the quality and determine the safety of packaged beef for consumers. Intelligent packaging has potential to indicate bacteria associated with foodborne illness.

Purpose: To develop and characterize a film with a color pH indicator coating using bio-based materials to determine spoilage of beef.

Methods: A film coating with a color pH indicator to determine spoilage of beef was successfully developed using the bio-based material pectin as a coating matrix and bromocresol green as the color pH indicator compound. Films were expected to change from yellow to blue. Control samples and treatment samples (i.e., film with a color pH indicator coating) were exposed to two different treatments: stored at 4 ± 1°C for and temperature abuse for 13 days. Beef samples were tested for pH (n = 100) and aerobic bacteria plate count (n = 100). Film samples were tested for color parameters (L^*, a^*, b^*, c^* and $h°$) (n = 100), mechanical properties (n = 50), water vapor transmission rate (n = 4), oxygen transmission rate (n = 4) and thermal properties (n = 4).

Results: The color pH indicator film detected spoilage of beef based on pH. The color change of the color pH indicator film was expected to activate upon change in pH level. The average b^* value changed significantly ($P < 0.001$), the film changed from yellow to dark blue during shelf life. There was not significant difference ($P < 0.05$) between control and treatment samples in aerobic bacteria plate count, mechanical properties and water and oxygen transmission rate, meaning the color pH indicator coating did not affect the preservation of beef.

Significance: This film with a color pH indicator coating for beef provides reliable and accurate information about the spoilage of beef.

P1-137 Occurrence and Distribution of Shiga Toxin-producing and Enterohemorrhagic *Escherichia coli* in Extensive and Intensive Beef Production Chain in Brazil

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Introduction: Shiga Toxin-producing *Escherichia coli* (STEC) can cause severe illness in humans, and specific strains can be characterized as Enterohemorrhagic *E. coli* (EHEC), usually with higher pathogenicity. Cattle are STEC and EHEC reservoirs, being important sources of contamination to beef.

Purpose: To characterize the occurrence and distribution of STEC and EHEC in extensive and intensive beef production in Brazil.

Methods: Animals from extensive (n = 100) and intensive (n = 100) beef production chains were sampled in four steps of production: feces (1), carcasses after bleeding (2), after evisceration (3) and after washing (4). Samples were subjected to STEC detection (ISO 13.136). Suspect isolates were screened for *stx* (STEC), and then for *eae* (EHEC), through PCR. EHEC isolates were subjected to XbaI digestion and Pulsed-field Gel Electrophoresis (PFGE).

Results: STEC were detected in 50 and 24 animals from extensive and intensive production, respectively, in at least one production step. STEC were more prevalent in feces in both extensive (94.0%) and intensive (95.8%) production. Based on *stx*, 251 isolates were characterized as STEC, from which 22 (8.8%) also harbored *eae* (EHEC), 3 from intensive and 19 from extensive production. PFGE analysis revealed a high clonal diversity among EHEC isolates, allowing the clustering of 3 different groups that shared 90.2%, 87.4% and 96.3% of band matching. The major group included 15 isolates, all from extensive production, 5 from carcasses and 10 from feces.

Significance: This study provides evidence regarding cattle extensive and intensive production as reservoirs of STEC, with relevance for EHEC in extensive production. Acknowledgments: CNPq, CAPES and FAPEMIG.

P1-138 Lethality of *Salmonella* during the Drying of Restructured Beef Jerky

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✦ ***Undergraduate Student Award Entrant***

Introduction: Drying is one of the oldest methods of food preservation. Jerky is a valuable product in the United States and an important shelf-stable protein source in many developing nations. When food is limited, consumers are particularly vulnerable to foodborne hazards. The drying process involves the dehydration of meat through the addition of salt, circulation of air, and thermal processing, which can be accomplished through natural or mechanical means. Mechanical failure or loss of power likely reduces the lethality of the thermal process.

Purpose: The purpose of this study is to investigate lethality of *Salmonella* on restructured beef jerky during a 6-h drying process. Data generated will provide information for jerky producers to accurately assess microbial risk associated with their product at various intervals during dehydration.

Methods: A trimmed eye of round was ground, seasoned with a commercial jerky seasoning (1.5% salt), and inoculated with five serotypes of *Salmonella enterica* grown with or without acid adaptation (1% glucose). Twenty-gram strips were formed and placed in a home-style dehydrator with a target temperature of 70°C. Samples were plated for enumeration at 0, 0.25, 0.5, 1, 2, 4 and 6 h of drying.

Results: Acid-adapted and non-adapted *Salmonella* display a non-linear, inverse sigmoidal inactivation curve during the 6-h drying process. The acid-adapted *Salmonella* reduced at a faster rate and to a further extent than the non-adapted organisms. After 6 hours of drying, a 6.14-log reduction of acid-adapted *Salmonella* and a 5.25-log reduction of non-adapted *Salmonella* were observed. Differences in final weight loss was not observed between treatments ($P = 0.67$) and strips achieved a final a_w of 0.741 ± 0.018.

Significance: Processors can utilize these results to identify appropriate risk mitigation strategies, should they experience a disruption in the jerky-making process. Under the described conditions, acid adaption did not increase *Salmonella* survivability.

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P1-139 *Escherichia coli* O157:H7 and *Salmonella* Occurrence in Raw Ground Beef Samples Collected at Retail

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Introduction: The Food Safety and Inspection Service (FSIS) collects raw ground beef (RGB) samples at retail and tests them for both *E. coli* O157:H7 and *Salmonella*. The agency uses the results from this sampling project to identify pathogen contamination of beef ground at retail and to facilitate the removal of adulterated product from commerce.

Purpose: To analyze *E. coli* O157:H7 and *Salmonella* data from RGB samples collected at retail to determine the potential risk to consumers.

Methods: The FSIS Office of Investigation, Enforcement and Audit (OIEA), Compliance and Investigations Division (CID), collected RGB samples from retail stores under the Agency's MT05 sampling project. Samples were collected using criteria in FSIS Directive 8010.1 and sent to FSIS laboratories for analysis. If a sample tested positive for *E. coli* O157:H7, FSIS may have recommended a recall and conducted follow-up testing. *Salmonella* positives were not subject to follow-up testing because *Salmonella* is not an adulterant in the product.

Results: FSIS analyzed MT05 samples obtained between August 2014 and December 2020. Samples were collected from retail locations in all 50 states and from Puerto Rico. There were 3,424 MT05 samples tested for *E. coli* O157:H7 and 3,428 samples tested for *Salmonella*. A total of five samples were positive for *E. coli* O157:H7 (0.15%), and 78 samples were positive for *Salmonella* (2.28%; 30 different serotypes). In contrast, the percentage of RGB samples collected from producing establishments (MT43 sampling project) during 2014-2020 positive for *E. coli* O157:H7 and *Salmonella* was 0.07% and 1.7%, respectively. No MT05 samples were positive for both pathogens.

Significance: Finding positive results can help protect public health by removing contaminated product from the marketplace and informing the Agency on the adequacy of food safety controls applied through commerce and retail.

P1-140 STEC Screening & Identification in Raw Beef: 8 Hour Foodproof® STEC Method Now AOAC-RI PTM Approved

Stefanie Wendrich, Hanna Hartenstein, Ivo Meier-Wiedenbach, Astrid Grönewald, **Cordt Grönewald** and Kornelia Berghof-Jäger

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Introduction: The **food**proof® STEC real-time PCR method enables a rapid and specific detection and identification of Shiga-toxin producing *Escherichia coli* (STEC) in food with only two PCR reactions. Following DNA extraction with the **food**proof® StarPrep Three Kit, the **food**proof® STEC Screening LyoKit detects *stx1, stx2,* and *eae* individually, and the **food**proof® STEC Identification LyoKit identifies eight major STEC serogroups in one single test using melting curve analysis. The assays are in accordance with ISO/TS 13136. The rapid enrichment protocols for fresh raw beef samples have received AOAC-RI Performance Tested Methods℠ (PTM) Certification (No. 102004) in October 2020.

Purpose: For a fast and cost-effective analysis of meat samples, we have developed rapid enrichment protocols for fresh raw beef.

Methods: Our STEC method was compared to the USDA reference method MLG 5C.00. 375-g samples were analyzed after 12 h and 20-24 h enrichment time and 25-g samples after 8 h and 20-24 h enrichment time. For inclusivity, 446 strains were tested. For exclusivity, 184 strains of closely related bacteria, non-STEC organisms, and STEC strains of known serogroups other than the eight major STEC O groups were analyzed.

Results: Our method demonstrated no significant differences between presumptive and confirmed results or between candidate and reference method results for 375-g test portions after 12 h and 20-24 h enrichment time and for 25-g test portions after 8 h and 20-24 h enrichment time. Inclusivity was 100%.

Significance: Our method is an effective alternative to the USDA reference method. An advantage is the rapid enrichment protocols, validated for 25-g test portions and 375-g test portions, respectively. The enrichment medium without antibiotics saves costs. The alternative confirmation procedure increases sensitivity and specificity, and reduces time to result and costs. For best convenience, safety, and sensitivity, the PCR reagents are lyophilized.

P1-141 Using a Limits Approach to Detect Specific Levels of *Salmonella* in Beef Trim and MicroTally™ Swabs with the Hygiena™ BAX® System

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Introduction: *Salmonella* often contaminates cattle during rearing and can remain attached to the hide or carcass during processing. If in-plant interventions and sanitary dressings do not effectively reduce levels of *Salmonella*, beef products including trim and ground beef can become contaminated. Due to the constant number of *Salmonella* outbreaks in ground beef over the past decade, some processors want to know how much *Salmonella* is present to effectively manage products posing a high public health risk.

Purpose: Using a 10 CFU/g threshold, the BAX® System was used in a limits approach to establish enrichment parameters to detect *Salmonella* in beef trim and MicroTally™ swabs.

Methods: Beef trim and MicroTally swabs were inoculated with a cold stressed *Salmonella* Typhimurium at 1,500 CFU (equivalent to 10 CFU/g). One-hundred fifty gram samples of beef trim (n = 30) were homogenized with 600 mL of pre-warmed MP media, while swabs were homogenized with 200 mL of either pre-warmed MP media (n = 30) or mTSB (n = 30). All samples were incubated at 42°C for 3-6 hours. Following enrichment, samples were analyzed every hour by real-time and standard PCR until all samples were positive.

Results: Both PCR assays had a positive detection rate of 100% at 5 hours for beef trim and 4 hours for swabs (MP and mTSB enrichments), indicating the concentration of *Salmonella* exceeded the 10 CFU/g threshold. All samples were also confirmed as positive following the USDA MLG reference culture method. Since PCR and culture were in complete agreement, dPOD analysis indicates there is no significant difference.

Significance: The BAX® System limits approach allows processors to identify combo bins of beef trim that contain levels of *Salmonella* that pose an increase risk to public health and take action before further processing to mitigate the risk of foodborne illness.

P1-142 Use of New Markers for Precise Detection of Pathogenic Shiga Toxin-producing *Escherichia coli*

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Introduction: The USDA-FSIS has indicated that only ~10% of samples that contain stx, eae, and a serogroup gene can be culture confirmed. Newly commercialized biomarkers (espK, and espV) that are present in potentially pathogenic *eae*-positive STEC can reduce the number of non-culture confirmable PCR screening results and increase the reliability of STEC screening assays.

Purpose: To evaluate the correlation between the presence of *stx*/*eae* genes from pathogenic STEC strains and the new biomarkers (NM) in STEC isolates.

Methods: Presence of *stx* and *eae*, and NM were determined using bioinformatics (3901 *E. coli* genomes with 775 pathogenic STECs) and GENE-UP® EH1 (*stx, eae*), and NM kits. A panel of 96 *E. coli* strains isolated from cattle, beef, and clinical cases were tested on 1) freshly grown, and 2) archived frozen stocks of the strains for the strain evaluation (total N = 192). Prequalified (USDA MLG Ch 5A&B/USMARC potential positive) archived beef enrichments (N = 288) were used for retrospective analysis. Data from EH1 and NM was compared to the culture methods as a reference. For a prospective analysis 45

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samples were chosen to assess the prevalence and EH1 and NM correlation.

Results: For the genomes and STECs strains, 99% (774/775) and >98% (162/165) correlation, respectively, was observed between NM and EH1. The retrospective analysis indicated 15 samples with eae-positive STEC isolated, and 96 negative for non-pathogenic *E. coli*. Sensitivity and specificity for NM+EH1 was 100% and 98.9% with the overall accuracy of ~99%. In the prospective analysis, out of 45 samples, 12 were NM+EH1+, 4 EH1- NM+, and 29 were EH1-NM-.

Significance: EH1+NM kits can provide precise detection of *eae*-positive STEC, while reducing presumptive positives sample numbers and implicate less product for diversion leading to significant time, cost savings.

P1-143 Fagecapsules, Micro-Encapsulated *Salmonella* Bacteriophages with Targeted Intestinal Release

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✦ ***Undergraduate Student Award Entrant***

Introduction: *Salmonella* represent a significant problem for poultry production, mainly associated with the consumption of chicken meat contaminated with *Salmonella* along with the possibility of transmitting antimicrobial resistant *Salmonella*. Within the enormous diversity of *Salmonella* serovars, Enteritidis, Typhimurium, and Infantis are recognized as being of major importance. Therefore, Bacteriophage (phage) appears as an alternative for the biocontrol of *Salmonella*, even antimicrobial resistant strains. To control *Salmonella* in chickens, phage cocktails need to be delivered to the intestine in an adequate concentration and viability.

Purpose: To micro-encapsulate a cocktail of lytic and specific bacteriophages against *S.* Enteritidis (Ph *SE*), *S.* Typhimurium (PH *ST*), and *S.* Infantis (PH *SI*) with the targeted intestinal release, for application in broiler chickens.

Methods: We sequenced and further characterized the 3 phages on their host range, their transduction ability, the One-Step, and their effective multiplicity of infection (MOI). Different formulations were tested to micro-encapsulate the phages, and targeted intestinal release was tested through *in vitro* stability assays on simulated gastric fluid (SGF) and phage release in simulated intestinal fluid (SIF) in comparison with non-encapsulated phages.

Results: The three phages belonged to different families (2 *Siphoviridae* and one *Myoviridae*). These phages were non-transducers of resistance genes, represented distinct host ranges and one step curves. The effective MOI was 100 for all of them. Formulation with liposomes showed efficiencies of encapsulation between 61-75%, while a second layer of alginate increased to 68-95% of effectiveness. Stability in SGF in comparison with non-encapsulated phages showed reductions in phage titer at 60 min in SGF from 0.5-4.1 logs for encapsulated and 1.5-5.9 logs non-encapsulated. Phage release in SIF achieved at 60 min was between 58-71% ($P < 0.05$).

Significance: Improving the strategies to control *Salmonella* is of great importance, as well as the development of new phage-based interventions that ensure the targeted release of phages.

Acknowledgment: FONDEF IDEA I+D: ID18I10235 FageCapsuleS, micro-encapsulated *Salmonella* bacteriophages with small and large intestine release technology.

P1-144 Biofilm Formation of *Salmonella* Serovars at Two Temperature Conditions

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Introduction: *Salmonella* has over 2,500 serovars that can express vastly different phenotypes and so create challenges regarding removal in processing plants. A significant phenotype is the ability to produce biofilms on surfaces and survive despite processing interventions. This persistence in the processing environment can lead to potential product contamination.

Purpose: The purpose of this study was to determine the differences in the biofilm forming ability of several different *Salmonella* serovars.

Methods: Twenty-two isolates of *Salmonella* belonging to 11 serovars that are commonly associated with food animals, especially poultry, were examined. The biofilm forming capabilities of the isolates on plastic and steel surfaces at two temperature conditions (25°C and 15°C) were assessed in triplicate. *Salmonella* was grown in plastic microtiter plates and steel coupons and biofilm assessed using crystal violet assay.

Results: Biofilm formation significantly differed ($P < 0.05$) among *Salmonella* isolates at both temperatures on plastic surface, but no differences were observed on steel surface. At 25°C, many of the isolates produced strong (9/22 isolates) and moderate (8/22) biofilm on the plastic surface compared to steel surface (3/22) and (14/22), respectively. At 15°C, a few isolates (6/22) were strong biofilm producers on plastic surface while there were none on steel surface. *Salmonella* serovar Schwarzengrund isolates consistently produced strong biofilm and serovar Newport isolates produced weak biofilms. As expected, the biofilm forming capability of some of the isolates was reduced at the lower temperature.

Significance: These results suggest that *Salmonella* serovars differ in their attachment to surfaces and subsequent biofilm production, which may influence the persistence of some strains in food processing environments. These differences in biofilm formation could provide useful information for mitigation of *Salmonella* in processing environments.

P1-145 Does Enriching in Modified Tryptic Soy Broth with Novobiocin Lead to Selection Bias in *Escherichia coli* Populations?

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Introduction: A much higher prevalence of the locus of heat resistance (LHR) was noted in generic *E. coli* recovered from combo bin via enriching in modified Tryptic Soya Broth supplemented with novobiocin (mTSB; 37.3%), a commonly used method for enriching *E. coli*, compared to *E. coli* from beef processing environments recovered by direct plating (1.6%).

Purpose: To determine whether enriching in mTSB with novobiocin favors the growth of *E. coli* harboring LHR.

Methods: A collection of 14 randomly selected *E. coli* strains (7-LHR positive and 7-LHR negative) were grown in mTSB with novobiocin in a 96-well microtiter plate ($n = 6$). The OD_{600nm} was monitored at 20 min intervals for 24 h using a plate reader held at 42°C. All strains were shotgun whole genome sequenced using an Illumina platform (150 x 2 cycles) and the LHR-positive strains were also sequenced by Nanopore technology for long reads. The short reads were quality checked, trimmed and filtered, assembled and annotated using FastQC v0.11.8, Trimmomatic v0.39, SPAdes v3.14.0 and Prokka, respectively. The long reads were quality checked, trimmed, assembled and polished using PycoQC v1.0, Porechop v0.2.4, flye v2.8.2 and Medaka, respectively. Genes associated with novobiocin resistance were made into a local database and searched against the pan genome parsed using Roary.

Results: The growth rates of *E. coli* in mTSB were different among the strains ($P < 0.05$), varying from 0.58 to 0.82 Log OD/h, and the LHR-positive group had a greater growth rate than the LHR-negative group ($P < 0.05$). Although strain variation in MaxOD was also found ($P < 0.05$), no correlation to LHR was observed ($P > 0.05$). No difference in the presence of novobiocin resistance genes or mutations in the DNA gyrase B were noted.

Significance: The findings show that the commonly used enrichment broth mTSB may favor the growth of the LHR-positive *E. coli* sub-populations.

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P1-146 Standardizing the Isolation Source Metadata for the Genomic Epidemiology of Foodborne Pathogens Using LexMapr

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Introduction: FDA's GenomeTrakr is a public/private genomic epidemiology network for foodborne pathogen surveillance, specifically targeting pathogens isolated from food or environmental sources. The raw genome plus a small set of associated metadata are made publicly available at the National Center for Biotechnology Information (NCBI). Metadata include organism name, geographical location, collection date, isolate contributor and isolation source. The isolation source field is currently a free text field, requiring no standard terminologies or structure. As the GenomeTrakr database grew to over 100K isolates and the diversity of isolation sources became more complex, this field became difficult to analyze and interpret using computational approaches.

Purpose: In order to maximize the use of GenomeTrakr data and make this resource FAIR (findable, accessible, interoperable and reusable), we have standardized the metadata for the isolation source of WGS data for publicly available GenomeTrakr records.

Methods: We evaluated and utilized LexMapr, a rule-based text-mining tool, to process the text from the isolation source and extract entities that are mapped to new standard ontology descriptors from relevant ontologies such as: FoodON, ENVO, and UBERON, among others. LexMapr is further deployed to evaluate these newly standardized descriptors, automating the curation of isolation source metadata and assigning categories from the expanded source categorization schema Interagency Food Safety Analytics Collaboration (IFSAC+) based on IFSAC categories.

Results: GenomeTrakr has a total of 9,452 unique isolation sources. LexMapr successfully processed 88% of these records, as determined by manual curation and verification. After the evaluation of LexMapr, 71,886 publicly available records were curated, assigned ontology terms, and categorized using the IFSAC+ categorization schema.

Significance: The use of standard terminologies in the context of metadata for WGS is essential to facilitate data exchange and generate machine-readable resources that can expand our understanding of the dynamics of pathogen transmission across the food chain.

P1-147 Genotypic Characterization of *Listeria monocytogenes* Isolates Collected through Provincial Dairy Inspection System in British Columbia, Canada from 2007 to 2017

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Introduction: In response to two listeriosis outbreaks in British Columbia (BC; Canada) linked to soft cheeses, a voluntary monthly testing of soft/mold-ripened cheeses and inspector-collected testing of environmental swabs for *L. monocytogenes* (*Lm*) were introduced in 2003. These activities have helped assess prevalence of *Lm*; however, there is a need to better understand strain characteristics, particularly those associated with persistent strains.

Purpose: Assess genomic diversity of *Lm* isolates from BC dairy facilities collected though provincial dairy inspection system.

Methods: *Lm* (*n* = 88) were recovered from 48 food and environmental swab samples in five BC dairy facilities in 2007-2017. Isolates were paired-end sequenced on Illumina HiSeq. Sequences were trimmed and *de novo* assembled with Trimmomatic (v0.39) and SPAdes (v3.14.1), respectively. Serogroups and multi-locus sequence type (ST) profiles were obtained *in silico* through *Listeria* Pasteur database. Draft genomes were screened for antimicrobial tolerance-associated genes (*bcrABC, emrE*, *emrC*, *qacC*, *qacH, tetR*, *tnpABC*) using BLASTN (≥80% identity/alignment).

Results: Excluding clonal isolates (same ST; *n* = 40) from each sample resulted in 48 representative strains. The majority of strains were lineage II (42/48), while 6/48 *Lm* belonged to lineage I. Isolates grouped into nine clonal complexes (CCs), with CC11 (29/48) being the most prevalent, followed by CC14 (12/48). Clones CC7, CC224, CC288 (1 isolate each) and hypervirulent CC1 (2/48), CC4 (1/48), and CC6 (1/48) were rare. Multiple CCs were seen in only one facility (#71), where CC11 and CC1 isolates were recovered from food and environmental samples intermittently in 2007-2016. All tested isolates possessed *tetR*, three carried *qacC* (WRLP45, WRLP67, WRLP95), and one had *bcrABC* (WRLP95).

Significance: Whole genome sequencing helped identify recurring and transient *Lm* clonal complexes within dairy facilities over several years. Genes associated with antimicrobial tolerance were found in a subset of strains, likely aiding their survival in food processing environments.

P1-148 Updated Prevalence and Persistence Evaluation of *Listeria monocytogenes* 4bV Subset

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Introduction: Previous work identified the association of *Listeria monocytogenes* (*Lm*) 4bV strains with fresh produce outbreaks, including caramel covered apples, bagged greens, and sprouts. These outbreaks raised questions about the prevalence of 4bV overall and its environmental prevalence and persistence.

Purpose: This work undertakes to determine if there is change in 4bV prevalence since prior studies and to evaluate data on sources of isolates to determine if there is evidence of environmental prevalence and persistence.

Methods: *Lm* for inclusion in the study were identified via BLAST queries of the NCBI database or isolated from environmental samples. Genomes were analyzed with the SNP Pipeline via GalaxyTrakr. SNP alignments were used to generate phylogenetic trees using the maximum likelihood method to identify clonal groups. SNP distance matrices were used to evaluate persistence.

Results: A total of 35,226 *Lm* NCBI submissions were evaluated using BLAST, identifying 2.2% (*n* = 790) as 4bV isolates (lower than observed in our prior study but in line with studies from others). Separately, unique environmental isolates from the eastern half of the US (*n* = 340) evaluated in collaboration with several research groups surveying the natural environment identified 23 4bV isolates. The prevalence in this dataset was roughly 6.8%, suggesting that 4bV is more likely to be present in the natural environment in samples collected from the eastern half of the US. WGS analysis indicates that some of these strains are persistent in the natural environment. Additional clonal groups were identified via WGS analysis.

Significance: This work showed that there is an environmental presence of 4bV strains on the eastern half of the US that could contribute to outbreak events. The distribution of 4bV strains is globally broad, though prevalence is low, with potential regional variation. Understanding its presence and potential circulation is important in preventing outbreaks from produce.

P1-149 Monitoring the Antimicrobial Resistance Dynamics of *Salmonella enterica* in Healthy Dairy Cattle Populations at the Individual Farm Level Using Whole-Genome Sequencing

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Introduction: Cattle are a potential reservoir for zoonotic foodborne pathogen *Salmonella enterica*; infected animals can shed *Salmonella*, regardless of whether they express clinical signs of salmonellosis or not.

Purpose: Whole-genome sequencing (WGS) was used to monitor the microevolution and antimicrobial resistance (AMR) dynamics of persistent *Salmo-*

nella in healthy dairy cattle. The accuracy and concordance of multiple *in silico* serotyping and AMR prediction tools were additionally evaluated.

Methods: *Salmonella enterica* isolates (15, 13, 36, 20, 1, 16, and 27 isolates representing serotypes Anatum, Cerro, Kentucky, Meleagridis, Minnesota, Newport, and Typhimurium, respectively) were obtained from repeated sampling of apparently healthy dairy cattle and their surrounding environments on 13 New York State farms between 2007 and 2009. Strains underwent serotyping, antimicrobial susceptibility testing (Sensititre System, Thermo Scientific), and WGS (Illumina HiSeq). Following quality control, assembly, and annotation, genomes were characterized *in silico* using (i) two serotyping tools (SISTR, SeqSero2) and (ii) combinations of five AMR determinant detection tools (ABRicate, AMRFinderPlus, ARIBA, BTyper, SRST2) and one to five AMR determinant databases (ARG-ANNOT, CARD, MEGARes, NCBI, ResFinder). Within-serotype time-scaled phylogenies were constructed using Snippy v4.3.6 and BEAST v2.5.0.

Results: High concordance was observed between (i) *in silico* serotyping tools (serotype agreement for 127/128 isolates, 99%) and (ii) all AMR determinant detection methods (≥98% agreement between susceptible/resistant assignments for all pipeline/database combinations; *n* = 1,920 total susceptible/resistant assignments per combination). Time-scaled phylogenies identified (i) farm-associated *S.* Anatum, Newport, Typhimurium, and Kentucky lineages and (ii) inter-farm *S.* Cerro, Meleagridis, and Kentucky lineages. Numerous AMR determinant acquisition and loss events were identified, including the acquisition of cephalosporin resistance-conferring bla_{CMY}- and $bla_{CTX\text{-}M}$-type beta-lactamases after the year 2000 among multiple serotype groups.

Significance: The results presented here highlight the strengths of WGS in tracking zoonotic pathogens and their associated AMR determinants at the individual farm level.

P1-150 Genomic Diversity of *Salmonella* Mississippi

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Introduction: *Salmonella enterica* subsp. *enterica* serovar Mississippi (*S.* Mississippi) is the 2nd and 14th leading cause of human clinical salmonellosis in the Australian island state of Tasmania, and the US, respectively. Despite its public health relevance, relatively little is known about this serovar.

Purpose: The goal of this study was to use whole-genome sequence (WGS) data to characterize the population structure of *S.* Mississippi.

Methods: WGS data for 364 *S.* Mississippi isolates were downloaded from the NCBI Pathogen Detection database, along with assemblies representing 317 additional serovars. Serotype information was confirmed using SISTR. Maximum-likelihood based phylogenies were inferred from alignments of core single nucleotide polymorphisms. blastn searches were used to extract genes encoding surface antigens. Genomes were annotated with Prokka, and gene presence/absence analyses were performed with Panaroo and Scoary.

Results: Comparison of WGS data of *S.* Mississippi isolates with 317 additional *S. enterica* serovars placed one clade of *S.* Mississippi within *S. enterica* clade B, and the other within section Typhi in *S. enterica* clade A, confirming this serovar's status as a polyphyletic serovar. Our analyses indicate that *S.* Mississippi isolates from Australia, the UK, and the US cluster geographically, with US and Australian isolates representing different subclades within clade A Mississippi, and clade B isolates representing the predominant *S.* Mississippi in the UK. Comparison of genes encoding surface antigens suggested that isolates in clade A and B Mississippi evolved independently. Intra-clade comparisons suggested that different mobile elements, some of which encode virulence factors, are responsible for observed differences in gene content among isolates within these clades.

Significance: Our results suggest that polyphyletic serovars arise via a combination of vertical evolution and horizontal gene transfer events. Furthermore, our comparative genomic analyses highlight the need for additional discriminatory methods to classify polyphyletic serovars to facilitate epidemiologic studies.

P1-151 Potential Antimicrobial Resistance Mitigation in Livestock Industry through Production System Management and Animal Breeding

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❖ ***Developing Scientist Entrant***

Introduction: The rapid spread of antimicrobial resistance (AMR) is an urgent global threat in public health. The gastrointestinal tract microbiota of farm animals is a reservoir of AMR, which can transfer to humans through the food chain. However, an effective AMR mitigation strategy is currently lacking at the pre-harvest level.

Purpose: The purpose of this study is to seek factors for AMR load in food-producing animals throughout the production lifecycle for developing potential AMR mitigation strategies.

Methods: We collected fecal samples from a beef cattle cohort (*n* = 278) raised without antibiotic exposure at different growth stages from birth, preweaning and postweaning stages on pasture, to the end of fattening stage in feedlot. The gut resistome was investigated using both culture-dependent and -independent (metagenomic sequencing) approaches. The gut microbiota composition was detected using the 16S rRNA gene amplicon sequencing.

Results: We found that AMR in the gut of newborns was highly concentrated but it was reduced in mature cattle grazing on pasture. However, AMR increased at the fattening stage in the feedlot. The change in the gut resistome was associated with the development of gut microbiota that was affected by mobile genetic elements, which greatly contributes to the AMR transmission. We identified critical bacteria for AMR increase in feedlot operation, which harbored specific transposons carrying antimicrobial resistance genes, such as tetQ and mefA. Notably, the prevalence of AMR bacteria was strongly influenced by host genetics and inversely interacted with bacteria that were enriched in cattle grazing on pasture.

Significance: Our results highlight the advantage of cattle grazing system on reducing the AMR in beef cattle, and shed light on AMR mitigation in feedlot operation by modification of microbiome through animal breeding and management.

P1-152 How Does Analytic Approach Impact Pathogen Population Structure When Analyzing Whole Genome Sequence Data?

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Introduction: Whole genome sequencing (WGS) data, approaches, and interpretations provide valuable information for effective pathogen surveillance, outbreak detection, and other food safety activities. Agencies involved in foodborne outbreak investigations use different analytical post-sequencing approaches and bioinformatic pipelines for WGS analysis. It is unclear how the differences in approaches may impact outbreak investigations and cluster detection.

Purpose: Undertake a systematic comparison of several WGS analysis approaches to characterize how differences in approach may impact subsequent pathogen population structure and cluster membership.

Methods: We utilized a systematic *in silico* experiment to generate WGS datasets from the NCBI' pathogen database for *Salmonella enterica*, *Escherichia/Shigella*, and *Listeria monocytogenes* isolates. Fifteen datasets were created to represent the following metadata variables: sample type, sequence quality, geographical source, host species, and a random selection of genomes for each pathogen. Each dataset was analyzed using core- and pan-genomes, in addition to each of the following four comparative approaches commonly used by public health agencies; SNP-based; k-mer-based; gene-by-gene allelic comparison, and finally a novel comparison based on virulence factor-related domains.

Results: For each approach and dataset, phylogenetic trees were generated. Concordance and discordance in phylogenetic relatedness and cluster membership of spiked in outbreak genomes were evaluated. Multivariable modeling indicated that important WGS decision points (pipeline, core- or

pan-genome, sample type, and host species) were significantly associated with differences in tree structure and shape, as measured by numerous tree summary statistics. All trees were paired to compare tree topology using the Robison-Foulds distance (RFD). Generally large RFD values were found, corresponding to a bigger difference in tree topology. Ongoing and near-future computing challenges in analyzing large genomic datasets were reported, including how they hamper WGS-based outbreak investigations.

Significance: Results will provide additional insight regarding interactions between analytical approach and WGS dataset. This insight can help contribute to development of a uniform framework for interpretation of WGS results, which could improve decision-making regarding foodborne pathogens.

P1-153 Direct Detection of *Salmonella* Serotypes from Food Samples – Complete Solution from Sample to Identification with Next-Generation Sequencing

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Introduction: Identification of *Salmonella* serotypes in food samples proposes a challenge to food producers as culturing methods can be inaccurate and open to interpretation errors, and whole-genome sequencing methods are expensive and involve time consuming sample handling.

Purpose: Proposed method applies targeted sequencing on Ion Torrent platform, enabling fast and accurate detection of more than 80 *Salmonella* serotypes simultaneously from multiple samples. The flexible sequencing workflow enables the use of both lysates from enrichment samples and plate cultures as a sample material. Allowing the use of direct lysates from enrichment samples allows the user to utilize this method as a next-step after a PCR-positive result without additional culturing or DNA extraction steps from the original enrichment sample.

Methods: Method was applied to food enrichment samples from minced beef and chicken spiked with 20 *Salmonella* serotypes. Enrichment protocol of validated *Salmonella* species detection method was followed (SureTect Salmonella spp.) and the presence of *Salmonella* was confirmed with PCR. NGS libraries from the lysates were prepared and analyzed on S5 Genestudio Food Protection instrument. Resulting sequences were mapped against *Salmonella* database for serotype identification.

Results: The results show that *Salmonella* serotyping by targeted NGS method can be applied directly to lysates from food enrichment samples as well as to pure cultures.

Significance: A complete solution from sample enrichment to *Salmonella* serotyping is proposed based on DNA analysis by PCR and NGS.

P1-154 Precision Metagenomics Using a Hybrid Assembly for Classification of Shiga Toxin-producing *Escherichia coli* in Enriched Agricultural Water

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Introduction: Culture-independent metagenomic sequencing of enriched agricultural water could expedite detection and virulotyping of Shiga toxin-producing *Escherichia coli* (STEC). We previously determined the limit of assembly of a complete fragmented metagenome-assembled genome (MAG) for O157:H7 in enriched agricultural water using long reads (nanopore) was 10^5 CFU/mL.

Purpose: We aimed to establish the limits of detection and assembly for STECs in enriched agricultural water by Illumima Miseq sequencing technology alone and in hybrid assembly with nanopore long read sequencing.

Methods: STEC-negative agricultural water was enriched overnight according to the FDA BAM Chapter 4A and 1 mL aliquots were artificially contaminated with 1 mL of a 10^9 – 10^4 CFU/mL dilution of pure, overnight *E. coli* EDL933 culture (6 samples). Short reads were generated using the Illumina Miseq system with three samples run in duplicate per cartridge and *de novo* assembled using CLC Genomics (Qiagen). Long reads for the same samples generated in a previous study were used for the hybrid assemblies with Spades v3.13.1 and OPERA-MS v19.07.01.

Results: The MiSeq sequencing output (1.6-2.8 Gb per sample) generated over 11,000 contigs for each sample. For enriched agricultural water samples spiked with 10^7 CFU/mL and above, a complete fragmented O157:H7 MAG was achieved. The O157:H7 serotype and all virulence genes present in the spiked strain were identified. The 10^6 CFU/mL and lower spiked concentrations produced incomplete MAGs with missing virulence genes and did not identify the O157:H7 serotype. Hybrid assembly using the short and long reads for the same samples produced variable results. None of the hybrid assemblers was able to recover a completely closed O157 MAG from any sample even with the highest inoculation level (10^8 CFU/mL).

Significance: Precision metagenomic sequencing can be used to generate fragmented or complete closed MAGs, but hybrid assemblies require higher coverage or sequencing depth from short read technologies.

P1-155 Copper Resistance in *Salmonella*: An Emerging Food Safety Issue

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❖ ***Developing Scientist Entrant***

Introduction: The Copper Homeostasis and Silver Resistance Island (CHASRI) is a mobile element found on plasmids and chromosomes of some Enterobacteria. It contains two operons which can protect against copper in aerobic, anaerobic and transitional conditions.

Purpose: Our goal is to measure the prevalence of the CHASRI in *Salmonella* from food and environmental sources to understand the impact of genetic variations on sensitivity of the host to copper.

Methods: NCBI Pathogen Detection browser was used to identify *Salmonella* isolates containing a CHASRI gene. Raw sequence data of 6,195 isolates was downloaded, and analyzed using a custom bioinformatic pipeline and screened with quality control parameters. The final amino acid sequences were aligned, concatenated, and a maximum likelihood tree with RAxML was generated. Isolates from different clades and controls (n = 25) were selected for growth inhibition (MIC) and bactericidal (MBC) testing in presence of copper sulfate (1300 – 4000 µg/mL).

Results: A total of 4,954 isolates belonging to 61 serovars dating from 1972 – 2020 and originating from 49 countries passed the criteria. Majority of isolates were derived from poultry products, environmental samples, and pork. The maximum likelihood tree partitioned into 10 clades. Isolates in clades V, VI, and VII were predominately from poultry sources. Clade IV contained most isolates (n = 1,895) and was more diverse in serovars (n = 39), sources (n = 21) and geographic spread (n=41). Some genes in the cassette are highly diverse (*i.e. pcoS, cusS)* and some are more conserved (i.e., *pcoC, cusF)* among the isolates. MIC of copper sulfate (2,000 µg/mL) did not change between isolates containing or lacking CHASRI. However, higher MBC was observed in majority of isolates (n = 19) containing CHASRI (2,200 to >4,000 µg/mL), compared to isolates lacking CHASRI (n = 4) (2,000 µg/mL).

Significance: This study identified variants between the CHASRI amino acid sequence and possibly increased survivability in copper solutions that is conferred by its acquisition.

P1-156 Changes in Bacterial and Fungal Components of the Gala Apple Microbiome during Long-Term Storage Conditions

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❖ ***Developing Scientist Entrant***

Introduction: Apples are one of the few ready-to-eat commodities which can be kept in storage for up to one year before packing and distribution. As storage time increases, apples are increasingly vulnerable to opportunities for contamination, such as the development of advanced postharvest rot, which has been shown to impact the growth of saprophytic foodborne pathogens like *Listeria monocytogenes*.

Purpose: To determine how the bacterial and fungal population dynamics of the microbiome of fresh apple surfaces change under long-term modified atmosphere cold storage conditions.

Methods: Whole Gala apples (*n* = 180) were harvested in September 2019 and the microbiomes of the fruit surface evaluated at time of harvest, 1, 3, 6, 9, and 11 months into storage. Apples were drenched in pyrimethanil and treated with 1-methylcyclopropene before storage under modified atmosphere conditions (1°C, 1% CO_2/2% O_2) to simulate conventional postharvest handling. At each time point, 10 subsamples were hand-massaged in 250 mL 1X Tris-EDTA buffer with 2% Tween 80 for 1 min before bath sonication for 5 min. Samples were centrifuged at 10,000 *x g* and pellet resuspended in 250 µL 1X TE before DNA extraction using the ZymoBIOMICS DNA Miniprep Kit. Genomic DNA was sequenced by Genewiz to identify bacterial and fungal populations via 16S (V3, V4, and V5) and ITS1 pathways, respectively.

Results: Microbial abundance is affected by time in storage, while community diversity analysis showed diversity across all samples at all timepoints. Samples at and across all timepoints up to three months in storage showed low abundance and high diversity (D_{bac} = 0.95; D_{fun} = 0.97), with increased abundance and continued high levels of diversity (D_{bac} = 0.96; D_{fun} = 0.97) from 6-11 months.

Significance: Community composition variance by timepoint in storage can give an indication of the core microbiota of the apple surface, which could be used to better understand food safety issues in storage.

P1-157 Benchmarking Different Metagenomic Laboratory Pathways Based on Biodiversity Analysis of Environmental Samples Collected from a Chicken Farm

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❖ ***Developing Scientist Entrant***

Introduction: Chicken could harbor enteric pathogens such as *Salmonella enterica* and shed them into surrounding environment. Shotgun metagenomic analysis provides unbiased sequencing and insights into microbial communities in environmental samples of interest, and therefore plays important roles in food safety surveillance.

Purpose: This study was to apply biodiversity analytic methods to investigate how different steps in metagenomic analysis pathways could cause variance in the final results and to investigate if any of the combinations of different methods could result in statistically significant interactions.

Methods: Environmental samples were collected from chicken feces and litter from a farm. Genomic DNA from both samples were extracted using Qiagen PowerFecal kit or Zymo Fecal/Soil Miniprep kit. DNA from ZymoBIOMICS Microbial Community Standards were extracted and served as the positive control. Library preparation was done using Nextera XT DNA Library Preparation kit or Truseq DNA nano kit. Shannon-Wiener index was selected to represent the alpha diversity in each sample. Three-way ANOVA (analysis of variance) was applied to different combinations of methods to investigate method reproducibility and to estimate variance introduced in each of the steps.

Results: A total of 36 samples were included, with three replicates for each possible combination in the pathway. It suggested that when using Shannon-Wiener index, there is no significant effect of DNA extraction but there were significant differences among sampling sources (p = 1.54e-23) and sequencing library preparation (p = 1e-3). Apart from single factorial effects, there were statistically significant two-way interactions between DNA extraction and library preparation ($F(1,24)$ = 4.702, p = 4e-2), and between DNA extraction and sampling sources ($F(2, 24)$ = 5.759, p = 9e-3).

Significance: This study compared how different laboratory pathways may lead to different estimates of microbial community diversity, therefore could help better interpret metagenomic data in the field of food safety.

P1-158 Differences in *Salmonella* Survival between Strains in Low Water Activity Environments is Only Partially Explained by Genome Differences

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* strains show different survival capabilities in low water activity foods, but the reasons for these differences is not clear.

Purpose: This research used next generation sequencing to compare several different strains of *Salmonella* to determine what role genomic differences may play in survival.

Methods: Six strains of *Salmonella* from 3 serotypes (Enteritidis, Montevideo, and Typhimurium) previously tested for survival in whole milk powder were investigated. Genomic DNA was extracted using the Promega Wizard Genomic DNA kit. Strains were sequenced using the Illumina MiSeq platform. Software used in sequence analysis included FastQC, Trimmomatic, SPAdes, QUAST, and SeroSeq2. All subsequent sequence analyses were conducted using GalaxyTrakr.

Results: Pairwise genomic comparisons found the Salmonella isolates differed by 15 to 830 SNPs, with the greatest difference being between the two strains of *Salmonella* Enteritidis. These strains also showed great differences in survival rates with strain *S*. Enteritidis BAA-1045 having rates of survival between -0.001 to -0.013 log CFU/day and *S*. Enteritidis H3527 having survival rates between -0.004 to -0.019 log CFU/day. The two *S*. Montevideo strains were the most closely related with a difference of 15 SNPs. These two strains were also very closely related to the *S*. Typhimurium FSIS O26 strain, separated by only 20 and 23 SNPs. SeroSeq2 results showed that this strain that was previously thought the be Typhimurium was most likely Montevideo. These Montevideo strains had very similar survival rates with rates ranging from -0.002 to -0.012 log CFU/day for *S*. Montevideo LJH 1234, -0.002 to -0.019 log CFU/day for *S*. Montevideo LJH 614, and -0.006 to -0.018 log CFU/day for *S*. Typhimurium FSIS O26.

Significance: Survival of *Salmonella* strains was strain dependent. The survivability of many of the strains varied greatly despite close genetic relatedness showing that survivability is only partially explained by genome differences.

P1-159 Genome Sequence Analysis of Stress Tolerant *Listeria monocytogenes* Isolated from Foods and Humans

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Introduction: Whole genome sequencing (WGS) was applied to characterize genetic information of opportunistic foodborne pathogen *Listeria monocytogenes.* The genetic information is important in improving the ability to detect, investigate, and control *L. monocytogenes* in foods.

Purpose: This study aims to investigate significant genetic characterization between stress (temperature, salinity, and pH) resistant and sensitive *L. monocytogenes* isolated from various foods, humans, and animal farm environmental sources.

Methods: Twenty-four stress-resistant *L. monocytogenes* were selected by testing 187 *Listeria* strains using a broth model under exotic conditions, pH (3), salinity (4%), and temperature (1°C). DNA extraction was performed using a DNeasy Blood & Tissue Kits according to the manufacturer's instruction and concentration was determined using a Qubit 3.0. The library of the 24 stress-resistant *L. monocytogenes* was generated using a Nextera XT sample prep kit. Whole genome sequencing was performed using an Illumina MiSeq platform and the sequencing data were analyzed for pan-genome, clusters of orthologous groups (COG), and Kyoto encyclopedia of genes and genomes (KEGG) analyses.

Results: The average genome length of 24 stress-resistant *L. monocytogenes* was 2,973,315 bp and the average GC content was 37.78%. The 24 stress-tolerant *L. monocytogenes* isolates were comparatively analyzed in conjunction with 243 publicly available genome sequences to determine the genetic diversity. A total of 267 *Listeria* genomes yielded the 6,289 pan-genome, 1,369 core genes, 3,868 accessory genes, and 1,052 unique genes, respectively. Among the 24 stress-tolerant *L. monocytogenes,* three strains (BL72, P2637, and FI3) had a multitude number of unique genes. For the functional annotation of pan-genome analysis using COG, core and accessory genes were enriched in the general function prediction only (R, 12.54% and 11.74%), and the unique genes were enriched in replication, recombination, repair (L, 17.32%), and transcription (K, 12.21%).

Significance: The research will contribute to the identification and tracking of stress-tolerant *L. monocytogenes* strains isolated from foods.

P1-160 Comparative Genomic Characterization of *Cronobacter* Species Obtained from a German Powdered Infant Formula Production Facility with Other Strains from Europe, Asia, and the United States

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Introduction: *Cronobacter* species cause infections in individuals of all ages. Infantile infections are linked to consumption of contaminated powdered infant formula (PIF).

Purpose: This study investigated the importance of environmental selection, dispersal, and persistence of *Cronobacter* within a PIF manufacturing facility.

Methods: One hundred and three *Cronobacter* strains were obtained during a year surveillance study and characterized using DNA microarray (MA), PCR, and whole genome sequencing (WGS) analyses. WGS was conducted using Illumina's MiSeq platform and Nextera XT chemistry. Multi-locus sequence typing (MLST) and antimicrobial resistance (AMR) genes were identified using CFSAN's GalaxyTrakr. Genomic comparisons were made with other *Cronobacter* strains from Europe, Asia, and the USA.

Results: Eighty-nine strains were identified as *C. sakazakii* (Csak, 86.4%), 12 strains as *C. malonaticus* (11.7%) and two strains were *C. muytjensii* (Cmuy, 1.9%). Ten different sequence types (ST) were found, preponderated by Csak ST4 and followed by Csak ST1, ST83, and ST64. PCR analysis showed that 99 strains (96%) possessed a virulence plasmid, pESA3/pCMA1/pJZ38-2. AMR analysis identified a β-lactamase resistance gene in all strains except Cmuy. Colistin resistance (*mcr*-9.1) was identified in several ST1, ST17 strains, and kasugamycin resistance (*aac (2')-IIa*) was found in several ST4 Csak. This report represents the first findings of these resistance genes being carried by *Cronobacter* associated with a PIF manufacturing facility. MA and WGS studies showed that the strains from multiple facility locations phylogenetically clustered according to species and ST lineages and grouped with strains from other countries of origin.

Significance: *Cronobacter* species isolated from PIF manufacturing environments phylogenetically share common attributes with other strains that have been associated with illness, PIF, dried plant-origin foods, and dairy and PIF manufacturing facilities in Europe, Republic of Korea, and USA. These results also demonstrate the ubiquitous and persistent nature, the extent by which, *Cronobacter* can colonize econiches within a built PIF production environment.

P1-161 Prevalence of Potentially Enterotoxin and Cereulide-producing *Bacillus cereus* in Selected Food Products

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Introduction: *Bacillus cereus* toxins are responsible for two types of gastrointestinal diseases: the diarrheal and emetic form. Diarrheal symptoms are linked to the hemolytic enterotoxin HBL, nonhemolytic enterotoxin NHE and cytotoxin K, while the emetic form is caused by the depsipeptide toxin cereulide. With the increasing number of outbreaks *B. cereus* toxins have been one of the leading causes of foodborne diseases with over 437 strong evidence-based outbreaks from 2007-2015 in the Europe Union. However, the exposure to potentially toxin-producing *B. cereus* from the different types of food products is still not known.

Purpose: To determine the prevalence of potentially toxin-producing *B. cereus* in selected food products originating from European market.

Methods: A total of 250 samples including rice, pasta, spices, teas, sprouts, soups, ready-to-eat mixed food, cereals and cereal products, mashed potatoes, milk powder and cream analogues (MPCA) were obtained from European market. Presumptive *B. cereus* communities were detected according to ISO 7932:2004 and subjected to the Polymerase Chain Reaction (PCR). *nheA, hblA, cytK-1* and *ces* toxin genes were targeted in the present study.

Results: Presumptive *B. cereus* communities were collected from 176/250 (70.4%) of tested samples. *nheA* gene was the most prevalent in rice (93.1%), MPCA and instants soups (84.6%), while *hblA* was the most prevalent in instant soups (100%), seeds (85.7%) and MPCA (76.9%). Cereulide synthetize gene *ces* was the most prevalent in *B. cereus* communities obtained from instant soups, followed by mashed potatoes and MPCA, with 53.8, 41.2 and 38.5%, respectively. Although CytK-1 toxin is considered as highly dangerous but rare, its coding gene was detected in even 10/176 of samples, mostly from mashed potatoes and MPCA.

Significance: This study provides important information about the prevalence of potentially pathogenic *B. cereus* in a broad array of food products obtained from European market.

P1-162 Lineage-Specific Differences Among *Salmonella enterica* Serovar Javiana Isolates Reveal Environmental Fitness

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Introduction: *Salmonella enterica* serovar Javiana infections have historically been concentrated in southern Florida. Javiana outbreaks within a variety of agricultural products grown in the Mid-Atlantic states are increasingly common, indicating that this serovar poses a major threat to agriculture in a wider geographical area. Additionally, Javiana is one of the few serovars in the United States harboring the CdtB toxin, which causes cell cycle arrest and enhances persistence in hosts. Despite the clear public health risk, little is known about the evolution of this ubiquitous serovar due, in part, to a lack of comprehensive studies into the phylogeny of these isolates.

Purpose: The purpose of this study was to identify selective advantages evolved among Javiana isolates, which may contribute to Javiana being a dominant agricultural contaminate.

Methods: Sequencing reads for Javiana isolates collected in the United States were obtained from NCBI, assembled using SPAdes, and annotated using Prokka. Complete genomes for a set of isolates were assembled using a combination of short and long read sequencing. Pangenome and whole genome alignments were constructed using Roary and Mauve, respectively. A maximum-likelihood tree was constructed using IQ Tree from variant sites within the genome. Tree manipulations and the identification of unique gene features were performed using R.

Results: The Javiana isolates separated into three distinct lineages, largely grouped by source and location. The majority of non-clinical isolates group into one lineage with a unique set of genetic features including accessory metabolic genes and genes key to biofilm formation, suggesting enhanced bacterial fitness in an agricultural environment.

Significance: This study is the first comprehensive analysis of Javiana genomes, allowing for better prediction of clinical impact and agricultural preventive controls.

P1-163 Identification of Microbial Flora in Dry-Aged Beef to Evaluate the Rancidity during Dry Aging

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Introduction: Consumption of dry-aged beef has increased because of their unique taste and flavor. Although dry-aging induces the favorite flavor and taste, the process also causes rancidity, which is harmful to human. During dry-aging, microbial flora in beef continuously change. Therefore, microbial flora in dry-aged beef could be used as an indicator for rancidity.

Purpose: The objective of this study was to analyze the correlation between microbial flora in beef and rancidity during dry-aging.

Methods: Round of beef (2.5-3 kg) was dry-aged under at 1.5 ± 1°C and 82 ± 5% of moisture during 17 weeks. Dry-aged beef were analyzed by pyro-sequencing and plated on agar media at appropriate time during dry-aging. Also, VBN (volatile basic nitrogen) and TBARS (thiobarbituric acid reacted substance) values in dry-aging beef were measured at the same sampling time as analyzing microbial flora. After bacteria growing as VBN and TBARS increased were identified, primers were designed to detect and quantify levels of the bacteria with quantitative RT-PCR.

Results: VBN and TBARS values in dry-aging beef were depreciated from week 11 of aging duration. In the pyrosequencing and the analysis with media for microbial flora, the quantitative levels of *Streptococcus* spp., *Pantoea* spp., and *Pseudomonas* spp. were significantly changed at around week 11 of dry-aging. Furthermore, qRT-PCR analysis with developed primers showed that levels of *Pantoea* spp. and *Streptococcus* spp. can be used to identify rancid meat during dry-aging.

Significance: These results indicate that among the microbial flora in dry-aged beef, *Pantoea* spp. and *Streptococcus* spp. could be used to determine the rancidity of dry-aged beef.

P1-164 Application of Metagenomic Methods to Define Microbial Diversity and Subtype *Listeria monocytogenes* in Dairy and Seafood Manufacturing Facilities

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Introduction: Microbes present in the environment within food manufacturing facilities can be diverse and may include human pathogens. Metagenomics enables the ability to determine microbiota diversity and provide valuable insights into the survival and persistence of pathogens within these environments.

Purpose: This work examined the microbiomes of environmental swab culture enrichments from seafood and dairy manufacturing facilities to determine microbiota diversity, subtype *Listeria monocytogenes*, when present, and assess congruence with isolate-based sequencing.

Methods: 16S rRNA amplicon and shotgun metagenomic sequencing was performed on 25 environmental swab enrichments from 5 seafood and 27 enrichments from 7 dairy facilities. Community analyses were performed using an in-house implementation of MAPseq for amplicon sequencing and an in-house kmer database for shotgun metagenomics. *Listeria* contigs were assembled using metaSPAdes and identified by BLAST (megablast) against reference genomes. Concatenated backbone SNPs were used for phylogenetic analysis.

Results: 16S rRNA gene sequencing demonstrated similar taxa (e.g., *Enterococcus*, *Pseudomonas*, *Carnobacterium*), with varied abundances in facility types. Shotgun sequencing allowed finer resolution and revealed the dominant bacterial species in dairy facilities as *Enterococcus casseliflavus* and *faecalis*, while seafood facilities were dominated by *Carnobacterium maltaromaticum* and *Pseudomonas fluorescens.* We observed 87.5% (28/32) congruence between metagenomic and culture-based methods for detection of *L. monocytogenes*. Conversely, 12.5% (4/32) of culture positive samples yielded insufficient reads to confidently detect *L. monocytogenes*. Approximately 86% (24/28) of *L. monocytogenes* metagenome assembled genomes (MAGs) resolved in the same cluster as the corresponding WGS isolate while 11% (3/28) of *L. monocytogenes* MAGs placed outside of the cluster, due to low genome.

Significance: This study demonstrated that metagenomic sequencing methods can provide valuable insights into microbiome composition and diversity within food manufacturing facilities. Additionally, shotgun metagenomic sequencing can be used for *Listeria monocytogenes* subtyping with equivalent resolution to isolate WGS, given adequate sequencing coverage.

P1-165 Prevalence and Genetic Diversity of *Listeria monocytogenes* Isolated from Whole Fresh Avocado Skins

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Introduction: *Listeria monocytogenes* can cause a severe disease in immunocompromised patients. In 2014-2016, a survey of *L. monocytogenes* recovered the pathogen on the skins of avocado samples. These isolates were subjected to whole-genome sequencing and the genomes were deposited in GenomeTrakr.

Purpose: The biodiversity (genetic lineages with each lineage containing multiple serogroups) and genetic features of the *L. monocytogenes* isolates was analyzed to understand the clonal distribution of this pathogen on avocados.

Methods: Genome data of 254 *L. monocytogenes* isolates were analyzed. *In silico* PCR-serogroup and clonal complex (CC) were determined according to the multilocus sequence typing (MLST) *Listeria* database. Core genome MLST and CFSAN Single Nucleotide Polymorphism Pipeline were used to determine the relatedness of these isolates. The presence of major virulence genes and stress resistance genes was also determined.

Results: The *L. monocytogenes* isolates were classified into lineages I (40%) and II (60%). There were 21 CCs in which the most prevalent CCs were CC14 (28%), CC392 (12%), and CC412 (8%). The serogroups included IIa (60%), followed by IIb (37%), and IVb (2%). *Listeria* stress islet (SSI)-1 was present in 32% of lineage I and II isolates and SSI-2 was not found in any isolates; *Listeria* Pathogenicity Island (LIPI)-3 was detected in 53% of lineage I isolates and 2% of lineage II isolates; LIPI-4 was found in 21% of lineage I isolates and 1% of lineage II isolates; one isolate of CC14 (lineage II) contained a cadmium resistance cassette, *cadA4C4*, and an arsenic resistance cassette; and one isolate of CC5 (lineage I) harbored a cadmium resistance cassette, *cadA2C2,* and a benzalkonium chloride resistance cassette, *bcrABC*.

Significance: The findings will contribute to our understanding of population structure and clonal spread of *L. monocytogenes* in avocados. This could also help with the understanding of source attribution of *L. monocytogenes* and risk-based analysis of *L. monocytogenes*.

P1-166 Evaluation of a Bait-Capture Method for Metagenomic Detection of Shiga Toxin-producing *Escherichia coli* in Environmental Samples

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Introduction: Metagenomic sequencing provides valuable information in environmental assessments involving STEC. Bait-capture techniques offer increased detection sensitivity that can aid detection of the typically low abundant STEC and other genes of interest in microbial communities.

Purpose: In this study, the impact on the sensitivity of detecting *E. coli* including serotyping and virulence gene information in environmental samples using a bait-capture method was assessed.

Methods: An Illumina Custom Enrichment Panel of 18,287 oligos including sequence for *E. coli* serotyping and virulence genes, MLST, and ECOR SNPs, along with ARGs and selected animal mtDNA was designed. DNA was extracted from soil samples spiked over a six-log range with STEC O157:H7 along with unspiked stacked dairy manure and field soil five days after manure application. Shotgun metagenomic sequencing was performed both with and without utilizing bait-capture for library preparation. Bioinformatic analyses were performed using custom programs and BLAST with public and privately curated databases.

Results: Utilizing the bait-capture method compared to no library enrichment for culture independent spiked soil samples resulted in a $2.2 \pm 0.4 \times 10^4$ increase in detection sensitivity for virulence genes carried in the spiked STEC, along with detection of serogroups in addition to O157. For manure and manure-amended soil, STEC virulence genes were present in bait-capture metagenomic datasets, but not unenriched libraries. Between seven and 53 *E. coli* serogroups were observed in the manure and amended field soil samples, while without bait-capture only zero to three were detected. Bovine mtDNA was not detected in traditional datasets but was in bait-capture datasets for the three manure and two of three manure-amended soil samples.

Significance: Utilizing the custom bait-capture oligos for metagenomic sequencing results in improved detection sensitivity for characterization of *E. coli* communities, thus provides a valuable tool for longitudinal studies and environmental assessments in agricultural regions associated with STEC outbreaks.

P1-167 Long Read Sequencing for Metagenomic Analysis and Detection of Stecs in Agricultural Water

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) contaminated agricultural water has been associated with an increase in produce-related illnesses and outbreaks, however current FDA protocols require approximately two weeks of analysis time, including single colony isolation for whole genome sequencing.

Purpose: We aimed to test a metagenomic analysis approach using long read nanopore sequencing for culture-independent, in-field detection and virulotyping of STECs in irrigation water.

Methods: Irrigation water samples (100 L) from multiple sites along a canal in the Southwestern US were filtered and backflushed (600 mL) and DNA was extracted from 10 mL. Sequencing libraries were prepared using the Oxford Nanopore rapid and field sequencing kits (RAD004 and LRK001) or the ligation kit (LSK109) and reads were classified by the EPI2ME WIMP workflow.

Results: Library preparation of DNA from the irrigation water samples using the RAD004 and LRK001 kits generated low output (0.3 – 1.7 M reads) and at least 50% reads failed initial quality standards. However, a DNA library prepared with the LSK109 kit increased yield and quality (2.2 M reads, 15% failed reads). Taxonomic classification of the reads from the first tested site identified 11 bacterial genera (>1% abundance), including *Synechococcus* (30-40%) and *Cyanobium* (4%). Three adjacent sites along the canal spanning 3.7 miles shared a similar bacterial composition. Despite microbiological STEC detection in enriched irrigation water, samples sequenced prior to enrichment failed to detect the presence of STECs or virulence genes from the reads identified as *Escherichia coli* (0.02% or ~100 reads).

Significance: While the STEC concentration in unenriched irrigation water is below accurate detection, the Oxford Nanopore field sequencing and ligation kits can be appropriate for identification of highly abundant microorganisms. Testing and development of culture-independent, in-field testing methods expedites pathogen detection and aids source tracking efforts.

P1-168 Utilization of Metagenomics for Evaluation of Three Enrichment Procedures for Detection and Isolation of *E. coli* O157:H7 in Mung Bean Sprout Irrigation Water

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Introduction: Spent irrigation water has been tested in compliance programs for detection of *E. coli* O157:H7 in sprouts. However, detection and isolation by standard cultural methods can be difficult due to the high background microflora associated with this matrix

Purpose: This study evaluated three procedures for rapid detection and isolation of *E. coli* O157:H7 from artificially contaminated mung bean sprout irrigation water samples.

Methods: Spent mung bean sprout irrigation water was inoculated with *E. coli* O157:H7 at low (0.075 CFU/mL) and high (0.75 CFU/mL) levels, and stored refrigerated (4°C) for 72 h. Three enrichment procedures were used: (1) FDA BAM procedure using static incubation in modified buffered peptone water + pyruvate (mBPWp) enrichment media for 5 h at 37°C, followed by addition of acriflavine (A), cefsulodin (C), and vancomycin and then further incubated with no shaking at 42°C to enhance selectivity; (2) mBPWp with CV held at 42°C with shaking and (3) mBPWp with CV held at 42°C without shaking. Samples were collected for metagenomic analysis at the onset of the enrichment, and after 5- and 24-h enrichment.

Results: The relative abundance of *E. coli* O157:H7 at the 24-h enrichment time point for both the low and high inoculum was found to be between 15 and 30% for test conditions (2) and (3) but less than 3% for test condition (1). Additional differences in relative abundances of other bacterial taxa in the enriched sprout irrigation water microbiomes were also observed.

Significance: This study demonstrates how metagenomics provides an additional tool for enrichment method development studies that can be used to improve current cultural methods used for pathogen detection in difficult matrices.

P1-169 Microflora Analysis of Bacterial Biofilms in a Meat Processing Facility over an Eight-Week Period

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Introduction: Traditional culture methods used for environmental sampling in food production facilities select for certain groups of microorganisms, and therefore may not always reflect the full range of microorganisms associated with food industry surfaces. Microorganisms existing in biofilm communities also often exist in a viable but non-culturable state, preventing accurate enumeration of biofilm-associated cells.

Purpose: 16S metagenomic analysis was conducted to determine the bacterial species composition of biofilms sampled from selected food contact and non-food contact surfaces within a raw meat production facility after cleaning and disinfection over an 8-week period.

Methods: Eight weekly sampling visits were made to a UK raw meat processing facility, post cleaning and disinfection. Areas containing biofilms were identified using commercially available biofilm detection sprays, biofilms were sampled using a cell scrapers and flocked swabs to maximize recovery. Samples were analyzed using Next Generation Sequencing (NGS) with the Thermo Scientific™ Ion GeneStudio™ S5 Food Protection System to determine species composition at each sampling point. Samples were also enumerated for Total viable count, Enterobacteriaceae, *Listeria* and *Salmonella.*

Results: Total viable counts from biofilm samples ranged from 2.42 to 5.32 log CFU/swab. Metagenomic analysis of swab samples showed a large diversity of organisms associated with food industry surfaces (alpha diversity: 20-160), with Enterobacteriaceae (predominantly *Serratia* spp.), Pseudomonads (*Pseudomonas, Acinetobacter* and *Aeromonas* spp.), Flavobacteriaceae (*Myroides* spp.) and *Shewanella* spp. uniformly representing the most abundant organisms within the biofilm samples assessed.

Significance: These results indicated that biofilm communities showed resistance to cleaning and disinfection. High species diversity was observed within the biofilms sampled from the raw meat facility over the 8-week period, although the predominant genera remained relatively conserved. As sequencing costs reduce, bacterial metagenomic analysis offers a greater understanding of resident flora present in food processing facilities, providing insights into factors influencing adverse changes to the microbiome.

P1-170 Development of a Bioinformatics Plasmid Search Engine for *Cronobacter* Species

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❖ ***Developing Scientist Entrant***

Introduction: *Cronobacter* spp. are foodborne pathogens that cause serious disease in neonates, infants, and adults.

Purpose: This study developed a bioinformatics plasmid-search engine to identify genomic attributes contained on *Cronobacter* plasmids.

Methods: A database containing 33 *Cronobacter* plasmid sequences from all seven *Cronobacter* species was developed. Another database containing 683 draft and closed genomes was also developed. Mutli-locus sequence typing (MLST) using CFSAN's GalaxyTrakr was used to identify each strain. An in-house BLAST+-python based script was used to perform a Linux-BLAST analysis to create a formatted %ID output matrix of plasmid genes.

Results: Each plasmid was sorted into four different categories based on their genetic attributes: virulence, conjugative-Type-IV, heavy-metal, and cryptic. Virulence plasmids (e.g., pCCO1, pCDU1, pCMA1, pESA3, CmuyJZ38_p2, pCUNV1, and pCTU1) contained a common origin of replication gene (*repA*), and two iron acquisition gene clusters, a siderophore (*iucABCD/iutA*) and ABC iron transporter (*eitCBAD*). Plasmids containing species-specific virulence factors included an omptin gene (*cpa*) in *C. sakazakii* and *C. universalis* and a filamentous hemagglutinin gene cluster in *C. malonaticus* and *C. turicensis.* Type VI secretion system gene clusters were seen in plasmids from *C. sakazakii, C. muytjensii*, and *C. dublinensis*. Conjugative Type-IV plasmids (pESA2 and pCTU2) were found in *C. sakazakii* and *C. turicensis*. Heavy-metal resistance plasmids such as pCMA2, pSP291-2, and pCTU3 contained genes encoding for arsenic (*arsABCDR*), copper (*pcoABCDERS)*, or silver (*silABCEPRS*) efflux. Lastly, several ~ 6 kB in size cryptic plasmids were identified which possessed several mobilization genes (*mob*). Other features found among these plasmids included both Type II (*hipAB*), and Type I (*hok*) toxin-antitoxin systems, phage or tyrosine-type recombinase/integrase and several prophage gene clusters.

Significance: This report represents the first bioinformatics plasmid-search engine developed for *Cronobacter* species. Understanding the role of plasmids in virulence and persistence underpins the development of future mitigation strategies for controlling this pathogen.

P1-171 Multidrug-resistant *Salmonella* from Swine Lymph Nodes in Different Brazilian States – A Genomic Approach

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* spp. remains a public health problem and is distributed around the world, causing outbreaks and spreading resistance genes among bacteria. The capacity to infect human and animals' hosts make these bacteria an important foodborne pathogen, generating huge economic losses annually.

Purpose: The purpose of this study was to investigate the genomic features by molecular techniques to understand the behavior of this genus.

Methods: A total of 27 Multidrug Resistant –MDR *Salmonella* from different serovars (*S*. Derby, *S*. Cerro, *S*. Give, *S*. Typhimurium, *S*. Panama, *S*. Infantis, *S*. Bradney, *S*. London, *S*. Bovimorficans and *S*. I 4,[5],12:i:-) isolated from swine lymph nodes and collected from three different states of Brazil (Minas Gerais, São Paulo and Paraná) were sequenced, assembled and noted, the main goals being the pathogenic islands, plasmids, resistance genes and MLST identification.

Results: All isolates presented a high diversity of antimicrobial resistance related genes (AMR), being the most prevalent the *blaTEM-1B, tet(A), aac(6')-Iaa* and *floR*. Based on MLST, isolates were predominantly ST 19 and ST 40. The main plasmids identified were IncFIB, followed by IncC and Col(pHAD28). A total of 13 pathogenic island profiles were characterized, being eight pathogenic islands identified (C63PI, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-13, SPI-14).

Significance: After all profiles analysis, the results revealed difference between Minas Gerais State from the Paraná and São Paulo State. The presence of MDR *Salmonella* in the pork production chain from different locations shows a continuous circulation of this pathogen. Acknowledgements - The authors are thankful to CAPES (Financial Code 001), Fundação de Amparo à Pesquisa do Estado de Minas Gerais - FAPEMIG and Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq.

P1-172 Genomic Insights into Divergent Evolution of Virulence and Fitness Traits in Shiga Toxin-Producing *Escherichia coli* O121

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) serotype O121:H19 is among the top six non-O157 serotypes that are frequently associated with human disease. We previously reported that, although clinical STEC O121:H19 strains are indistinguishable from the environmental ones based solely upon virulence gene content or MLST typing, they differ in carbon utilization profiles and other fitness traits.

Purpose: Identify the molecular determinants underlying phenotypic divergence between clinical and environmental STEC O121:H19 isolates and evaluate pathogenicity potential of other O121 serotypes.

Methods: STEC O121 strains were sequenced using SMRT technology on a PacBio RSII instrument. The genomes were assembled with RS_HGAP_Assembly.3 and annotated using Prokaryotic Genome Annotation Pipelines. Comparative genomics were carried out in Edgar. Virulence genes and antibiotic resistance genes were identified by searching VFDB and CARD, respectively.

Results: Pan genome analysis revealed 3760 core Coding DNA Sequences (CDSs), 1708 dispensable CDSs, and 1441 strain-specific CDSs. This large set of dispensable genes were mainly attributed to environmental strains RM8082 (O121:H7) and RM10740 (O121:H10). The environmental isolate RM8352 (O121:H19) exhibited the highest similarity with 16-9255, a clinical isolate linked to the large 2016 outbreak associated with flour in Canada. All O121:H19 strains carry key virulence genes of enterohemorrhagic *E. coli* and genes encoding TTSS, TTSS effectors, and T6SS. Both non-O121:H19 strains lack LEE and possess a few TTSS effector genes. RM8082 lacks the chromosome-bore hemolysin gene *hlyE*, T6SS genes, and *paa*, but carries K88 and P fimbriae genes. RM10740 carries an additional 14 SCI-1 T6SS genes and a homolog of *Yersinia* virulence gene *ail*. Analyses of the O121:H19 strain-specific genes revealed that they mainly encode functions related to antibiotic resistance, carbon and energy metabolism, transport, and stress resistance.

Significance: These data revealed the genetic loci determining strain specific traits in STEC O121:H19 and demonstrated distinct evolutionary linages and pathogenicity potential among serotypes O121:H19, O121:H7, and O121:H10.

P1-173 Intracompany Proficiency Trial for Whole Genome Sequencing of *Listeria monocytogenes* and *Salmonella enterica*

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Introduction: The use of whole genome sequencing (WGS) as a tool to identify sources of contamination in food manufacturing equipment and facilities is increasing. Quality of WGS data and consistency in performance by WGS service providers are important features to consider when building databases and drawing conclusions from WGS data.

Purpose: An intracompany proficiency trial (PT) was organized to compare the performance of three labs in the global Mérieux Nutrisciences network that provide WGS services: Silliker Food Science Centers in USA and China, and Biofortis Europe in France. The main objectives were to check performance against standardized protocols for the entire WGS workflow, understand gaps, and make appropriate corrections.

Methods: Four *Salmonella enterica* and three *Listeria monocytogenes* strains were used in the PT. Isolates were chosen, prepared and known only to the R&D team; samples were blinded to the three participant labs. Each lab processed blinded samples according to their internal procedures and protocols. WGS was performed either on an Illumina MiSeq or iSeq, but all used the same Nextera XT workflow. BioNumerics software was used to analyze the data by assessing serotypes, wgMLST, cgMLST, MLST, and wgSNP. Each participant lab was required to return the same deliverables to the R&D team, including raw data, sequencing quality indicators, dendrograms, MLST/SNP tables, and customer reports. R&D compared results from all labs and independently analyzed the wgMLST and wgSNP data.

Results: Overall, participants were able to perform WGS to a satisfactory level. Statistical analyses, which included Mantel test and cophenetic distance, were performed and showed similarity.

Significance: An internal PT, like the one performed here, provides a deeper approach to check a lab's protocols and a company's procedures, to ensure consistent results are delivered to their customers, regardless of where or when samples are analyzed. This PT will help to identify and fill the gaps that may have an impact on WGS results.

P1-174 Longitudinal Metagenomic Study Correlating Soil Microbial Community and Abiotic Properties with STEC Survival in Soils with and without Untreated Dairy Manure Amendment

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Introduction: Addition of untreated manure to agricultural soils alters the taxonomical and functional composition of soil, impacting the survival of pathogens such as Shiga toxin-producing *E. coli* (STEC). Metagenomic sequencing of amended and unamended soil combined with *E. coli* population characterization and abiotic soil properties during temporal studies enables correlation of multiple factors with STEC survival.

Purpose: The purpose of this study was to examine the temporal microbial impacts of untreated manure application on the *E. coli* community, including naturally occurring STEC, in agricultural soil.

Methods: Two farms, one using untreated dairy manure amendment, the other using no biological amendment, were used for longitudinal soil sampling. DNA was extracted from 45 5g-culture independent and 50g-*E. coli* enriched soil samples and used for shotgun metagenomic sequencing. Microbial taxonomy was determined using an in-house k-mer-based program. *E. coli* serogroup, toxin, and resistance genes were identified using publicly available and privately curated databases. Soil and manure physical/chemical property measurements were also obtained.

Results: Characterization of the *E. coli* community revealed that the diversity of *E. coli* strains increased fivefold following manure application, averaging 27 to 144 serogroups, more closely reflecting the amendment community than the unamended soil. The STEC toxin genes *subAB, stx1a, stx2,* and *ehxA*, were detected in the metagenomic data following amendment application, but not in unamended soil. Both abundance of toxin genes and serogroup diversity decreased over time. Taxonomic and functional analyses of the metagenomes identified shifts in the microbiome that can be correlated with STEC persistence results.

Significance: This longitudinal study demonstrates that utilizing metagenomics to determine soil microbiome alterations in conjunction with *E. coli* community characterization and physical/chemical soil properties after addition of untreated dairy manure provides valuable insights into STEC survival. Furthermore, the data and results generated can be used to improve risk assessment models.

P1-175 Genetic Relatedness of Salmonella enterica Serovar Corvallis from Environmental Isolates from Cambodia and Clinical Cases in the United Kingdom

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Introduction: The pathogenic serovar *Salmonella enterica* subspecies *enterica* serovar Corvallis (*S.* Corvallis) has been identified as a human pathogen and as a food contaminate. Diarrheal disease is a common diagnosis in foreign tourists visiting Southeast Asia, often with unknown etiology. However, numerous foreign public health institutes have identified *Salmonella* as a common causative agent when consuming contaminated food and water.

Purpose: This study reports on the genetic relatedness between isolates of *S.* Corvallis from informal market environments in Cambodia and historical clinical cases in the United Kingdom using Whole Genome Sequencing (WGS).

Methods: Genomic data from isolates collected from environmental surfaces in a Cambodian informal market were uploaded into the National Center for Biotechnology Information (NCBI) platform allowing the novel sequences to be compared to global WGS archives. A maximum likelihood tree based on core Single Nucleotide Polymorphisms (SNPs; variations where a single nucleotide in the genome may differ from strain to strain) was generated of 70 *S.* Corvallis genomes received by the Public Health of England (PHE) from April 2014 that cluster monophyletically with four isolates recovered from a Cambodian informal market. Genomes of *S.* Corvallis environmental isolates were also compared to 270 isolates of *S.* Corvallis received from routine surveillance in England.

Results: The SNP analysis revealed that two human clinical cases from England and several of the environmental isolates were closely related with an average SNP difference of one (0–3 SNPs). The environmental isolates clustered into a broader phylogenetic group within the *S.* Corvallis population containing 70 additional human isolates, of which 43 reported recent foreign travel almost exclusively to Southeast Asia.

Significance: The environmental isolates of *S.* Corvallis isolated from an informal market in Cambodia are concerning to public health due to their genetic similarity to strains with known human virulence and pathogenicity. This study emphasizes the benefits of global and public data sharing of pathogen genomes.

P1-176 Impacts of Manure-derived Fertilizer Application on the Bacterial Community in Raspberry Fields

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Introduction: Manure-derived fertilizers are enriched with nutrients for crops and show potential for use as soil amendments in the red raspberries in Washington State. The application of manure-derived fertilizers could directly or indirectly alter bacterial communities in soil, which has not been examined.

Purpose: To investigate the impacts of the manure-derived fertilizer applications on bacterial community structure in soil sampled from a commercial red raspberry field in Whatcom County, Washington.

Methods: Soil samples were collected from the raspberry field after amending with manure straight lagoon (SL), composted dairy manure (COM), or a standard synthetic fertilizer (used as the control, CON). Soil bacterial abundance was analyzed using Illumina MiSeq dual-barcoded two-step PCR amplicon sequencing.

Results: There are 35 phyla detected in soil samples; *Proteobacteria* (42.2-46.4%), *Acidobacteria* (8.7-14.6%), *Actinobacteria* (6.3-12.8%), and *Bacteroidetes* (6.9-9.9%) are most abundant phyla. Manure-derived fertilizer applications impacted soil bacterial ecology at phyla, family, and genus levels. Soil amended with COM had higher bacterial diversity compared to those with CON and SL. SL increased ($P < 0.05$) *Chlamydiae* and COM increased ($P < 0.05$) *Acidobacteria*, *Bacteroidetes,* and *Chloroflexi* phyla when compared to CON. The abundance of *Actinobacteria* and *Gemmatimonadetes* ($P < 0.05$) was decreased ($P < 0.05$) in COM compared to CON and SL. At the family level, SL increased ($P < 0.05$) *Acidobacteria* Gp3/6, *Planctomycetaceae*, *Comamonadaceae* and *Reyranellaceae*, and COM increased ($P < 0.05$) *Microbacteriaceae*, *Flavobacteriaceae* and *Anaerolineaceae* level compared to that with CON. At the genus level, compared to CON, the abundance of *Acidobacteria genus Gp6* and *Nakamurella* was increased ($p<0.05$) in SL; the abundance of *Ohtaekwangia*, *Pedobacter*, *Planomicrobium*, *Hyphomicrobium, Devosia,* and *Massilia* was increased ($P < 0.05$) in COM, while the abundance of *Gemmatimonas*, *Rhizomibrobium*, *Bradyrhizobium, Burkholderia* was decreased ($P < 0.05$) in COM.

Significance: This study provides useful information to guide future manure-derived fertilizer development to maximize the diversity of soil microbial community and the health condition of the soil.

Poster

P1-177 A *S. enterica* Isolate Persists in an *In Vitro* Simulator of the Human Intestinal Microbial Ecosystem (SHIME®) Model and Disrupts the Gut Metabolome

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Introduction: *Salmonella enterica* is a global pathogen which accounts for 80 million cases of foodborne gastroenteritis and 155,000 deaths annually. The effect of *S. enterica* on commensal intestinal microbiota and the gastrointestinal tract (GIT) metabolome remains unknown.

Purpose: This study investigated the survival of *S.* Heidelberg in a GIT model and its effects on the metabolome of a stabilized fecal sample.

Methods: Ten mL of liquid egg and *S.* Heidelberg (log 11) culture (1:1 ratio) was inoculated into the stomach/small intestine compartment of a modified triple L-SHIME® under parameters mimicking a healthy adult GIT. Samples were collected from the proximal (PC) and distal (DC) colon compartments before inoculation and every 6 h during a 48-h period (excluding 42 h) and were plated onto *Salmonella* Brilliance agar. The profiles of 26 GIT metabolites were characterized using ^{1}H-NMR spectrometry and analyzed using the Chenomx software.

Results: *S.* Heidelberg decreased from log 8.48 to log 7.48 (between 0 h – 12 h) in the PC, and from log 7.31 to log 6.53 (between 0 h – 6 h) in the DC compartment. By 48 h, *S.* Heidelberg decreased to log 6.75 (PC) and log 5.98 (DC). In the PC compartment, 5 metabolites (i.e., 4-hydroxyphenylacetate, alanine, formate, methanol, propionate) had significant differences in at least 5 of the 9 sample timepoints. These metabolites (excluding 4-hydroxyphenylacetate) increased in concentration over time when compared to the control. In the DC compartment, 12 metabolites had significant differences in at least 5 of 9 timepoints. Six of these metabolites (i.e., 4-hydroxyphenylacetate, alanine, butyrate, cholate, malonate, tryptophan) decreased in concentration over time when compared to the control, while 5-aminopentanoate, formate, glycine, propionate, trimethylamine, and valerate increased in comparison.

Significance: *S. enterica* changes the phenotype of the GIT metabolome. These findings improve our understanding of *S. enterica* survival in models of human digestion and the effect of *S. enterica* on the activity of the intestinal microbiota.

P1-178 Evaluation of *Salmonella* Serotype Prediction with Multiplex Nanopore Sequencing

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Introduction: Whole genome sequencing (WGS) data generated by the long-read sequencing platform Oxford Nanopore Technologies (ONT) has been shown to provide reliable results for *Salmonella* serotype prediction. Our previous study evaluated ONT sequencing for the prediction of individual isolates, whereas the performance of multiplexing of several isolates in one flow cell still needs assessment.

Purpose: To evaluate the accuracy and cost-efficiency for the food industry of ONT multiplexing sequencing for *Salmonella* confirmation and serotype classification.

Methods: Three, four, five, seven or ten *Salmonella* isolates (each represented one *Salmonella* serotype) were pooled in one R9.4.1 flow cell. Each multiplexing strategy was repeated five times in separate flow cells, and five loaded samples were sequenced simultaneously in a GridION sequencer for 48 hours. Sequencing data were used for *in silico* serotype prediction by SeqSero2 (for raw reads and genome assemblies) and SISTR (for genome assemblies) software suites.

Results: An average of 10.63 Gbp of clean data was obtained per flow cell in 48 hours. Both SeqSero2 and SISTR accurately predicted all the multiplexed isolates under each multiplexing strategy using genome assemblies with genome coverage ≥ 50 × for each isolate. We identified that cross-sample barcode assignment was a major cause of prediction errors when using raw sequencing data, and the inequality of data yield for each multiplexed isolate was a major barrier for shortening sequencing time. When five *Salmonella* isolates were multiplexed, genome coverage at ≥ 50 × per isolate could be achieved within an average of six hours of sequencing, and the cost per isolate could be reduced to 23% of that incurred with single isolate sequencing.

Significance: This study is a starting point for further validation of multiplex ONT WGS as a cost-efficient and rapid *Salmonella* confirmation and serotype classification tool for the food industry.

P1-179 Evaluation of Nanopore Sequencing Technology to Identify *Salmonella enterica* Choleraesuis Var. Kunzendorf and Orion Var. 15+, 34+

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Introduction: Our previous study demonstrated that whole genome sequencing data generated by Oxford Nanopore Technologies (ONT) can be used for rapid and accurate prediction of selected *Salmonella* serotypes. However, one limitation is that established methods utilizing data from either ONT or Illumina could not differentiate certain serotypes and serotype variants with the same or closely related antigenic formulae.

Purpose: This study aimed to evaluate ONT sequencing and additional data analysis for differentiation of these serotypes and serotype variants, thus overcoming this limitation.

Methods: The latest chemicals and basecalling models were applied, including R9.5 and R10.0 flow cells, 1D^{2} library construction kit, and modified basecalling modeling, to test if these new inventions met the needs of this study by improving data accuracy. Five workflows that combined different flow cells, library construction methods and basecalling models were compared. *S. enterica* Choleraesuis var. Kunzendorf and Orion var. 15^{+}, 34^{+}, were sequenced using these workflows. Downstream Single Nucleotide Polymorphism (SNP) analysis by the CFSAN SNP pipeline and *in silico* prophage prediction by PHASTER were also performed for these two strains.

Results: The workflow comprising the R9.4 flow cell, rapid ligation kit and guppy basecaller with base modified model performed best for SNP analysis. A 99.98% matching identity was achieved with this workflow between assembled genomes from ONT and those from Illumina. Less than five high quality SNPs differed when sequencing data from ONT and Illumina were compared. SNP typing successfully identified Choleraesuis var. Kunzendorf, while prophage prediction further differentiated Orion var. 15^{+}, 34^{+} from the other two Orion variants. The methods established were a proof of principle study and wider validation will be needed.

Significance: Our study improves the readiness of ONT as a *Salmonella* subtyping and source tracking tool for food industry applications.

P1-180 Pathogenic Characterization of *Listeria monocytogenes* Isolates from Enoki Mushroom and Sequences of *L. monocytogenes* SMFM2019-FV16 Whole Genome

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❖ ***Developing Scientist Entrant***

Introduction: There were foodborne outbreaks by consuming raw enoki mushrooms (*Flammulina velutipes*) contaminated with *Listeria monocytogenes*. Thus, pathogenic characterization for the *L. monocytogenes* isolate is necessary to improve the food safety management for enoki mushroom.

Purpose: This study analyzed the pathogenicity of *L. monocytogenes* isolated from raw enoki mushroom, and sequenced the whole genome of *L. monocytogenes* SMFM2019-FV16.

Methods: Enoki mushroom samples (*N* = 79) were purchased from markets in Seoul, Korea to isolate *L. monocytogenes*. The presence of pathogenic

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genes, hemolytic property, heat resistance, and antibiotic resistance for the *L. monocytogenes* isolates were analyzed to characterize the pathogenicity. The whole genome of the *L. monocytogenes* isolate showed the highest pathogenicity was sequenced with PacBio® RS II platform, and the sequence was analyzed using a CLC genomics workbench program.

Results: *L. monocytogenes* (8.8%) was contaminated in raw enoki mushroom, and 7 *L. monocytogenes* strains were isolated. All isolates had pathogenic genes (*inlA*, *inlB*, *actA*, *plcB*, and *hlyA*) and showed hemolytic property (β-hemolysis). The cell counts of *L. monocytogenes* decreased by 90% after heating at 56°C for 10-28 min and 60°C for 2-3 min. Among the isolates, 2 isolates had multi-drug resistance for antibiotics. Taken together, the *L. monocytogenes* isolate showed the highest pathogenicity, *L. monocytogenes* SMFM2019-FV16, consists of 3,090,672 bp, 3,042 coding sequence, 18 rRNA, and 67 tRNA. Also, *L. monocytogenes* SMFM2019-FV16 has 43 genes for virulence factors and 14 genes for antibiotic resistances. Moreover, 35 single nucleotide variants (SNVs) and 11 single nucleotide polymorphisms (SNPs) were identified in the 43 genes of virulence factors. Therefore, the genomic difference of the genes of virulence factors may cause highly pathogenic *L. monocytogenes*.

Significance: The results indicate that the consumptions of raw enoki mushroom may cause *L. monocytogenes* foodborne illness, and *L. monocytogenes* SMFM2019-FV16 isolate is highly pathogenic because it has many SNVs and SNPs in the virulence factors.

P1-181 Phenotypic and Genotypic Characterization of *Salmonella* Resistance within the U.S. Food and Drug Administration's Foods Program

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Introduction: Antimicrobial-resistant nontyphoidal *Salmonella* poses a One Health threat as it can spread between people, animal, and environment. Surveillance programs such as the National Antimicrobial Resistance Monitoring System (NARMS) play a critical role in combating antimicrobial resistance in this important foodborne pathogen.

Purpose: This study aimed to evaluate the current status of antimicrobial resistance among nontyphoidal *Salmonella* within human and animal food samples collected by the U.S. Food and Drug Administration (FDA)'s Foods program between 2002 and 2020.

Methods: As part of the FDA's routine surveillance and compliance programs, FDA field investigators analyzed human food, animal food, and environmental samples for *Salmonella* contamination. Confirmed *Salmonella* isolates were subsequently characterized phenotypically by antimicrobial susceptibility testing and genotypically by whole-genome sequencing (WGS). This study reports on findings from 4,043 *Salmonella* isolates recovered from 3,200 samples.

Results: The 4,043 *Salmonella* isolates belonged to 373 serovars with leading ones identified as Weltevreden, Newport, and Senftenberg. Phenotypic resistance was most commonly observed to tetracycline (6.6%), followed by nalidixic acid (3.4%), chloramphenicol (2.6%), trimethoprim-sulfonamide (2.5%), and ampicillin (1.9%). The majority (82.1%) of isolates did not have any detectable resistance mechanisms by WGS. Among identified genes, the most common were *fosA7* (9.0%), *tet(A)* (4.9%), and *aadA* genes (3.4%), conferring resistance to fosfomycin, tetracycline, and streptomycin, respectively. One *Salmonella* Indiana isolate from a frozen tilapia had four different extended spectrum beta-lactamases, along with genes conferring resistance to macrolides and fluoroquinolones. These resistance mechanisms encompass all major drug classes used to treat *Salmonella* infections in humans.

Significance: These data demonstrated that diverse *Salmonella* serovars were present among human food, animal food, and environmental samples collected within the FDA's Foods program from 2002 to 2020, but antimicrobial resistance was less common compared to those reported in the NARMS retail samples. WGS provided a powerful tool for in-depth characterization of antimicrobial resistance and other genetic traits for *Salmonella*. Continued monitoring of the resistance trend is warranted.

P1-182 Characterization of Microbial Community Dynamics and Detection of *Listeria* in Food Manufacturing Facilities Using 16S rRNA Gene Sequencing

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Introduction: Microorganisms frequently colonize surfaces and equipment within food manufacturing facilities which can have a substantial impact on food quality, food safety, and human health. 16S rRNA gene sequencing can determine microbiome composition including pathogens and commensals.

Purpose: This work investigated the microbiomes of environmental swab culture enrichments from various food manufacturing facilities to evaluate microbiota diversity, define distinct microbial community profiles and to detect *Listeria*.

Methods: 16S rRNA gene sequencing was performed on 983 environmental swab culture enrichments from 41 food manufacturing facilities. Swab samples were collected from various areas of the food manufacturing environment and were categorized into 6 different classifications based upon the food commodities produced (seafood, dairy, confection, sauce/condiments, meat and produce). Community analysis was performed using an in-house implementation of MAPseq for amplicon sequencing. All samples were tested for *Listeria* following standard culture-based protocols.

Results: 16S rRNA gene sequencing identified unique microbial taxa based upon the food facility classification such as: *Weissella* and *Leuconostoc* in samples from the confection facility; *Vagococcus* in the meat, produce and seafood facilities; *Citrobacter* and *Carnobacterium* in seafood facilities; and *Pantoea, Clostridium* and *Fusobacterium* in the sauce/condiment facility. *Enterococcus* and *Pseudomonas* were found in 83% (5/6) of the facilities. We observed 84.6% (832/983) agreement between 16S rRNA and culture-based methods for detection of *Listeria*. Conversely, 15.3% (151/983) of culture-positive samples were below the threshold (1% Relative Abundance) to confidently detect *Listeria*. Predominant taxa in *Listeria* culture positive samples were *Enterococcus, Lactococcus* and *Carnobacterium* while *Pseudomonas, Staphylococcus* and *Brocothrix* were observed in culture-negative samples.

Significance: Our study provides valuable insights into the composition and diversity of microbiomes within various food manufacturing facilities producing distinct food commodities. This study demonstrates that 16S rRNA sequencing is a valuable tool which can identify the co-occurrence of bacterial taxa in samples cultured for *Listeria*.

P1-183 Application of a Mitochondrial Sequence Profiling Tool (MitoKmer) to Identify Imported Seafood from Metagenomic Sequences

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Introduction: Methods for identifying and confirming labeling of seafoods provides essential data to support regulatory responses aimed at seafood safety and misbranding (or economic adulteration). Complete mitochondrial genomes have demonstrated utility for high-resolution phylogenetic discrimination of closely related species. MitoKmer is a publicly-accessible tool that uses complete mitochondrial genomes to detect and identify eukaryotic species in food commodities.

Purpose: This work investigated the microbiomes and mitochondrial sequences of unenriched and enriched imported seafoods to determine microbiota diversity and confirm labeling of seafoods when compared to fish DNA barcoding.

Methods: Shotgun metagenomic sequencing was performed on a total of 20 seafood commodities originating from 11 countries. 25 g X 15 subs of seafoods are taken (total of 375 g) in 3,375 mL UPB incubated for 24 h. Unenriched (1 h enrichment) and culture enriched (*Listeria, Salmonella)* samples were extracted for DNA using Zymo Mini prep kit and the libraries were sequenced using an Illumina Miseq. Raw sequencing reads were analyzed using MitoKmer – an in-house bacterial kmer analysis pipeline.

Results: MitoKmer analysis on shotgun sequences of both unenriched and enriched seafoods samples identified 90% (18/20) of samples when compared to their corresponding label. MitoKmer correctly identified a wide variety of seafoods including octopus (*Octopus sp*.), mussels (*Mytilus sp*.), shrimp (*Litopenaeus vannamei*), tilapia (*Oreochromis sp*.), croaker (*Collichthys niveatus*, *Larimichthys polyactis*), smelt (*Osmerus mordax*), tuna (*Thunnus albacares*), cobia (*Rachycentron canadum*) and trout (*Brycon orbignyanus*). Ground tuna from Indonesia was identified as chicken (*Gallus gallus*) by MitoKmer and did not identify tuna. MitoKmer also did not identify the seafood component in breaded grouper fingers. In accordance with traditional fish DNA barcoding, the salmon sample was identified as *Salmo salar*. Bacterial kmer identified predominant bacterial species as *Carnobacterium maltaromaticum*, *Pseudomonas fluorescens, Citrobacter sp,* and *Psychrobacter sp*.

Significance: Our study demonstrates the feasibility of shotgun metagenomic sequencing for seafood. Metagenomics and MitoKmer hold promise as tools for facilitating public health management decisions in seafood safety and economic adulteration.

P1-184 Detection and Survival of *Listeria monocytogenes* on Seaweed (Sugar Kelp) during Storage

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❖ ***Developing Scientist Entrant***

Introduction: Numerous bacterial pathogens persist in coastal waters and can potentially contaminate edible seaweeds. Moreover, pathogens such as *Listeria monocytogenes* and Shigatoxigenic *Escherichia coli* (STEC) are occasional contaminants of fresh produce and can present a serious health risk in minimally processed vegetables including sugar kelp (*Laminaria saccharina*). Although kelps have some bacteria inhibitory potential, they can also harbor pathogens due to their rich nutritional components and high moisture content.

Purpose: The aim of this study was to evaluate the survival of STEC and *L. monocytogenes* on sugar kelp during post-harvest storage.

Methods: Sugar kelp (whole blade and shredded slaw) was inoculated with 6.0 log CFU/g of two strains each of *L. monocytogenes* and STEC. Samples were sealed either with or without vacuum and stored at ~ 4°C and ~ 22°C for 7 days. Microbiological analyses were performed daily on each treatment using FDA-BAM standard protocols. One-way ANOVA ($P < 0.05$) was performed to evaluate effects of the kelp form and storage temperature on pathogen survival.

Results: *L. monocytogenes* populations were reduced by 3 log CFU from 5.5 to 2.2 log CFU/g for whole blades and from 5.5 to 2.7 log CFU/g for shredded slaw immediately after an hour of inoculation and before refrigerated storage. *L. monocytogenes* populations were below detection limits from day 1 through day 7 of refrigerated storage of kelp. However, *L. monocytogenes* was detected on kelp samples during storage when subjected to microbial enrichment protocols.

Significance: The reduction in *L. monocytogenes* counts to below detection limits during storage suggests bacteriostatic nature of sugar kelp but results from the enrichment study imply the need to optimize postharvest processing practices. Thus, supplemental measures, such as blanching to kill pathogens should be considered to enhance the safety of sugar kelp.

P1-185 The Effect of Dry Salting and Brining on the Physicochemical and Microbial Properties of Sugar Kelp

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❖ ***Developing Scientist Entrant***

Introduction: Sugar kelp (*Saccharina latissima*) is a marine alga that is consumed for its nutritional benefits. Food preservation techniques such as drying are used to extend kelp shelf life. Salting and brining have been used on other algae, but their effects on sugar kelp have not been characterized.

Purpose: In this study, the effect of salting and brining treatments on the physicochemical and microbial properties of sugar kelp were analyzed throughout storage for up to 90 days.

Methods: Fresh sugar kelp was blast frozen and stored at -20°C after vacuum packaging. Frozen samples were thawed at 4°C for 24 h before experiments. Salting (30% w/w) and brining (40% NaCl w/vol solution) treatments were applied to kelp until water activity reached ≤ 0.7. Salted kelp was stored at ambient temperature or 4°C for up to 90 days. Physicochemical properties (water activity, moisture, color), and microbial quality (aerobic mesophilic, psychrotrophic, marine bacteria count) were assessed on days 1, 30, 60 and 90. Two-way ANOVA ($P < 0.05$) was used to evaluate the effects of treatment, storage temperature and time on characteristics of kelp.

Results: Salting and brining significantly reduced the water activity and moisture content of sugar kelp. Storage temperature and time both had significant effects on the color of the salted and brined kelp. Salting and brining significantly increased the darkness of kelp, regardless of storage temperature. No microbial counts exceeded 5.0 log CFU/g for any sample, but highest counts observed were for psychrotrophic organisms on brined samples stored at 22°C for 90 days. Storage at 4°C resulted in all counts below 2.75 log CFU/g regardless of time or treatment, again with counts slightly, but not significantly higher in brined samples.

Significance: Results indicate that dry salting and brining can be used for preservation of high quality sugar kelp.

P1-186 Population Dynamics of *Vibrio*, Oyster Microbiome and Effects of Aquaculture Practices

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❖ ***Developing Scientist Entrant***

Introduction: Oyster aquaculture is a primary approach that offers a sustainable and reliable food supply. Although the Controlling Plans are enforced by states and industries, oyster-related illnesses caused by pathogenic *Vibrio parahaemolyticus* (*Vp*) and *Vibrio vulnificus* (*Vv*) continue to occur and are on the rise.

Purpose: The purpose of this study was to assess the effects of the aquaculture practices, on the bottom and water floating cages, on the oyster-associated microbiome, and their interaction with the pathogenic *Vibrio* species.

Methods: Three oysters and 1L of water from each aquaculture system were collected monthly from June to November 2019, from the Chesapeake Bay. Physico-chemical water quality parameters were recorded per event. Oysters were abused at 25°C for 24 h before processing. Water samples and the temperature-abused oysters were examined for the levels of total and pathogenic *Vp* and *Vv* using three-tube MPN-qPCR method. Five hundred milliliters from each aquaculture-system water sample were filtered through a 0.2µm Sterivex. DNA from each temperature abused oyster homogenate, water filters were extracted, and shotgun metagenomic sequencing was conducted using HiSeq 4000 platform. One-way ANOVA and Pearson correlation tests were performed to assess the differences between samples and correlation between variables, respectively.

Results: The abundance score of *Vp* and *Vv* were higher among oysters that were grown in the floating cages. *Vv* dominated samples collected in August and September, while *Synechococcus* sp. CB0101 and *Synechococcus* sp. WH 8016 were the dominant species in July and October samples. Unlike *Synechococcus*, the abundance score of *Vibrio* from water and oyster samples was not proportional.

Significance: Results indicate that aquaculture-growing methods can significantly affect oyster's microbiome. It also indicates that *Vibrio* does not thrive during the first half of the summer as environmental conditions may become more favorable for other species in the first phase.

P1-187 Qualitative Application of Fourier Transform Near-Infrared (FT-NIR) for Freshness Assessment of Fresh Shrimps (*Litopenaeus setiferus*)

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Introduction: Fourier Transform Near-infrared (FT-NIR) spectroscopy utilizes a non-destructive approach and is suitable for solid, semi-solid, and liquid samples. In the present study, Total Volatile Basic Nitrogen (TVB-N) and trimethylamine (TMA) formation in fresh shrimp was monitored over time in order to develop an FT-NIR-based model that could be able to predict the freshness of shrimp stored at refrigerated temperature.

Purpose: Using a non-destructive method (FT-NIR) to create a freshness evaluation model depicting spoilage levels in shrimp (Litopenaeus setiferus).

Methods: Fresh shrimp (Litopenaeus setiferus) samples were washed and stored under refrigeration (4°C). Freshness parameters (TVB-N and TMA) were measured every day, along with transmittance spectra via FT-NIR (32 scans, 8 nm resolution, 12000 cm^{-1} to 4000 cm^{-1}). Data were collected over 5 days. Additionally, pH and TPC (log CFU/mL) were taken simultaneously. A deep learning algorithm will be used to develop and prediction model using Tensor flow architecture.

Results: The pH range was within 7.8 - 8.3 during the duration of the experiment. TPC ranged between 7.54 - 8.86 log CFU/mL. Spectra differs over the course of 5 days; around 5400 cm^{-1} - 4600 cm^{-1} peaks narrowed and broad peaks lessened around 7400 cm^{-1} - 6200 cm^{-1}.

Significance: FT-NIR as a non-destructive method, when coupled with advanced modeling techniques, helps rapidly assess shrimp freshness in a retail environment.

P1-188 Bacteriophages Reduce *Listeria* Contamination in RTE Seafoods

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Introduction: *Listeria* contamination of seafood can occur naturally, yet it can also be introduced post lethality. Hence, processors are in need of food safety interventions in order to ensure regulatory compliance and to mitigate human illness. The use of bacteriophages can help processors to kill *Listeria* on incoming raw materials and RTE products.

Purpose: Assess the ability of a commercially available intervention (PhageGuard Listex™) to reduce *Listeria* contaminations in RTE shrimp and on salmon roe.

Methods: Unpeeled cooked shrimp and salmon roe samples were dip treated in phage solution ($1x10^9$ PFU/mL) for 5 minutes. After drain, samples were inoculated with one *Listeria monocytogenes* strain at a level of $1x10^5$ CFU/g. After contamination, samples were stored at 40°F. After 24 hours, samples were retrieved to enumerate *Listeria*. To rule out that phages kill *Listeria* during retrieval, residual activity was measured by adding one sample that was inoculated and another sample that was phage treated into the same retrieval bag followed by the standard retrieval method. All data presented are the mean values of three individual experiments statistically analyzed by a two-tailed paired *t*-test.

Results: In contaminated cooked shrimp, phage dip treatment in $1x10^9$ PFU/mL reduced *Listeria* significantly by 1.5 log ($P < 0.05$) after 24 hours. Similar *Listeria* reductions (1.1 log, $P < 0.05$) were obtained with salmon roe after 24 hours. Thus, a dip approach resulted in an effective way to treat food matrices such as shrimp and salmon roe. There was no statistical difference ($P > 0.05$) found when control and treated samples were retrieved together, showing that there was no residual phage activity during retrieval.

Significance: Since *Listeria* is able to grow at refrigeration temperatures, steps to eliminate *Listeria* should be taken by processors. Application of PhageGuard Listex™ to unpeeled cooked shrimp and salmon roe is an effective way to control *Listeria*.

P1-189 Optimization of *Carcinus maenas* Fermentation

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❖ ***Developing Scientist Entrant***

Introduction: *Carcinus maenas,* is an aggressive invasive species inhabiting the Pacific and Atlantic coasts of North America. Marketable uses for this species may help in establishment of a fishery that can reduce ecosystem damage and predation. Previous research has supported the viable use of *Carcinus maenas* for a value-added fermented sauce product but the effects of fermentation temperature and starter culture inoculation are unknown.

Purpose: The purpose of this study was to investigate the effect of fermentation temperature and the use of starter cultures on the amine nitrogen concentration and histamine content.

Methods: Green crabs trapped off the coast of Maine were euthanized by freezing. Crabs were crushed in a Hobart grinder, combined with 20% (w/w) salt in triplicate and fermented at 24°C, 30°C (with and without *Tetragenococcus halophilus* inoculation), 37°C (with and without *Staphylococcus carnosus* inoculation), and 50°C. Sampling occurred after 15, 30, 60, and 90 days of fermentation. Amine N was quantified by formol titration. Histamine content was determined by HPLC using Waters AccQ·Tag. Data were analyzed by Shapiro test for normality, ANOVA or Kruskal Wallis test for variance, followed by Tukey's HSD post hoc test in R studio.

Results: No significant differences ($P > 0.05$) in histamine content (average 6.7 ± 0.9 mg/100 mL) among treatments were observed. Amine N at fermentation temperatures of 30, 37, and 50°C was significantly higher than the 24°C treatment throughout the fermentation, indicating a greater extent of

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desirable proteolysis in these treatments. The treatments reached a maximum amine N of 423.9, 682.9, 679.8, and 515.7 mgN/100 mL at 24°C, 30°C, 37°C, and 50°C respectively, on the final day (d 90).

Significance: This data shows that *S. carnosus* and *T. halophilus* are ineffective in reducing histamine in a fermented crab condiment. Temperatures ranging from 30-37°C appear to be ideal for production of a fermented crab condiment.

P1-190 The Power of Good Bacteria: A Natural Food Safety Hurdle for *Listeria monocytogenes* Inhibition on Smoked Salmon

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Introduction: C*ontamination with Listeria monocytogenes is reasonably likely to occur in cold smoked salmon.* A cocktail of lactic acid bacteria (LAB) has shown its efficacy as a natural hurdle to help suppress the growth of this pathogenic bacteria on this sensitive ready-to-eat fish product.

Purpose: The purpose was to evaluate if the addition of a specific cocktail of LAB (*Leuconostoc carnosum* + *Lactococcus lactis*) can inhibit the growth of *L. monocytogenes* on 6 recipes of smoked salmon without negatively impacting their sensory properties.

Methods: Nine challenge tests (6 different recipes/suppliers) were performed. The LAB were added (concentration between 6.5 and 7.0 log CFU/g) either into the brine (when the fish was injected) or by spray-on application just after the salting phase or during slicing (when the fish was dry salted). The slices were vacuum or MAP packed and stored until their use-by-date considering part of the storage at abuse temperature. *L. monocytogenes* concentrations were measured at D_0, $D_{1/3}$ and $D_{\text{end of shelf life}}$ (3 samples per sampling time). At the same sampling times, pH and LAB concentrations were measured. Sensory evaluation was also performed by a panel of consumers (hedonistic + triangular test).

Results: The *L. monocytogenes* growth potential -GP- (Concentration at D_x - Concentration at D_0) was always below 0.5 log CFU/g when LAB were added. In the control samples the GP was always above 0.9 and up to 6.3 log CFU/g. The LAB cocktail was able to grow in all recipes without impacting on the flavor of the smoked salmon.

Significance: The *Leuconostoc carnosum* + *Lactococcus lactis* cocktail is an efficient natural food safety hurdle to help suppress the *L. monocytogenes* growth in cold smoked salmon without compromising the taste of the product.

P1-191 Efficacy of Phage Intervention Against *Salmonella* on Salt and Fresh-Water Fish

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Introduction: The presence of *Salmonella* in raw seafood provides a risk to human health and promotes efforts by the supply chain to mitigate these risks. This is particularly critical for fish that is eaten raw or is cold smoked. Novel interventions which can reduce or eliminate *Salmonella* from seafood products without affecting finished product quality are needed by the industry.

Purpose: Determine the efficacy of a commercially available bacteriophage product against a cocktail of *Salmonella* strains on raw seafood including both saltwater and fresh-water fish.

Methods: Salt and fresh-water fish samples were inoculated with a cocktail containing five of the most prevalent *Salmonella* strains in seafood at $2x10^5$ CFU/cm^2. Subsequently, samples were treated in duplicate with phage concentrations of $1x10^7$, $7x10^7$ PFU/cm^2 or water. Samples were stored at 39.2°F for 0, 1 or 24 hours before retrieval and bacterial enumeration on selective agar plates. To rule out phage activity during retrieval, one fish sample was inoculated while another was treated, then added to the same retrieval bag and retrieved as normal. The trials were repeated independently three times and a two-way ANOVA was performed.

Results: Phage application to salt-water fish gave reductions of 0.9 and 1.3 log, respectively, ($P < 0.05$) after 24 hours. Similar results were obtained in fresh-water fish (1.0 and 1.4 log, respectively [$P < 0.05$]). Most of the phage activity was observed in the first hour, with slight further reductions after 24 hours. Overall, a dose response was observed. Furthermore, there was no difference ($P > 0.05$) in bacterial concentration between control samples versus the samples that were separately inoculated and treated, but retrieved together, showing that no residual phage activity occurred during retrieval.

Significance: Phage treatment is an effective intervention for reducing Salmonella on seafood and provides increased consumer safety. The tested phage solution can reduce *Salmonella* contamination on different fish types by 1.3 to 1.4 log.

P1-192 Prevalence of Antibiotic-resistant Bacteria in Retail Shrimp

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Introduction: In the US, imported farm-raised shrimp accounts for ~80% of the total market share which are cultivated as monoculture at a high density. Application of unapproved antibiotics as prophylactic and therapeutic agents can result in the development of antibiotic-resistant bacteria in farm-raised shrimp.

Purpose: To this end, we herein aimed to characterize the prevalence of antibiotic-resistant bacteria and the gut microbiome communities in commercially available shrimp.

Methods: Thirty-one raw and cooked shrimp samples were purchased from supermarkets in Florida and Georgia between March-September 2019. The samples were selectively enriched in tryptic soy broth with cefotaxime, and the enriched samples were streaked on MacConkey agar with antibiotics. Isolates obtained were identified by 16S rDNA gene sequencing and were characterized using an array of molecular and antibiotic-susceptibility tests.

Results: A total of 110 isolates, spread across 18 genera comprised of coliforms and opportunistic pathogens, were isolated. Aerobic plate counts of the cooked samples (n = 13) varied from <25 to 6.2 log CFU/g. Interestingly, isolates from cooked shrimp showed higher resistance towards chloramphenicol (18.6%) and tetracycline (20%), while those from raw shrimp exhibited low levels of resistance towards nalidixic acid (10%) and tetracycline (8.2%). Compared to wild-caught shrimp, the imported farm-raised shrimp harbored distinct gut microbiota communities, i.e., by a higher proportion of Proteobacteria*,* Alteromonadales, Rhizobiales, Synechococcaceae, Myxococcales, and Planctomyces. In contrast, Ecuador's shrimps were characterized by a higher abundance of Tenericutes*, Mollicutes,* Flavobacteriales*, Desulfovibrio,* Oscillatoriales, *Mycobacterium,* and Bacteroidales. The Ecuador shrimp samples microbiota showed a higher prevalence of antibiotic-resistance genes in their gut.

Significance: The presence of multidrug-resistant bacterial strains and opportunistic pathogens in imported cooked shrimp products may threaten human health and calls for the need for standardization of effective cooking time-temperature combinations for potential mitigation of antibiotic-resistant strains in cooked shrimp samples.

P2-01 Multi-Mycotoxin Occurrence in Asia Spices

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Introduction: Mycotoxins represent a risk to the food supply chain with an impact to human health. Food samples have been reported to be contaminated with more than one mycotoxin. In most cases, the concentrations were low enough to ensure compliance with the European Union (EU) guidance values or maximum admitted levels. However, mycotoxin co-contamination might still exert adverse effects on humans due to synergistic effect. And also, if the food samples with mycotoxins presence are exported to Regulated countries like European Union, United States, the companies will be blacklisted.

Purpose: Many countries in South East Asia and South Asia are only regulated for Aflatoxin and food spices are only analyzed with multi mycotoxin if they are exported overseas. In south and south east Asia, spices are consumed daily by people. In this study, we analyzed different types of different types

of food spices for multi-mycotoxin.

Methods: Many different types of spices are analyzed by Romer Labs ISO 17025 accredited Analytical Service Lab in Singapore using LCMS/MS. This includes ground pepper, ground garlic, turmeric, cumic mustard, and cinnamon. The mycotoxins analyzed are Aflatoxin, B1, B2, G1 and G2, ochratoxin, Zearalenone, deoxynivalenol, fumonisins, T2 and T2/ HT2.

Results: The results for most spices analyzed are <LOD for most mycotoxins. Aflatoxin are found are in some spices but all are below 5 ppb. Ochratoxin at 11.9 ppb are found are to be present in paprika. This is above the regulation limit in Europe. There are also several mycotoxins found in Nutmeg, at 59.6 ppn level for aflatoxin, 13.9 ppb for Ochratoxin and 27 ppb for Zearalenone. Mycotoxin co-contamination might still exert adverse effects on humans due to synergistic effect on 2 or more mycotoxin found in food spices.

Significance: Food spices are recommended to be analyzed for mycotoxin regularly.

P2-02 Multideterminationof Nitrofurans and Chloramphenicol in Food and By Enzyme-Linked Immunosorbent Assay

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Introduction: Veterinary drugs such as nitrofurans and chloramphenicol are used in aquaculture products to control disease and improve food safety. However, the occurrence of residues in drinking water and food and feed poses major public health concerns when consumed by humans and animals. This makes it all the more necessary to have a fast, effective, affordable testing regimen such as ELISA in place.

Purpose: To harmonize the extraction protocols of AgraQuant® Nitrofurans (AHD, SEM, AOZ Plus and AMOZ Plus) and Chloramphenicol plus for ELISA screening purposes for shrimp so that one sample extract can be used for all five kits. To validate ELISA Drug Residue kits ((AHD, SEM, AOZ Plus and AMOZ Plus) and Chloramphenicol by comparing to the LCMS/MS reference method for the analysis of drinking water, honey, eggs and feed.

Methods: Honey and eggs and drinking water are obtained from supermarket. Feed was obtained from customer. All the samples were sent for LCMS/MS analysis in Nitrofurans (AHD, SEM, AOZ and AMOZ) and determined to be non-detectable. Twenty non-detectable shrimp samples of 1 g each were either fortified with 0.5 ppb AHD, 0.5 ppb SEM, 0.5 ppb AOZ, 0.5 ppb AMOZ or 0.15 ppb Chloramphenicol according to the half of MRPL (minimum required performance limits). This limit was set by European Union Commission Decision 2003/181/EC. The spiking controls used were analyzed by LCMS/MS to confirm the concentration. Another 20 blank samples were used as control. The samples were extracted using ethyl acetate and analyzed using the ELISA.

Results: The mean recovery of all the samples is 88.0% (0.44 ppb) for AMOZ, 86.6% (0.433 ppb) for AOZ, 123.0% (0.615 ppb) for SEM, 95.4% (0.477 ppb) for AHD and 99.0% (0.495 ppb) for Chloramphenicol. No false positives were found in the blank samples.

Significance: The AgraQuant® Nitrofurans and Chloramphenicol plus offers a rapid and reliable tool for testing drug residues in food, feed and water.

P2-03 Withdrawn

P2-04 Changes in Sensitivity to Quaternary Ammonium Compound (QAC) in Seven *Listeria monocytogenes* Strains after Exposure to Gradually Increasing Concentrations

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Introduction: Although the in-use concentrations of quaternary ammonium compound (QAC) in food industries are lethal for most foodborne pathogens, some conditions can create sublethal concentrations which can be advantageous for growth and survival of these pathogens.

Purpose: The objective of this study is to determine the survival of *L. monocytogenes* in QAC after adaptation to gradually increasing concentration of QAC.

Methods: Twenty-five strains of *L. monocytogenes* were selected on TSAYE containing QAC of 2, 5 and 10 µg/mL after 48 h of incubation at 37°C. The single colonies isolated from TSAYE-10 µg/mL QAC were selected. Adaptive response was measured by determining the changes in short-range MIC of QAC for QAC-adapted phenotypes versus non-adapted cells using broth dilution assay. Also, growth curve analysis of QAC-adapted phenotype and non-adapted cells was determined in 2 µg/mL of QAC at OD_{600nm}; and survivals of QAC-adapted and non-adapted cells was determined on TSAYE-5 µg/mL QAC plates. The experiments were repeated thrice, and results were analyzed using ANOVA and means separated using Duncan's multiple range test with significance level $P < 0.05$.

Results: Of the 25 *L. monocytogenes* strains tested, 7 strains were adapted to gradually increasing concentration of QAC. A significant increase in MIC by 1.4 to 1.8 fold (= 2.3 to 3.3 µg/mL changes in MIC) was observed for all QAC-adapted phenotypes of *L. monocytogenes* ($P < 0.05$). Also, growth rate of QAC-adapted phenotypes was faster in 2 µg/mL of QAC at OD_{600nm} ($P < 0.05$). Lag phase of *L. monocytogenes* was decreased by 4-8 h in 2 µg/mL of QAC for QAC-adapted cells as compared to non-adapted cells. Survival of QAC-adapted cells was increased significantly by 2-5 log CFU/mL ($P < 0.05$) for all strains on TSAYE-5 µg/mL QAC plates compared to non-adapted cells.

Significance: These findings suggest that the low-level tolerance to QAC may allow the survival of *L. monocytogenes* in environment where QAC might be widely.

P2-05 Developing Standard Reference Libraries of *Listeria monocytogenes* and *Escherichia coli* O157:H7 Using an Affordable Custom Assembled Hyper Spectral Imaging System

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❖ ***Developing Scientist Entrant***

Introduction: With the increased incidences of foodborne diseases and outbreaks in the recent past, there is a need for pathogen detection and identification system which is rapid and inexpensive. Hyperspectral imaging (HSI) is one such novel technique that amalgamates imaging and spectroscopy methods to capture both spatial and spectral features of bacterial cells.

Purpose: To obtain hyperspectral data of individual bacterial cells grown on nutrient and selective agars using a custom assembled HSI system and to create reference libraries using environment for visualizing images (ENVI) software.

Methods: This study was designed as a randomized complete block design with three replications. Three strains of *Listeria monocytogenes* (LM) and *Escherichia coli O157:H7* (EC) were used in this study. Brain heart infusion agar (BHI) was employed as nutrient media, and PALCAM and Sorbitol MacConkey agars were used as selective media for LM and EC, respectively. Freshly prepared single and mixed strains of respective pathogens were streaked for isolation on the respective media. A single colony of the desired pathogen was selected and mixed in 1 mL of HPLC-grade water and vortexed for 1 minute. Subsequently, using a sterile loop, slide preparation was performed, and then analyzed using HSI setup. Acquired images were imported into ENVI software and 3-regions of interest (ROI) were selected for each image. The hyperspectral data was imported into R-software for statistical analysis.

Results: This HSI system was assembled using $20,946, which is about a fifth of the cost of pre-assembled and pre-programmed commercial HSI microscope system. Difference between scattering intensities of LM and EC were discernable at wavelength ranges of 500-700 and 900-1025 nm, where scattering intensities of LM were lower as compared to EC. Future work will focus on improving magnification power of HSI system and strengthening reference libraries.

Significance: The data from reference libraries can be utilized for rapid identification of various pathogens in variety of food matrices.

Poster

P2-06 Understanding Bacteria Adhesion and Biofilm Formation on Different Surfaces Using a Center for Disease Control and Prevention (CDC) Biofilm Reactor

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Undergraduate Student Award Entrant

Introduction: *Listeria monocytogenes* is one of the top food safety challenges for the produce industry due to its ability to form biofilms. The prevalence and persistence of *Listeria* biofilms on food contact surfaces increases throughout time. After the biofilm is formed, it becomes difficult to disrupt. Understanding the properties of bacterial adhesion and biofilm formation on different surfaces, will offer a better insight to the effectiveness of antimicrobial treatments.

Purpose: The objective of this research was to assess the formation and growth of mixed-species biofilms on different materials using a CDC Biofilm Reactor.

Methods: A CDC biofilm reactor was used to grow a multi-strain *Listeria* biofilm over a 4-day period. Different materials were evaluated for biofilm growth: stainless steel, polycarbonate, wood, and nylon. All biofilms were grown at 22 ˚C in Tryptic Soy Broth (TSB). Biofilm adhesion, growth and development was observed overtime by enumeration and microscopy methods (Laser Scanning Confocal Microscopy, LSCM).

Results: High cell counts (~ 10^6) were recovered on stainless steel, while low counts were obtained on nylon (~ 10^4) ($P < 0.05$). An interaction between material and biofilm cell count was observed ($P < 0.05$). When biofilms were characterized by LSCM, cells appeared to spread throughout the surface with no significant differences among materials during initial attachment (day 0). After 24 hours differences in aggregation and clustering were visible: larger colonies were present on nylon.

Significance: The results obtained in this research offer a preliminary understanding of *L. monocytogenes* multi-strain biofilm growth, adhesion and development on different surfaces commonly used in the produce industry using a CDC Biofilm Reactor. This information could be used to develop effective antimicrobial treatment to control biofilm in produce environment.

P2-07 Changes in Sensitivity to Ciprofloxacin in Seven *Listeria monocytogenes* Strains after Exposure to Gradually Increasing Concentration of Quaternary Ammonium Compound

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Introduction: Evidence is slowly emerging that sublethal stresses encountered in food processing environments can contribute towards heterologous cross-protective response against antibiotics.

Purpose: The objective of this study is to investigate the heterologous stress-response in *L. monocytogenes* strains to ciprofloxacin after adaptation to gradually increasing concentration of QAC in an agar model.

Methods: A total of 25 *L. monocytogenes* strains were selected on TSAYE containing gradually increasing QAC at 2, 5 and 10 µg/mL after incubation at 37°C/48 h. The single colonies isolated from TSAYE-10 µg/mL QAC plates were selected. The heterologous stress response was determined by the changes in short-range MIC of ciprofloxacin for QAC-adapted phenotypes versus non-adapted cells using broth dilution assay. The growth rate of QAC-adapted phenotype and non-adapted cells was determined in 2 µg/mL of ciprofloxacin at OD_{600nm}; and survivals of QAC-adapted and non-adapted cells was determined on TSAYE-2 µg/mL ciprofloxacin plates. The experiments were repeated thrice, and results were analyzed using ANOVA and means separated using Duncan's multiple range test with significance level $P < 0.05$.

Results: Of the 25 *L. monocytogenes* strains tested, 7 strains were adapted to gradually increasing concentration of QAC. A 1.5- to 2.4-fold increase (= 2-5.3 µg/mL increase) in MIC of QAC was observed for all QAC-adapted phenotypes as compared to the non-adapted strains ($P < 0.05$). As compared to the non-adapted cells, the QAC-adapted cells showed faster growth in 2 µg/mL of ciprofloxacin at OD_{600nm} ($P < 0.05$). Also, the lag phase of *L. monocytogenes* was decreased by 6-8 h in 2 µg/mL of ciprofloxacin for QAC-adapted cells as compared to non-adapted cells. There was an increase in survival by 3 log CFU/mL for all QAC-adapted cells ($P < 0.05$) TSAYE- 2 µg/mL ciprofloxacin.

Significance: These findings illustrate the potential for occurrence of sub-populations of *L. monocytogenes* strains tolerant to ciprofloxacin as a result of gradual sublethal adaptive response to QAC in conditions where QAC may be used widely.

P2-08 Sanitizer Tolerance and Attachment Capacity of Non-Outbreak and Outbreak-Associated *Salmonella enterica* Isolates from Multiple U.S. Outbreaks

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Introduction: Non-typhoidal *Salmonella enterica* causes 1.35 million illnesses annually in the United States, with 26,500 hospitalizations and 420 deaths. We previously found that isolates from the 2013-2014 *Salmonella* Heidelberg outbreak in chicken had increased stress tolerance compared to non-outbreak isolates.

Purpose: The objective of this study was to compare the attachment capacity and sanitizer tolerance of non-outbreak (NOA) and outbreak associated (OA) isolates of monophasic *Salmonella* I 4,[5]12:i- to *Salmonella* Heidelberg isolates.

Methods: We analyzed 21 OA and four NOA isolates using crystal violet assays to test attachment at 24, 72, and 120 hours in 1X and 1/20X TSB at 22°C and 4°C. Minimum inhibitory concentrations (MICs) were determined for bleach and peroxyacetic acid sanitizer Inspexx-250 in 1X and 1/20X TSB at 24 hours, 22°C using OD_{600}. Resfinder (v. 4.1) and PlasmidFinder (v. 2.1) identified antibiotic resistance elements and plasmids. Statistically significant differences between isolates were determined via analysis of variance with Tukey's HSD test ($P_{adj} < 0.05$).

Results: In 1/20X TSB at 4°C, three *Salmonella* Heidelberg OA, two monophasic OA, and one NOA monophasic isolate attached significantly better than ≥3 others. At 22°C, five Heidelberg OA, two monophasic OA, and one NOA monophasic isolate attached significantly better than ≥3 others. In 1X TSB at 4°C and 22°C, only one OA Heidelberg attached significantly better than ≥3 others. No monophasic OA isolate had plasmids, while all NOA monophasic isolates had ≥1 plasmid. All OA and three NOA monophasic isolates had *blaTEM-1B, sul2*, and aminoglycoside resistance genes. MICs for bleach, 1X TSB were >200ppm and >100ppm in 1/20X TSB ($p_{adj} < 0.01$). MICs for PAA, 1X TSB were >175ppm vs 25.9ppm in 1/20X TSB ($p_{adj} < 0.01$).

Significance: Understanding the varying ability of OA and non-OA isolates from serovar I 4,[5]12:i- to form biofilms and tolerate sanitizers is vital for the prevention of future outbreaks.

P2-09 Cold Shock Domain Family Proteins: Investigation of Phenotypes and Regulons in *Listeria monocytogenes*

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Developing Scientist Entrant

Introduction: Cold shock proteins CspA, CspB, and CspD play important roles in *Listeria monocytogenes* (*Lm*) stress resistance and virulence.

Purpose: Evaluation of Csp-dependent phenotypes and regulons in food and clinically relevant *Lm* strains.

Poster

Methods: *Lm* lineage I, II, and III strains (*n* = 7) and their corresponding *csp* deletion mutants (*n* = 24) were characterized on phenotype microarrays (PM), targeted stress (0-8% NaCl and 0-12.5 mg/mL β-phenylethylamine), motility, and virulence (zebrafish embryos microinjection) assays and transcriptome analysis. Data were analyzed using ANOVA.

Results: On PM, significantly ($P < 0.05$) reduced carbon-source utilization and increased osmotic, pH, chemical, and antimicrobial stress sensitivity was observed upon deletion of *csp* genes. A Δ*cspABD* mutant devoid of Csps demonstrated increased sensitivity on 56/240 tested PM-stress conditions. PM data was further corroborated through targeted phenotypic assays. For instance, this confirmed that Δ*cspABD* mutant growth was impaired on glucose, glycerol, and rhamnose as sole carbon sources. Δ*cspABD* and Δ*cspD* mutants (4-fold growth rate reduction) were less tolerant to 4% NaCl stress compared to wild-type strains, while Δ*cspA* and Δ*cspAB* (1.24- and 1.14-fold area under growth curve increase, respectively) were more resistant to β-phenylethylamine stress. Complete motility attenuation occurred in Δ*cspABD* mutants, whereas single Csp expressing mutants displayed a hierarchical motility trend of CspA>CspB>CspD indicating varying individual Csp contribution to *Lm* motility. Virulence analysis revealed that CspB expression alone was sufficient to retain wild-type strain virulence levels (60-100% mortality [*n* = 30 embryos/strain] 24 h post-infection), whereas individual expression of CspA and CspD caused lower (0-20% mortality) pathogenicity than the wild-type strains. Transcriptomics revealed Csp regulons that include rhamnose utilization genes consistent with impaired rhamnose utilization observed among *csp* mutants.

Significance: Our study has revealed important Csp roles in *Lm* stress tolerance and virulence, traits central to its public health and food safety impacts. Such knowledge provides a basis for novel and improved *Lm* control strategies.

P2-10 Identification and Characterization of a *Salmonella enterica* Plasmid That Confers Increased Resistance to Bacteriophages

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Introduction: *Salmonella enterica* is a major cause of foodborne illness from numerous sources including meat, eggs, poultry, and fresh produce. A method utilized to lyse bacterial cells on these foods and associated processing surfaces is the application of bacteriophages. However, some bacteria possess genetic systems that enhance resistance to bacteriophages which could reduce their efficacy. This study identified a foodborne *S. enterica* plasmid with a putative abortive infection system and tested its role in phage resistance.

Purpose: The purpose of this study was to evaluate whether a plasmid from *S. enterica* could increase resistance against bacteriophages.

Methods: The polyvalent bacteriophage, FelixO1, was tested via plaque assay on several strains of *Salmonella*. The genomes of some strains of *S. enterica* with reduced susceptibility to phage lysis were analyzed and a putative plasmid encoded phage abortive infection system identified. Using genetic recombination, the genes were transformed into *S. enterica* serovar Typhimurium (STM). A combination of plaque, adsorption and efficiency of plating assays were used to test phage sensitivity of STM transformed with the abortive infection system or an empty vector.

Results: FelixO1 lysed all strains of *Salmonella* tested in this study although plaques were only visible on ~54% of them (25 of 46 strains). The most resistant strains had plasmids with an abortive infection system that was subcloned into STM. The genes for the abortive infection system enhanced resistance to FelixO1 by reducing plaque forming unit size and number. The efficiency of plating was significantly reduced ($n = 3$; 0.14 ± 0.04, $P = 0.0001$) whilst the adsorption efficiency was not ($n = 3$). This indicates that FelixO1 phage replication is inhibited at a stage post-adsorption by the *Salmonella* abortive infection system identified in this study.

Significance: These data indicate that mechanisms of phage resistance naturally present within species of *S. enterica* may inhibit polyvalent bacteriophages such as those used commercially in the food industry.

P2-11 Efficacy of Different Bacteriophage Multiplicities of Infection Against *Salmonella enterica*

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❖ ***Developing Scientist Entrant***

Introduction: In the past 5 years, there have been 41 *Salmonella* outbreaks in food within North America. Bacteriophages have been utilized against *Salmonella* in numerous studies but the efficacy of different multiplicities of infection (MOI) of bacteriophage viruses against *Salmonella enterica* (*S. enterica*) is unclear.

Purpose: To determine if there is an optimal bacteriophage MOI against *S. enterica*.

Methods: Four *S. enterica* strains (*S.* Typhimurium 536, *S.* Enteritidis 483, *S.* Newport 639 and *S.* Muenchen 504) were tested against four bacteriophages (SF5, SF6, SE14, and SE20) separately and in two different bacteriophage cocktails: 1) SF5, SF6, SE14 and 2) SF6, SF14, SE20. The MOIs tested were 1, 10, 100, 1,000 and 10,000 with a microplate assay for 48 hours at 25°C. Six biological replicates were conducted for each MOI.

Results: At the same MOI, bacteriophage efficacy is dependent on the *S. enterica* strain. Cocktail 2 significantly reduced growth across all four strains. In contrast, cocktail 1 was not effective against *S.* Muenchen 504 and *S.* Newport 639, but MOIs 1,000 and 10,000 both significantly reduced the highest population of *S.* Typhimurium 536, followed by MOIs 100, 10 and 1 respectively ($P < 0.05$). MOIs 1,000 and 10,000 of SE14 significantly reduced growth on all strains ($P < 0.05$). SF5 delayed *S.* Enteritidis 483 growth at all MOIs until 16 hours and continued to significantly reduce growth compared to the control at 48 hours ($P < 0.05$). The cocktail at MOI 10,000 at SF5 was most effective at delaying and reducing *S.* Typhimurium 536 growth and similar results were shown with bacteriophage SF6 against the strain.

Significance: Understanding the optimal MOI of individual bacteriophages and cocktails and the *S. enterica* strains susceptible to bacteriophage infection is invaluable to the utilization of bacteriophage as a biocontrol method against foodborne pathogens in food.

P2-12 Novel Approach Based on Mathematical Modeling for Determination of the Lytic Capacity of Two Bacteriophages in a Model of *Salmonella* Infantis

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✦ ***Undergraduate Student Award Entrant***

Introduction: *Salmonella* is an important foodborne pathogen. In recent years, its serovar *S.* Infantis, has significantly emerged worldwide. Viruses that infect bacteria, bacteriophages (phages), can eventually cause their death, and due to this, they have been used in the biocontrol of *Salmonella*. While there have been recent advances in the use of phages as a *Salmonella* control, there are still challenges remaining, such as selecting the best candidates for the therapeutical applications of this pathogen.

Purpose: To develop and test a mathematical model that predicts, based on kinetic parameters of the bacteriophage, phage's lytic capacity against a strain of *Salmonella* Infantis.

Methods: Kinetic characterization of two phages vB_Si_35FD and vB_Si_SF20-2, was performed (adsorption constant, latency period, and burst size) in a *S.* Infantis host. A mathematical model was created, which was later fed with the determined experimental data. The simulations were computed in Berkeley Madonna version 10.1.2. As standard methodology for lytic capacity was used, killing curve of both phages for 12 h with *S.* Infantis was used, with a statistical analysis of *t*-test performed obtaining a *P*-value < 0.005.

Results: The mathematical model was solved based on the kinetic parameters obtained experimentally, the simulations demonstrated that the phage vB_Si_35FD had a greater lytic capacity than the phage vB_Si_SF20-2. Experimentally, it was obtained that the phage 35FD presented a higher adsorption constant, lower latency period (20 min) than the phage SF20-2 (40 min) and the burst size in the phage 35FD (90 particles/mL) was three times higher than

the SF20-2.

Significance: These results suggest that in a first approach, the mathematical model could predict the lytic capacity of a bacteriophage based on its kinetic parameters, as an alternative of large process of selection.

P2-13 A Comparison of *Salmonella* Survival and Detection Using an Enrichment Technique in Dry and Wet Inoculated Rendered Chicken Fat Treated with Sodium Bisulfate (SBS)

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Introduction: Coating of dry pet food with rendered chicken fat is a common practice to increase energy density and enhance palatability. This occurs after the established kill step. If the fat gets contaminated with *Salmonella* from dust or residual water in storage tanks or during transport could become a source of contamination.

Purpose: To determine the effect of dry vs wet inoculum on the recovery of *Salmonella* from rendered chicken fat treated with sodium bisulfate (SBS).

Methods: Sterile rendered chicken fats were inoculated (~7 logs) with *S*. Enteritidis, *S*. Heidelberg, and *S*. Typhimurium cocktail using dry and wet inoculum followed by treatment with 0.5% aqueous SBS and incubated at 40°C. An aliquot from both the fat and aqueous phases were analyzed at different time intervals (0, 2, 6, 12, and 24 h) by plating on TSA agar plates. The plates were incubated at 37°C for 24 h, all in triplicates. The 24-h fat samples with no growth on TSA agar were enriched for *Salmonella* isolation followed by PCR confirmation using primers for the *invA* gene.

Results: The untreated controls in the aqueous phase had a consistent level of *Salmonella* (~7 logs) in both inoculum types. In the SBS treated aqueous phase, *Salmonella* was not detectable after 6 h and 24 h in wet inoculated and dry inoculated samples, respectively. *Salmonella* was detected for up to 2 h and 6 h in wet inoculated and dry inoculated fat phases, respectively, in the SBS treated samples. Upon molecular confirmation, both SBS-treated and control samples from the dry inoculated, and the inoculated control from the wet inoculated fat tested positive for *Salmonella*.

Significance: The results suggest the development of potential VBNC states of *Salmonella* or the presence of injured cells in rendered chicken fat. This would indicate the need for enrichment and appropriate molecular confirmation rather than a determination by agar plating alone.

P2-14 Evaluation of an Ozonated Water Spray on Microbial Decontamination of Domestic Kitchen Surfaces

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Introduction: During household preparation of food, utensils can become contaminated from food or food handlers. Contaminated utensils can lead to food safety concerns if they are not cleaned and sanitized after use. Aqueous ozone is an attractive sanitizing option for household use, with no detectable residues and potential antimicrobial effects.

Purpose: This study aims to evaluate the effect of ozonated water spray in reducing different bacteria on polypropylene cutting boards and stainless-steel knives.

Methods: Bacterial suspensions (10^8 CFU/mL) of *E. coli*, *Pseudomonas* spp., *Listeria innocua* and *Staphylococcus aureus* were spot inoculated within a 100 cm^2 area on a cutting board and allowed to air dry for 25 minutes. Ozonated water spray (o3waterworks® sanitizing spray bottle, 2 ppm ozone) was applied following the manufacturer's instructions, allowing 30 s before sampling. Surfaces were swabbed using polyurethane sponge samplers with D/E neutralizing broth. *E. coli* counts were determined on MacConkey agar, *Pseudomonas* on LB agar, *Listeria* on Brain Heart Infusion agar, and *Staphylococcus* on Tryptic Soy agar. Untreated surfaces were used as a positive control. Experiments were performed in triplicate (one board/per replicate).

Results: Bacteria counts (log CFU/cm^2) on the untreated cutting boards were 4.0 ± 0.1 for *E. coli*, 4.4 ± 0.5 for *Pseudomonas* and 4.5 ± 0.3 for *Listeria* and *Staphylococcus*. The ozone treated boards had counts (log CFU/cm^2) of 4.0 ± 0.3 for *E. coli*, 4.3 ± 0.5 for *Pseudomonas*, 4.4 ± 0.4 for *Listeria* and 4.4 ± 0.0 for *Staphylococcus*. There was no significant difference between ozone treated and untreated cutting boards.

Significance: There was no significant change in counts on cutting boards after the ozone spray treatment while following the manufacturer's instructions. Further experiments will be carried out with longer exposure times and on stainless steel knives. It is important to validate commercial decontamination systems advertised for household use.

P2-15 Validation of the Baking Step to Control *Salmonella* and *Listeria monocytogenes* in Brownies

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Introduction: *Salmonella* and *Listeria monocytogenes* can survive under the dry environment of flour for extended periods of time and could multiply when flour is hydrated to prepare batter or dough. Therefore, inactivation of these pathogens during the cooking/baking step is vital to ensure the microbiological safety of bakery products such as brownies.

Purpose: To validate a simulated commercial brownie baking process as a kill-step for controlling *Salmonella* and *L. monocytogenes*.

Methods: Independent studies were conducted in a complete randomized block design with three replications. All-purpose flour was inoculated with a 5-serovar *Salmonella* or 3-strain *L. monocytogenes* cocktails and dried back to original pre-inoculation water activity (a_w). Brownie batter was prepared from inoculated flour and baked in a conventional oven at 176.7°C for 40 min in a 12" by 12" pan, followed by 15 min ambient-air cooling. Samples (~15 g) were taken at 5 min intervals during baking and after the 15 min cooling period for microbial enumeration, pH, and a_w analyses. *Salmonella* and *L. monocytogenes* populations were enumerated using injury-recovery media (brain heart infusion agar (BHI) overlaid with xylose lysine deoxycholate (XLD), or PALCAM agar, respectively). When the crust and crumb were formed during baking, a_w and pH of brownies were measured separately.

Results: Initial *Salmonella* and *L. monocytogenes* populations in brownie batter were 6.9 and 6.5 log CFU/g, respectively, and fell below the detection limit (0.6 log CFU/g) after baking for 30 and 35 min, respectively. The pH was similar throughout the baking process, and the a_w significantly decreased over the entire baking process.

Significance: This study validated that the typical commercial brownie baking process utilizing an oven temperature of 176.7°C and baking for at least 40 min would achieve >5-log reductions in *Salmonella* and *L. monocytogenes* populations. However, validation studies should be individually conducted for brownie products with different baking parameters and ingredients.

P2-16 Microbiological Load of Edible Insects Sold in Southeastern Brazil

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Introduction: Edible insects have gained increasing attention in the world, as they are considered an alternative source of protein for human and animal consumption, with less environmental impact. However, they may contain microbial hazards, thus being a concern for producers and consumers. In Brazil, this market is small and mainly aimed at animal feeding.

Purpose: The aim of this study was to report results on the microbiological load of edible insects sold in the city of Valinhos, SP, southeastern Brazil.

Methods: A total of six packages of edible insects [three of mealworm larvae (*Tenebrio molitor*) and three of house crickets (*Acheta domesticus*)] were obtained from local producers. They were divided into 30 portions (of 10 g each) of either raw, frozen or boiled insects (five of each type) and analyzed for

mesophilic aerobic bacteria, fungi, total *Enterobacteriaceae*, total coliforms, generic *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp.

Results: All the samples of boiled insects showed microbial results below the detection level of the methods applied. On the other hand, the mean counts for samples of mealworm larvae (ML) and house crickets (HC), raw and frozen, respectively, were as follows: mesophilic aerobic bacteria (ML: 7.3 ± 0.4 and 7.2 ± 0.5; HC: 8.4 ± 2.6 and 7.5 ± 0.2 log CFU/g), fungi (ML: 4.1 ± 0.4 and 4.8 ± 0.4; HC: 5.5 ± 0.2 and 6.6 log CFU/g), total *Enterobacteriaceae* (ML: 6.5 ± 0.3 and 6.0 ± 0.4; HC: 6.7 ± 0.5 and 6.9 ± 0.3 log CFU/g) and total coliforms (ML: 3.0 and 3.0; HC: 2.7 ± 0.5 and 3.0 log MPN/g). Generic *E. coli* was detected in all raw and frozen samples for both insects, while none of the samples were positive for *S. aureus* and *Salmonella*.

Significance: These results suggest that a proper heat treatment of edible insects is essential to reduce their microbial load and, consequently, provide safer products for human and animal consumption. *Acknowledgements:* FAPESP (#2013/07914-8) and PUB-USP (#1440/2020).

P2-17 Ensuring *Escherichia coli* Possessing Colibactin Genes (*clb*) Linked to Colorectal Cancer Do Not Become a Food Safety Problem for Beef

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Introduction: *E. coli* carrying colibactin genes (*clb*) induce mutations that lead to colorectal cancer (CRC). Red meat consumption is arguably associated with CRC.

Purpose: To establish the prevalence of $clb^{+\,E.\,coli}$ in beef.

Methods: A multiplex-PCR that targeted *clbA* and *clbQ* genes was used to screen generic *E. coli* for Clb genes. These *E. coli* were previously isolated from 72 nine to ten head lots of cattle-at-harvest (n = 1430); 599 retail ground beef products from six different US cities (n = 1074); and 184 fed cattle (3 lots) at seven stages of beef processing feedlot to whole muscle cuts (n = 232). Prevalences were compared using Fisher's exact test. Efficacy of current processing interventions in controlling $clb^{+\,E.\,coli}$ was evaluated by survival after treatment with hot water, 2% lactic acid, 300 ppm bromine, and 200 ppm peroxyacetic acid.

Results: Overall, 11% of *E. coli* isolated from cattle-at-harvest carried *clb* in their genome. Fifty of the 72 lots possessed cattle carrying clb^{+} *E. coli*, with the lot-to-lot prevalence ranging from 4.5% to 50%. Among ground beef *E. coli*, *clb* was detected in 4.1% (44) isolates that came from 28 ground beef samples. clb^{+} *E. coli* were detected in samples from all 6 US cities (1% to 12.5% samples/city) representing 3 geographical regions. Finally, prevalence of $clb^{+\,E.\,coli}$ in the beef processing continuum was established to be extremely low (2/232) and $clb^{+\,E.\,coli}$ were present only in early stages of continuum; feedlot feces (1/36) and harvest hide (1/36). Current meat processing interventions effectively control meat borne $clb^{+\,E.\,coli.}$

Significance: The prevalence level of 4.1% in finished ground beef suggests that it may act as a vehicle of $clb^{+\,E.\,coli}$. Identifying efficacious processing interventions that reduce $clb^{+\,E.\,coli}$ can improve beef safety and impact CRC.

P2-18 Growth of *Staphylococcus aureus* in Raw Fish over Time at Non-Refrigerated Conditions

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Introduction: The safety and quality of fish products are top concern to the seafood industry. Pathogenic bacteria, including *Staphylococcus aureus* can enter the process stream via raw material, but also during processing from the air, personnel handling, insanitary conditions, and cross-contamination between raw and cooked product. Understanding the growth of *S. aureus* over time can help implement appropriate programs to control the growth and subsequent toxin production.

Purpose: The objective of this study was to evaluate the growth of *S. aureus* and other indicator organisms in raw, white-meat fish during simulated production conditions to provide scientific support for appropriate preventive controls for the factory's HACCP Plan.

Methods: Frozen cut raw white-meat fish was weighed out into 25-g samples in sterile bags. Samples were defrosted at refrigeration (4°C), inoculated with *S. aureus*, and frozen. Target *S. aureus* inoculum in samples was estimated to be around two-log CFU/g. Samples were stored at 50°F, 70°F, and 80°F. Triplicate samples were enumerated at 0, 8, 16, 24, 32, 48, and 72 hours. Samples were also enumerated for APC, generic *E. coli*, coliforms, and lactic acid bacteria using scientifically valid methods. *S. aureus* was enumerated using Baird-Parker spread plate agar. Limit of food safety was determined to be 10^5 CFU/g. Data was log-transformed and analyzed using ANOVA to determine if *S. aureus* growth significantly changed at different storage temperatures.

Results: Overall, *S. aureus* counts were significantly ($P < 0.05$) below the food safety limit at all the evaluated conditions. At 70°F and 80°F, *S. aureus* counts were at the limit of detection (1.00 log CFU/g) by the end of 72 hours.

Significance: The findings of the study indicate that there is no significant food safety risk associated with the current production practices. The data generated in this study provide scientific basis for the factory's HACCP plan in compliance with FDA guidelines.

P2-19 Efficacy of Peracetic Acid (PAA) on Agricultural Irrigation Water to Reduce Microbiological Pathogens and Indicators

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Introduction: In response to recent foodborne outbreaks, surface water used for overhead irrigation of leafy greens must now be treated. Chlorine, an industry standard, may be less effective in the presence of fertilizers, can increase soil salinity, and has the potential for phytotoxicity. Peracetic acid (PAA) could be an important alternative to treat irrigation water as efficacy may not be as greatly impacted by these same factors. However, limited guidance exists on treatment efficacy against human health pathogens.

Purpose: The purpose of this study was to evaluate the efficacy of five PAA-based sanitizers against a suite of pathogenic microorganisms significant to the produce industry in irrigation water.

Methods: Agricultural irrigation water was collected and inoculated with either *E. coli* TVS 353 (gEC), *E. coli* O157:H7 (#700728), or *Salmonella* (BAA-2828) between 10^2 and 10^5 CFU/100 mL. Five PAA products, plus an industry standard of 6% sodium hypochlorite (chlorine), were assessed at varying product dilutions from 2 to 26 ppm against naturally occurring Total Coliform bacteria (TC) and each inoculated organism. Reactions were neutralized after five minutes of contact time and evaluated by spread plate to determine log reductions of each organism and sanitizer pair.

Results: *E. coli* O157:H7 and *Salmonella* were more sensitive to PAA treatments than TC and gEC. PAA concentrations of ≤3 ppm showed <1 log reduction against TC and gEC and between 2-4 log reductions against O157:H7 and *Salmonella*, similar to chlorine treatments at 2 ppm against all organisms. At high PAA concentrations (≥6 ppm) all PAA treatment pairs showed between 2-4 log reductions.

Significance: The results of this study support new EPA FDA acceptance criteria where a product must demonstrate ≥3 log reduction of foodborne pathogens in irrigation water. This will further provide industry with specific guidance on using PAA to reduce contamination in agricultural water to protect public health.

Poster

P2-20 Characterization of Novel *Salmonella* Bacteriophages Isolated from Wastewater for Use in Food Protection

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Introduction: *Salmonella enterica* is a leading cause of foodborne illness-related hospitalizations and deaths. With increasing concerns about antibiotic resistance, and consumer preferences for "natural" and "clean label" products, interest is growing in using bacteriophages to lyse *S. enterica* cells present in foods and on food-contact surfaces. This study identified a unique mixture of novel bacteriophage which lyse a wide range of *S. enterica* serotypes and identified their receptors for a tailored approach to application.
Purpose: The purpose of this study was to isolate novel bacteriophage which are effective against *S. enterica* and to characterize their host range and receptors.
Methods: Six novel bacteriophages were enriched from wastewater collected from local facilities and streaked out for purification. Susceptibility of 17 different *S. enterica* serotypes to these phages was assessed using a plaque assay. Lysogeny testing was performed for these bacteriophages using plaque assays on phage-resistant *S. enterica* cultures. Putative bacteriophage receptors and other essential host factors were identified by selecting bacteriophage-resistant transposon insertion mutants and DNA sequencing of the transposon insertion sites.
Results: Each of the 17 *S. enterica* serotypes was lysed by at least one of the six novel bacteriophages, indicating that the combination of these phages may be synergistic in reducing *S. enterica* on food matrices and food-contact surfaces. Lysogeny tests were negative, suggesting that the novel bacteriophages may be classified as virulent. Putative receptors for the novel bacteriophages were identified through transposon insertion site sequencing and analyzed through BLASTN searches against NCBI genomic data to infer phage host-range.
Significance: These host range and genetic susceptibility data for novel bacteriophages identified potential synergistic combinations for use as food processing aids in reducing *S. enterica* and may allow for improved tailoring of bacteriophages for specific serotypes of concern.

P2-21 Quasi-Metagenomic Comparison of Lactose and Universal Preenrichment Broths for *Salmonella* Detection in Spent Sprout Irrigation Water

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Introduction: In the last 25 years, there have been 41 *Salmonella* outbreaks associated with sprout consumption in the U.S. Sprouts and spent sprout irrigation water (SSIW) presents a unique challenge for the development of *Salmonella* detection method in food matrices.
Purpose: This study is designed to compare the relative effectiveness of lactose broth (LB, the current BAM culture medium for SSIW) and universal preenrichment broth (UPB) for the detection of *Salmonella* and identify the associated co-enriching microbiota with different sprout varieties using a quasi-metagenomics approach.
Methods: Alfalfa, broccoli and mung bean SSIW samples were inoculated with a single *Salmonella* serovar, ranging from 0.7 to 1.5 CFU per 375 mL SSIW test portion. SSIW test portions were pre-enriched using UPB (1:3 sample-to-broth ratio) and LB (1:9 sample-to-broth ratio), respectively. The BAM *Salmonella* culture method was followed thereafter. DNA from selected 24-hour (H24) preenrichment samples were extracted and sequenced using an Illumina NextSeq platform. The sequencing reads were annotated using BactiKmer, an in-house bacterial kmer database.
Results: The relative abundance (RA) of *Salmonella enterica* was low throughout all varieties of SSIW, ranging from 0-9% in inoculated H24 preenrichment samples. For all sprout varieties, however, *Salmonella* RA was much higher in UPB than in LB. The composition and RA of H24 co-enriching microbiota was dependent on both sprout variety and preenrichment broth. For example, alfalfa SSIW co-enrichers were mostly comprised of *Leclerica, Bacillus* and *Pseudomonas* species. Broccoli SSIW was comprised primarily of *Bacillus* and *Pantoea* species and Mung bean SSIW primarily of *Leclercia, Citrobacter* and *Enterococcus* in LB and *Pseudomonas* in UPB.
Significance: This work demonstrated the utility of UPB, regardless of the co-enriching microbiota, in *Salmonella* isolation. The observation of potential exclusion of *Salmonella* in preenrichment provided novel information that may be used to further optimize preenrichment formulations.

P2-22 Growth Kinetics of *Salmonella enterica* on Heat-treated Potatoes during Storage

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Introduction: Heat treated plant foods have high water activities conductive to the proliferation of foodborne pathogens, including *Salmonella enterica*. A product assessment is required to determine the extent to which heat treated plant foods support the growth of *S. enterica*.
Purpose: To determine the growth kinetics of *S. enterica* on heat treated potatoes during storage at 5, 10, and 25°C.
Methods: Potatoes were cut into 1.3 cm^3 pieces and heat treated at 57°C for 30 min. Potatoes were cooled to 5°C for 1 h and then 30-g aliquots were portioned into deli containers. Potatoes were spot inoculated with a four-strain cocktail of *S. enterica* at 3 log CFU/g, air dried for 10 min, and stored at 5, 10, or 25°C for 7 d. At 0, 1, 3, 5, and 7 d, 10-g potatoes, in triplicate, were homogenized 1:10 with BPB and the homogenate was plated onto TSA with XLD overlay for enumeration of *S. enterica*. Three independent trials were conducted. Growth rates and lag phases were determined using DMFit and data were statistically analyzed using Student's *t*-test ($\alpha = 0.05$).
Results: The population of *S. enterica* on the heat treated potatoes after initial inoculation was 2.85 ± 0.21 log CFU/g. The population of *S. enterica* on the potatoes at 5°C did not significantly change during storage and no growth rate was determined. During storage at 10 and 25°C, the growth rate of *S. enterica* was 0.45 ± 0.09 and 1.20 ± 0.11 log CFU/g per d, respectively. The calculated time for the pathogen to reach a 1 log CFU/g increase was 3.25 d (including a 1.00 d lag phase) at 10°C and 0.84 d (20.16 h) at 25°C.
Significance: The results of this study can aid in determining appropriate storage time and temperature for heat treated potatoes.

P2-23 Can Probiotics be Used As Biotechnological Tools to Increase Bioaccessible Phenolics in Soursop Fruit Pulp?

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Introduction: Probiotics can metabolize fruit phenolic compounds (PC) and affect their bioavailability and bioactivity. *Annona muricata* L. (soursop) is a Brazilian fruit primarily consumed as pulp. Little is known about the effects of probiotics on bioaccessibility and antioxidant activity of PC in soursop pulp.
Purpose: To evaluate the effects of *Lactobacillus acidophilus* LA-05 and *Lactocaseibacillus casei* (LC1) metabolism on PC's bioaccessibility in soursop pulp and their antioxidant activity under simulated physiological conditions
Methods: The soursop fruits ($n = 10$) were pulped, homogenized in an industrial blender, and pasteurized (65°C, 30 min). Pulp samples were inoculated LA-05 and LC1 in single, or mixed inocula (1:1; ~8 log CFU/mL) and incubated at 37°C for 48 h under anaerobiosis. Soursop pulp without probiotics was

submitted to similar conditions (control). Pulps (with or without probiotics) were digested simulating physiological gastrointestinal conditions in three sequential phases oral, gastric and intestinal. Phenolics were identified in the pulps and their intestinal fraction (bioaccessible) using High Performance Liquid Chromatography by comparison with standards PC. Antioxidant activity was measured by oxygen radical absorbance capacity assay in pulps and their bioaccessible fraction.

Results: *PC profile was similar in all pulps including* epicatechin gallate*,* epigallocatechin gallate*,* procyanidin B1 and B2, hesperidin, and chlorogenic acid, while rutin was only detected in LA-05-added soursop pulp. Probiotics in single or mixed inocula increased the bioaccessibility of procyanidin B2 in soursop pulp. In addition, *L. casei* LC1 in single inoculum increased the contents of bioaccessible catechin in soursop pulp. Antioxidant activity of control pulp was higher than probiotics-added pulps before digestion, but similar among all pulps when only bioaccessible PC were tested.

Significance: Effects of probiotics on soursop pulp PC depend on the strain but did not compromise the antioxidant activity of the bioaccessible fraction besides possible increases in specific PC.

P2-24 Microbial Diversity in Naturally Fermented Fruits from Brazilian Caatinga Biome

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Introduction: Fruits have been explored as a source of lactic acid bacteria (LAB) and yeasts with biotechnological application. Little is known about the microbial diversity in underexploited fruits from Brazilian Caatinga biome.

Purpose: To assess LAB and yeast diversity over spontaneous fermentation of barbados cherry, cashew, soursop, and umbu and select strains with potential application in food systems.

Methods: Fruits were fermented at room temperature for 48 h. Samples were collected at zero, 24, and 48 h. Aliquots are plating in selective media MRS agar supplemented with chloramphenicol, and YPG agar supplemented with nystatin to isolate LAB and yeast, respectively. Strains were evaluated morphological characteristics (size, edge structure, relief, brightness and color) Gram and catalase tests, and carbohydrate fermentation. Selected isolates were identified through Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry.

Results: A total of 1,220 isolates were isolated over the fermentation. Forty-five and fifty-six isolates morphologically and biochemically identified as LAB and yeasts, respectively, were identified through MALDI-TOF analysis. *Lactobacillus* was the LAB genus predominant in fermented acerola cherry, cashew, and soursop. *L. plantarum*, *L. fermentum* and *Leuconostoc pseudomesenteroides* and, *Weissella confusa* comprised the identified species. Yeasts species of *Hanseniaspora* (*H. opuntiae, H. lachancei*) predominated in fermented-fruits, followed by *Issatchenkia terricola*. *Candida tropicalis*, *C. carpophila, C. guilliermondii*, *Saccharomyces cerevisiae* and, *Kodamaea ohmeri* were also identified and their abundance varied with the fermented fruit. Fermented soursop showed the highest yeast diversity.

Significance: Results show LAB and yeast diversity in different fruits from Brazil that may be exploited as source of yeasts with application in biotechnological fermentative systems.

P2-25 Presence of *Clostridium difficile* in Fresh Mushrooms at Retail Stores in Spain

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Introduction: *Clostridium difficile* is an opportunistic intestinal pathogen, mainly associated with nosocomial infections. The ecological niche of the bacterium are soils, and therefore *C. difficile* spores are often found in animals and foods, suggesting a potential for foodborne transmission. Microbiological studies in cultivated mushrooms are scarce and none of them have been focused on the presence of toxigenic *C. difficile.*

Purpose: The aim of this study was to investigate for the first time the prevalence of *C. difficile* in fresh mushrooms in Spain, and to characterize phenotypically and genotypically the *C. difficile* isolates in terms of toxin production, PCR-ribotyping and antimicrobial resistant rates.

Methods: Fifty samples were collected mainly from 5 different local markets who in turn worked with suppliers from different regions of Spain. Sampling was performed over 5 months. *C. difficile* was isolated by direct and enrichment culture, using the selective medium CCFT. Suspected colonies were identified using a latex agglutination test. Confirmation was performed by detection of *tpi* gene and the toxin *genes tcdA, tcdB* and *cdtA* by classical PCR. PCR-ribotyping based on capillary gel electrophoresis was also performed.

Results: A total of 40 samples were directly collected from local markets, yielding 3 *C. difficile* isolates and an overall prevalence of 7.5%. The isolates were identified as toxigenic and classified as rare profiles using Webribo database. Resistances to clindamycin and metronidazole were detected in two isolates.

Significance: Mushrooms are an important ingredient in the Mediterranean diet, which are often eaten fresh without cooking in salads and side dishes. The findings of this study reveal a low contamination of fresh mushrooms sold in retail markets in Spain. However, the isolates were toxigenic and exhibited resistances to antibiotics, underlining the need of a general recommendation "to wash properly before consumption" in the product label.

P2-26 Withdrawn

P2-27 Surrogate and Baking Validation of *Salmonella enterica, Listeria monocytogenes, and Enterococcus faecium* in Sunflower Seed Crackers

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Introduction: Sunflower seeds have been linked to outbreaks and recalls due to both *Salmonella* and *Listeria monocytogenes.* Thermal treatments can help reduce the pathogen hazard.

Purpose: Sunflower seeds were coinoculated with *Enterococcus faecium* and *Salmonella* or *L. monocytogenes* to validate *E. faecium* as a surrogate in laboratory studies. *E. faecium* inoculated sunflower seeds were then used in a pilot plant oven to validate the baking parameters of untreated sunflower seeds in crackers.

Methods: To validate *E. faecium* as a surrogate, two types of sunflower seeds (oleic and regular) were coinoculated with *E. faecium* and either *L. monocytogenes* or a 3-strain *Salmonella* cocktail. The inoculated seeds were incorporated into cracker dough and baked for 8 minutes at 200°C in a laboratory setting. Samples (n = 3) were enumerated on tryptic soy agar overlayed with selective media. For the baking validation, the sunflower seeds were inoculated with *E. faecium*, incorporated into cracker dough, and baked on the line of a commercial oven for 8 minutes. Samples (n = 10) were enumerated on tryptic soy agar and m-Enterococcus Agar.

Results: Overall, *Salmonella* and *L. monocytogenes* had a higher reduction compared to *E. faecium*. *L. monocytogenes* and *E. faecium* reduction on both types of sunflower seeds was between 3.26 to 4.33 log CFU/g and 1.41 to 1.64 log CFU/g, respectively. *Salmonella* and *E. faecium* reduction on both types of sunflower seeds was between 1.43 to 2.70 log CFU/g and 0.98 to 1.33 log CFU/g, respectively. For the baking validation completed at a pilot plant, the average log reduction of *E. faecium* on oleic and regular sunflower seeds was 4.33 ± 0.08 and 4.24 ± 0.07 log CFU/g, respectively.

Significance: *E. faecium* was found to be an appropriate surrogate for *Salmonella and L. monocytogenes* on sunflower seeds. The thermal treatment applied to the crackers produced >4 log CFU/g reduction of *E. faecium* on sunflower seeds in a cracker dough.

P2-28 Effect of Extended Storage on the Survivability and Thermal Resistance of *Listeria monocytogenes* in Dry and Hydrated Milk Powders

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Introduction: *Listeria monocytogenes (*LM*)* has a unique ability to survive in low water activity (a_w) conditions for prolonged time periods and can cause severe health concerns if post-pasteurization contamination occurs in milk powders.

Purpose: To determine survivability and thermal resistance of LM in dry and hydrated nonfat dry milk (NFDM) and whole milk powder (WMP) during storage of 4 months.

Methods: This study was designed as a two factorial (storage and powder type) randomized complete block design with three replications. Milk powders were inoculated with a 3-strain cocktail of LM and dried back to original a_w levels. The *D*- and *z*-values study were conducted every 30th day, starting on day one for both dry and hydrated powders. Five (g or mL) of respective samples were transferred into thermal-death-time (TDT) disks, sealed, and placed in the water baths set at 75, 80, and 85°C for NFDM and WMP, and 54, 57, and 60°C for hydrated NFDM and WMP. Samples were heat treated from 0 to 40 minutes and then taken out at predetermined time intervals and transferred immediately to an ice water bath. Samples were enumerated using injury-recovery media, and *D*- and *z*-values were calculated. Two-way ANOVA at $P \leq 0.05$ was used for statistical analysis.

Results: *D*-values of LM in NFDM for day one were 13.1, 6.0, and 4.0 min at 75, 80, and 85°C, respectively. Whereas *D*-values of LM in WMP for day one were 12.0, 6.3, and 3.3 min at 75, 80, and 85°C, respectively. There was no significant interaction of the main effects for *D*- and *z*-values of LM in dry and hydrated milk powders. However, the main effect (storage-day) was significant for *D*-values at 75, 80 and 85°C where it increased with time.

Significance: *D*- and *z*-values from this study provide basic information about the effect of storage time and milk powder type on heat resistance of LM in milk powders.

P2-29 Effect of Water Droplet Size and pH on the Growth and Survival of *Enterococcus faecium* in Margarine and Spread Products

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Introduction: Water-in-oil emulsions (W/O) are structured foods with a disperse aqueous phase in the form of water droplets distributed over a continuous lipid phase. Growth of microorganisms will occur within the water droplets, limited by space and/or nutrients availability. Microbial stability of margarine and spread products relies on compartmentalization of the aqueous phase in fine water droplets, because any contaminating bacteria will be confined within these droplets.

Purpose: The purpose of this study was to determine survival and growth of *E. faecium* in W/O emulsion products affected by Water Droplet Size ($D_{3,3}$), water phase pH, and storage temperature.

Methods: *E. faecium*, a non-pathogenic surrogate that has been widely used for validation studies was inoculated in water phase of emulsion products at a target level of 10^6 CFU/g. A total of eight sample variables with combinations of two different fat contents (55% and 80%) and two different water phase pH levels (≤4.6 and ≥7.0) were prepared using pilot plant equipment to produce W/O emulsion samples with different ranges of $D_{3,3}$. Each sample variable was prepared in triplicate and stored at 40°F, 45°F and 55°F for up to 45 days. All samples were plated in Tryptic Soy Agar (TSA) in duplicate.

Results: Samples were produced with $D_{3,3}$ ranging between 4 µm and 28 µm. Results showed no growth of *E. faecium* in all samples stored at 40°F. The growth of *E. faecium* in samples with 55% fat content, pH ≥7.0, and $D_{3,3}$ ≥15 µm increased by 2 log within 7 and 5 days when stored at 45° and 55°F, respectively. Samples with 80% fat content, pH ≤4.6, and $D_{3,3}$ ≤10 µm showed a significant decreased of *E. faecium* population when stored at 40°F, 45°F, and 55°F.

Significance: This study indicates that $D_{3,3}$ and pH are critical to control the growth of *E. faecium* in W/O emulsion products.

P2-30 Testing an Affordable Hyperspectral Imaging System for Rapid Identification of Pathogens in Dairy Products

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❖ ***Developing Scientist Entrant***

Introduction: With the increased concerns about food safety and defense, hyperspectral imaging (HSI) can serve as a potential novel tool for rapid, reliable, and non-destructive identification method for foodborne pathogens. This development with an affordable HSI system using a compound microscope and HSI camera offers the possibility of other researchers to explore this technology.

Purpose: To evaluate the efficacy of a custom-built, affordable HSI system to identify single and mixed strains of foodborne pathogens in dairy products.

Methods: The study was conducted as a completely randomized design with three replications. Three strains of *Listeria monocytogenes* and *Escherichia coli* O157:H7 were evaluated as single or mixed strains with an affordable HSI system in whole milk, cottage and cheddar cheeses. Dairy products were inoculated with single or mixed strains of respective cultures grown in brain heart infusion (BHI) broth at 37°C for 24 h. The inoculated samples were refrigerated for 24 h and then streaked on selective media (PALCAM and/or Sorbitol MacConkey agar) for isolation. After incubation, a colony was selected and mixed with 1-mL of HPLC water, vortexed for 1-min, and spread over a microscope slide. Images were captured at 2000X magnification, and three cells were selected as regions of interest to obtain hyperspectral signatures. Cells were classified by their hyperspectral signatures as either *L. monocytogenes* or *E. coli* O157:H7 using *k*-nearest neighbor (*k*NN) and cross-validation technique in R-software.

Results: With the implementation of *k*NN (k = 3), classification accuracies obtained in the inoculated dairy products were 58.33% and 61.11% for *E. coli* O157:H7 and *L. monocytogenes*, respectively. Further work will be performed to improve the classification accuracy and build stronger reference libraries of foodborne pathogenic bacteria.

Significance: The development of an affordable HSI system using a compound microscope and HSI camera offers the possibility of rapidly identifying foodborne pathogens, at least at presumptive levels.

P2-31 Prevalence and Antibiotic Susceptibility of *Salmonella enterica* Isolated from Meats and Their Related Samples in a One-Health Concept

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Introduction: The occurrence of antibiotic resistant foodborne pathogens in meats, meat products and their related samples is a concern worldwide, and a one-health approach to tackling this phenomenon is warranted.

Purpose: This study determined the presence and antibiotic susceptibility of *Salmonella enterica* isolated from meats, and it related samples in Ghana using a one-health concept.

Methods: The isolation of *Salmonella* species was done following the procedure in the US Food and Drugs Administration, Bacteriological Analytical Manuel (Andrews et al., 2018). Ten (10) each of fresh and ready-to-eat (RTE) beef, chevon, guinea fowl, mutton, pork and chicken, and hand, knife, table and utensil swabs of fresh and RTE meat sellers. The antimicrobial resistance test was done using the disk diffusion method (Bauer et al., 1996) and the results were interpreted using CLSI (2017).

Results: Of the 200 meat and their related samples examined, 22.5% were positive for *Salmonella enterica*. *Salmonella enterica* were highest in knife swabs from fresh meat sellers (70.0%), followed by knife swabs from RTE meat sellers (50%) and fresh beef samples (50%). *Salmonella enterica* were not detected in RTE chicken, utensil swabs from fresh meat sellers and table swabs from RTE meat sellers. Multidrug resistance (resistance to ≥3 different classes of antibiotics) was found in 35% (16) of the isolates. The percentage resistance to one and two antibiotics were 28.9% and 33.3%, respectively. Resistant to tetracycline (37.8%) and amoxicillin (35.6%) was observed. Intermediate resistance was 26.7% for ceftriaxone and 20% for gentamicin. One *Salmonella enterica* isolated from RTE chicken was resistant to as many as 8 (amoxicillin-azithromycin-ceftriaxone-ciprofloxacin-teicoplanin-trimethoprim-tetracycline-imipenem) different antibiotics. Susceptibility was high for imipenem (95.6%), chloramphenicol (84.4%), trimethoprim (82.2%), ciprofloxacin (77.8%) and gentamicin (75.6%).

Significance: This study revealed that meats and meat products were contaminated with *Salmonella enterica,* some of which were resistant to 3 or more different classes of antibiotics. The consumption of meats obtained from Ghana possess a health risk to consumers. Proper cooking or re-heating is required prior to consumption, and observance of personal hygiene by meat sellers is required.

P2-32 Antimicrobial Activities and Genotyping of Probiotic Lactobacilli in Nigerian Fermented Condiments as Potential Starter Cultures for Improved Food Safety

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❖ ***Developing Scientist Entrant***

Introduction: Locally fermented condiments [Locust beans "Iru" or "Dawadawa" (*Parkia biglobosa*); Oil beans "Ugba" (*Pentaclethra macrophylla*); Castor oil "Ogiri" (*Ricinus Communis*)] are plant-based food spices consumed for daily dietary protein and alleviate malnutrition in Nigeria. These seeds are locally fermented but usually result in poor quality with various bacteria and fungi contaminants. Previous reports suggested the use of starter cultures from locally fermented condiments in fermentation processes to ensure health-promoting benefits, improved quality, shelf life and organoleptic properties for the achievement of healthy nutrition, safe and quality food.

Purpose: The study aimed to genotype potential lactobacilli from locally fermented condiments and substantiates their potential use as starter cultures in fermentation processes.

Methods: A total of 185 lactobacilli obtained from fermented condiments (n = 328) purchased from popular food markets in southwest Nigeria were profiled for probiotic activities, hemolysis assay, antibiotics sensitivity and assayed for inhibitory activities against food pathogens and spoilage bacteria. Interesting strains were characterized with 16S rRNA sequencing and further evaluated for phylogenetic diversity with other globally used starter cultures.

Results: Four anaerobic *Lactobacillus* species exhibited significant probiotics, γ-hemolysis, anti-spoilage and anti-listerial activities ($P < 0.05$) with tolerable safety antimicrobial susceptibility profiles. Genotyped strains identified as *Lactiplantibacillus plajomi* YD001 (MW280136), *Lactiplantibacillus plantarum* YD002 (MW280139), *Lactiplantibacillus plantarum* YD003 (MW280137), *Lacticaseibacillus paracasei* YD004 (MW280138) possessed 50.75, 50.61, 50.75 and 52.54 mol% DNA G+C contents, respectively. These strains were further clustered into different phylogroups with high clonal relatedness with other potential lactobacilli meta-data (≥ 96.80%) obtained from the Genbank database.

Significance: Obtained genotyped *Lactobacillus* strains are potential starter cultures for improved fermentation processes, control of food pathogens and spoilage organisms. This research contributes to the enhancement of sustainable development in Nigeria through the provision of healthy nutrition, employment opportunities, and empowerment initiatives for the low-skilled women who happen to be major local producers of these condiments.

P2-33 Fate of *Listeria monocytogenes* on Citric Acid-treated Hard-cooked Eggs

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❖ ***Developing Scientist Entrant***

Introduction: Commercially-prepared hard-cooked eggs are available for food service and to the public in retail grocers. Potential contamination with *Listeria monocytogenes* after the cooking and peeling steps is of concern since this pathogen can proliferate at refrigeration temperatures.

Purpose: To evaluate the efficacy of citric acid treatment of hard-cooked peeled eggs to reduce the population levels of *L. monocytogenes*.

Methods: Fresh eggs were boiled for 12 min, cooled to 4°C, peeled, and stored at 4°C for 24 h prior to experiments. Hard-cooked eggs were dip inoculated with a 4-strain cocktail of rifampicin-resistant *L. monocytogenes* resulting in either 4 (low) or 8 (high) log CFU/egg. Eggs were air dried 10 min, followed by treatment with pH 2.5 citric acid (3.84 g/L) at 5 or 25°C for 24 h. *L. monocytogenes* populations were enumerated at intervals up to 24 h by homogenization of eggs with BLEB and cultivation on BHI/rifampicin agar. Triplicate eggs were assessed for each timepoint and three independent trials were conducted.

Results: At a low inoculation level, the *L. monocytogenes* population on the eggs was 3.95 ± 0.69 and 3.74 ± 0.76 log CFU/egg prior to treatment with 5 or 25°C citric acid, respectively. After 1 h, *L. monocytogenes* was reduced by 0.56 and 0.18 log CFU/egg when treated at 5 or 25°C, respectively; after 24 h, reductions were 0.73 and 0.86 log CFU/egg. At a high inoculation level, the *L. monocytogenes* population on the eggs was 7.93 ± 0.26 and 8.04 ± 0.47 log CFU/egg prior to treatment with 5 or 25°C citric acid, respectively. After 1 h, *L. monocytogenes* was reduced by 0.48 and 0.67 log CFU/egg when treated at 5 or 25°C, respectively; after 24 h, reductions were 1.23 and 1.16 log CFU/egg.

Significance: The data obtained in this study can be used to determine the efficacy of citric acid against *L. monocytogenes* during treatment of hard cooked eggs.

P2-34 AOAC Emergency Response Validation of the TEMPO YM® Method for Enumeration of Yeast and Mold in Cannabis Flower: AOAC *Performance Tested Method*℠ 041001

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Introduction: TEMPO® YM is an AOAC® *Performance Tested Method*℠ (PTM) approved for the automated enumeration of yeasts and molds in a variety of foods. The method utilizes a selective dehydrated culture medium and a Yeast and Mold (YM) enumeration card containing 48 wells across three dilutions for the automatic determination of the Most Probable Number (MPN).

Purpose: As part of the AOAC® Research Institute's Emergency Response Validation (ERV) Program, the alternative method was compared to the FDA Bacteriological Analytical Manual (BAM) to add cannabis flower (delta 9-tetrahydrocannabinol >0.3%; 10 g test sample) as a PTM matrix extension.

Methods: The TEMPO YM was validated following AOAC *Official Methods of Analysis*℠ Appendix J validation guidelines and the AOAC ERV protocol for total yeast and mold in cannabis flower. Five replicates at 3 levels of contamination (low (<1,000), medium (1,000-10,000), and high (10,000-100,000)) were evaluated.

Results: The 90% confidence interval of the difference between the means of the TEMPO YM and Dichloran Rose Bengal Chloramphenicol agar obtained in the method comparison study demonstrated statistical equivalence between the candidate method and the confirmation plating. Inclusivity and exclusivity results showed the TEMPO YM method is highly specific in discriminating target organisms found in cannabis flower from non-target organisms.

Significance: The TEMPO YM method is a rapid, easy-to-use automated alternative to traditional plating procedures for the enumeration of yeast and molds in cannabis flower.

P2-35 The Fit-for-Purpose Evaluation of the Bact/ALERT System for the Detection of Microbial Detection in a Variety of Plant-based Alternative Dairy Beverages

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Introduction: Much is known about the spoilage of traditional dairy but little information is available about the microbial spoilage of the very popular plant-based alternative dairy options.
Purpose: A fit-for-purpose evaluation was performed on Coconut, Hazelnut, Oat and Almond plant-based beverages by spiking with potential spoilage organisms and evaluating at different timed intervals.
Methods: Oat, Almond, Hazelnut and Coconut beverages (12.7 oz to 46 oz) were spiked with low levels (< 25 CFU/package) of *Bacillus*, *Pseudomonas* and *Staphylococcus*. Inoculated packages were incubated at 32-35°C and tested in triplicate at 24, 48, 72 hours and at 5 days to determine the survivability of the different organisms in the different product types. After incubation, 10 mL was inoculated into the BTA aerobic bottle and CO_2 production was monitored in the BTA system. Plate count and pH were also performed at each interval.
Results: *Bacillus* and *Staphylococcus* grew and detected in all products after only 1 day pre-incubation. The first trial with *Pseudomonas* at 1 CFU/package was intermittently detected during the 5 days of testing. It was uncertain if the intermittent growth was due to survivability in the product or if laws of distribution applied and *Pseudomonas* was not present in all packages. *Pseudomonas* spiking was repeated in the Almond variety in the largest 46 oz container size. All 5 individual spiked containers were positive after only 24 hour pre-Incubation suggesting it was laws of distribution.
Significance: The spiking studies demonstrated the fit-for-purpose of the BacT/ALERT for early detection of potential contamination in plant-based alternative dairy beverages. BTA Detection was supported by plate confirmation and in some instances was more sensitive than plate due to the BTA 10 mL test volume.

P2-36 Quantification of *Campylobacter jejuni* in Poultry Processing Rinses Utilizing Shortened Enrichment Times and RT-PCR

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Introduction: Currently, prevalence-based data is used to determine process control during animal harvest; however, this does not provide information on the level and subsequent risk of *Campylobacter* contamination. Therefore, it is imperative to be able to detect and quantify *Campylobacter* contamination throughout the harvest process.

Purpose: The objective was to develop and optimize the rapid quantification of *Campylobacter jejuni* (CampyQuant) in post-chill poultry rinsates using the BAX® Q7 RT-PCR System.

Methods: Bulk post-chill poultry rinsates (*n* = 13; 30 mL) were aliquoted to 24 oz Whirl-Pak bags inoculated with 1 to 4 log CFU/mL of *C. jejuni* (*n* = 3 samples/inoculation level) with one uninoculated sample. After inoculation, 30 mL of pre-warmed (42°C) 2× Bolton's Broth was added to each sample and incubated at 42°C for 18 h then tested in quintuple with the BAX® Q7 RT-PCR system. Simultaneously, samples were formalin-fixed, and *C. jejuni* was enumerated using a Petroff-Hausser counter. These results were then compared to determine *C. jejuni* growth in relation to the initial inoculation levels.

Results: A linear fit equation was generated using the cycle threshold (CT) values of *C. jejuni* from the BAX® System to estimate pre-enrichment log CFU/mL of rinsates yielding a R^2 of 0.95 and log RMSE of 0.25. Rinsates inoculated with 2 - 4 log CFU/mL of *C. jejuni* levels were enumerated post-enrichment using the Petroff-Hausser estimating 5.84, 6.30, and 7.50 log CFU/mL, respectively.

Significance: The study suggests that the BAX® Q7 RT-PCR system can provide the food industry with a rapid, accurate, and efficient alternative method for *C. jejuni* enumeration to ensure that process controls are working adequately to provide safe products to consumers.

P2-37 Determination of Detection Limits of a Commercial RT-PCR for *Campylobacter jejuni* in Poultry Rinsates

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❖ ***Developing Scientist Entrant***

Introduction: *Campylobacter jejuni* is a foodborne pathogen associated with the consumption of undercooked poultry. The European Union allows poultry carcasses to possess <3.00 Log_{10}CFU/g of *Campylobacter*. Therefore, a rapid and accurate *C. jejuni* detection method is critical to improve current surveillance technologies.

Purpose: The aim was to determine the limit of detection (LOD) of *C. jejuni* in poultry rinse samples using the BAX® Q7 RT-PCR.

Methods: Bulk post-chill poultry rinsates (N=32; 30 mL) were aliquoted to 24oz Whirl-Pak bags inoculated at a targeted 0.00, 1.00 and 2.00 Log_{10}CFU/mL of *C. jejuni* (n=5 samples/inoculation level; 1 non-inoculated sample/enrichment time). Subsequently, 30 mL of pre-warmed (42˚C) 2´ Blood-Free Bolton's Broth with 2× antibiotics was added to each sample and incubated at 42˚C for 16h and 18h. At each enrichment time, samples were removed from the incubator and ran on the BAX® Q7 system with 8 technical replicates per sample at each timepoint. Detection differences between enrichment times and inoculation levels were explored using chi-square and Mann-Whitney U test in R ($P \leq 0.05$).

Results: The inoculation levels were determined to be 0.58, 1.58, and 2.58 Log_{10}CFU/mL. After 16h of enrichment, *C. jejuni* was detected at 18, 70, and 100% among rinsates inoculated at 0.58, 1.58, and 2.58 Log_{10}CFU/mL of *C. jejuni*, respectively. After 18h of enrichment, the detectable percentages increased to 73, 100, and 100% for each inoculation level. There was a difference in detectable *C. jejuni* between different enrichment times ($P<0.05$). Furthermore, there was a difference in detection between inoculation levels 0.58 and 1.58 at 18 hours ($P<0.05$).

Significance: The results suggest that 18h of enrichment is required to achieve the limit of detection for 1.58 Log_{10}CFU/mL of *C. jejuni* in poultry rinses when using the BAX® system. These results provide the industry a semi-quantitative insight into threshold compliance for *C. jejuni*.

P2-38 Evaluation of the Hygiena™ BAX® System for the Detection of *Salmonella* and *L. monocytogenes* in Plant-based Meat Substitutes

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Introduction: Plant-based meat substitutes are environmentally more sustainable with the same practicality, safety and stability as conventional meat products. However, along with their health benefits, plant-based foods carry contamination risks just like those of animal products.

Purpose: As these products are gaining consumer popularity and are increasingly being incorporated into diets, scientifically robust methods should be used to assess their food safety. Therefore, an evaluation of the BAX® System was conducted for *Salmonella* and *L. monocytogenes* in plant-based meat products and compared to the U.S. FDA BAM reference method.

Methods: Verification studies of 3 plant-based meat products; ground, breakfast patties and sausage, were prepared following the FDA's "Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds, Edition 3." Twelve test portions (25 g) were artificially inoculated with a fractionally low level of *Salmonella* or *L. monocytogenes* and 12 samples were left uninoculated for controls. After food stabilization, test method samples for *Salmonella* were enriched in BPW and *L. monocytogenes* samples were enriched in 24 LEB Complete while FDA BAM reference samples were enriched according to their respective chapters. PCR was performed for test method samples and all samples (test and reference) were culture confirmed.

Results: Real-time and standard PCR results for both organisms were identical to culture with 100% sensitivity and 100% specificity for all product types. When compared to the reference method, the difference in probability of detection (dPOD) indicated no significant difference since the 95% confidence interval contains 0 in all cases.

Significance: The results of these studies demonstrate that the BAX® System method is sensitive, specific, and accurate for the detection of *Salmonella* and *L. monocytogenes* in plant-based meat products with no statistical difference compared to the reference method.

P2-39 Method Validation of Shelled Pecans for *Salmonella* and *Listeria monocytogenes* Using the Hygiena™ BAX® System

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Introduction: The harvesting process of tree nuts puts them at risk for potential contamination. Nuts are swept from the ground after coming into contact with harmful microorganisms that could be present in the soil or composted manure. Many different tree nuts including almonds, pistachios and walnuts have all been associated with foodborne outbreaks and recalls and since they are often consumed raw, manufacturers need to ensure the safety of these items.

Purpose: The performance of the BAX® System real-time PCR assays were compared to the U. S. FDA BAM reference method in an unpaired validation for the detection of *Salmonella* and *Listeria monocytogenes* in shelled pecans.

Methods: In two separate validations, 60 unpaired samples were inoculated with *Salmonella* Typhimurium or *L. monocytogenes* at a low level and a high level and held at 25°C for 14 days to equilibrate. Ten additional samples were left un-inoculated. Test method samples for *Salmonella* (375 g) were enriched in BPW and *L. monocytogenes* samples (125 g) were enriched in Actero™ *Listeria* Enrichment media. Samples were incubated, tested by real-time PCR and confirmed by culture. Reference method samples (25 g) for each organism were enriched and confirmed according to their respective procedures in the FDA BAM.

Results: Test method samples analyzed by real-time PCR returned fractional positives for low-inoculated *Salmonella* (11/20) and *L. monocytogenes* (13/20) with complete agreement to culture. Reference method samples returned culture positive result that also met fractional recovery for *Salmonella* (10/20) and *L. monocytogenes* (6/20). All high spiked samples (5/5) were positive for both organisms and all methods. For the method comparison, dPOD analysis indicated no significant difference for *Salmonella* (0.05; CI -0.12, 0.77), but a significant difference for *Listeria* (0.35; CI 0.04, 0.58) where the test

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method recovered a higher proportion.

Significance: The BAX® System is an effective and accurate method for the detection *Salmonella* and *L. monocytogenes* in shelled pecans.

P2-40 Validation of a PCR Workflow Combining Wet-Pooling and Real-Time PCR for *Salmonella* Detection in Large Test Portions of Cocoa and Chocolate Products

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Introduction: Although pathogens cannot grow in low-moisture foods, they can survive for months, if not years! The survival ability of several *Salmonella* in cocoa and chocolate products is very well-known. Proper sampling plans should be developed when combining both composite and wet pooling strategies for cocoa-containing matrices.

Purpose: PATHATRIX® 10-pooling *Salmonella* spp. kit with MicroSEQ™ *Salmonella* spp enables post-enrichment pooling of 375 g test portions of cocoa and chocolate products. The workflow was evaluated through an AOAC-PTM matrix study. The FDA/BAM Chapter 5 was used as the Reference Method.

Methods: Four challenging matrices were tested: cocoa powder, cocoa butter, cocoa liquor and > 70% dark chocolate. Two contaminations were run with bulk inoculation: one low level to achieve fractional recovery for 20 test portions, and one higher inoculation level for 5 test positions. Non-inoculated test portions were as well included. Large sample sizes of 375 g were tested and pooled together. Two enrichment procedures were used for the candidate method: pre-warmed Non-Fat Dry Milk and pre-warmed BPW. Post-enrichment pooling was run combining one inoculated test portion from the inoculated bulk with four non-inoculated test portions using the PATHATRIX™ Auto Instrument to get a pool of 5 test portions. After the sample preparation, MicroSEQ PCR assay was run. The FDA/BAM Chapter 5 method was performed on single samples of 25 g. The probability of detection (POD) was calculated as the number of positive outcomes divided by the total number of trials.

Results: No false positive results and no PCR inhibition was observed with the candidate method. The POD analysis demonstrated that there were no statistically significant differences between the candidate workflow and the reference methods for all 4 matrices tested.

Significance: PATHATRIX® combined with MicroSEQ™ Real Time PCR enables sensitive detection of *Salmonella* after post-enrichment pooling of five test portions of 375 g cocoa and chocolate products within one day.

P2-41 AOAC Validation Study of a Real-Time PCR Workflow for *Salmonella* Detection in Large Test Portions of Cocoa and Chocolate Products

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Introduction: Over the past two decades *Salmonella* has been associated with several outbreaks due to the consumption of various low-moisture foods, including cocoa and chocolate products. The control of this foodborne pathogen in low-moisture foods and the dry food production environments remains a significant challenge, and composite pooling is usually run to improve the sampling plan.

Purpose: The purpose of this study was to evaluate the Thermo Scientific™ SureTect™ Salmonella PCR Assay (candidate method) for detection of *Salmonella* from large test portions of cocoa and chocolate products according to the AOAC® Performance Tested Methods℠ program.

Methods: The candidate method was compared to the FDA/BAM Chapter 5 reference method using a 25 g sample size within a paired and an unpaired data study. Large sample sizes of 375 g were tested for the candidate method, with two enrichment procedures: pre-warmed non-fat dry milk as described in the FDA/BAM Chapter 5 and pre-warmed BPW.

Results: Four challenging matrices, raw cocoa powder, unrefined cocoa butter, cocoa liquor and dark chocolate (70% cocoa), were tested. No false positive PCR results and no PCR inhibition was observed with the candidate method. The probability of detection (POD) was calculated as the number of positive outcomes divided by the total number of trials. There were no statistically significant differences between the number of positive samples detected by the candidate workflow and the reference methods despite the difference in test portion sizes.

Significance: The candidate method enabled sensitive detection of *Salmonella* from 375 g test portions of cocoa and chocolate products within one day. Flexibility is offered to end-users with the choice of two non-proprietary enrichment media. The positive PCR data was easily confirmed by direct streaking onto Thermo Scientific™ *Brilliance*™ Salmonella or XLD agars.

P2-42 Emergency AOAC PTM Certification of a Method to Detect for SARS-CoV-2 from Environmental Surfaces

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Introduction: A significant number of SARS-CoV-2 outbreaks have been traced back to food processing plants, and numerous studies have shown that the virus remains viable on environmental surfaces for extended periods, leading to concerns that surface-to-human transmission may be occurring.

Purpose: The Thermo Scientific™ Real-Time PCR Detection of SARS-CoV-2 on Food Packaging and Environmental Surfaces Assay (candidate method) was submitted for emergency validation in accordance with the AOAC Research Institute Performance Tested Methods℠ Program for the detection of SARS-CoV-2 on environmental surfaces.

Methods: *In silico* analysis for target specificity was performed through comparison to 15,764 SARS-CoV-2 sequences and 65 exclusivity organisms identified in the GISAID and GenBank Viral NCBI databases. Probability of detection (POD) was evaluated in an unpaired study with the U.S. Centers for Disease Control and Prevention 2019-Novel Coronavirus (2019-nCoV) RT-PCR Diagnostic Panel (reference method). Two-by-two-inch stainless steel test areas were contaminated with SARS-CoV-2: five with a high level (POD=1), twenty with a low level (POD=0.5) and five uncontaminated (POD=0). Samples were subjected to transportation conditions and analyzed twenty-four hours after sampling. The candidate method utilizes RNA extraction and reverse transcription qPCR with an option of two different extraction devices and two different thermal cyclers, and all combinations were used evaluated in the study.

Results: *In silico* analysis showed that primers and probes used in the candidate method matched ninety-nine percent of the SARS-CoV-2 sequences analyzed; none of the exclusivity sequences showed sequence matches. In the matrix study, for all combinations of extraction device and thermal cycler, the candidate method showed comparable detection to the reference method.

Significance: These results demonstrate that the candidate method offers a specific and sensitive option to detecting SAR-CoV-2 on environmental surfaces, allowing directed hygiene intervention strategies to reduce potential health risks and plant shutdowns.

P2-43 Evaluation of Hygiena's New BAX® System Real-Time PCR Assay for *E. coli* O157:H7 Exact in Meat, Produce and Raw Dairy Matrices

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Introduction: *E. coli* O157:H7 is a pathogenic serotype of Enterohemorrhagic *E. coli* and is associated with several food and waterborne outbreaks worldwide. It is regulated as an adulterant by the U.S. Department of Agriculture (USDA) with a zero-tolerance standard. The evaluation of this single-target real-time *E. coli* O157:H7 PCR assay offers an alternative to dual-target assays, with faster results, more validated matrices and a more user-friendly method than traditional reference methods.

Purpose: This study aimed to assess a real-time PCR assay for screening and colony confirmation of virulent *E. coli* O157:H7 in beef trim, ground beef, leafy greens and raw dairy products. Inclusivity, exclusivity and sensitivity were used to measure the assay's performance vs. USDA, FDA and ISO reference

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methods.

Methods: Overnight cultures of *E. coli* O157:H7 (*n* = 75 for detection and confirmation methods) isolated from various sources were prepared at 10^6 CFU/mL for inclusivity testing and non-target *E. coli* O157:H7 strains (*n* = 99 for both methods) were prepared for exclusivity testing. For sensitivity testing, all matrices were tested using various enrichment protocols and samples sizes, then tested on the real-time PCR assay at both Q Labs and internally.

Results: Inclusivity testing demonstrated 100% detection of target organisms and 100% exclusivity of non-target strains. The sensitivity of the assay was determined to be ≤ 1 x 10^4 CFU/mL in all matrices. All PCR colony confirmations matched reference method confirmations.

Significance: The new BAX® System Real-Time PCR Assay for *E. coli* O157:H7 EXACT provides users with a single-target chemistry to detect *E. coli* O157:H7 with robust performance. The evaluation yielded a new method for testing raw dairy products and shorter enrichments for quicker time to results for ground beef, beef trim and leafy greens over previous dual target assays. The alternate PCR colony confirmation method offers yet another avenue to increase shelf life, by decreasing product release time.

P2-44 Internal Validation of the Hygiena™ BAX® System for *E. coli* O157:H7 and *Salmonella* from Ready-to-Eat Chicken Sausage

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Introduction: Ready-to-eat (RTE) meat and poultry products such as hot dogs and cold cuts are in an edible form that do not require any additional preparation by the consumer. These products are processed by the manufacturer by cooking, smoking, fermenting, or other lethality treatment to ensure product safety. Despite these processing techniques, RTE foods are still at risk for contamination and often implicated in product recalls.

Purpose: This study was designed to validate the use of a real-time and standard PCR assay for the detection of *E. coli* O157:H7 and *Salmonella* compared to the USDA FSIS reference methods in unpaired samples of RTE chicken sausage.

Methods: Chicken sausages were co-inoculated with *E. coli* O157:H7 and *Salmonella* to create 60 low fractional samples and 15 high samples, along with 15 negative controls. Following a 4°C equilibration period for 48-72 hours, test method samples (375 g – 5 negatives, 20 low, 5 high) were enriched in pre-warmed (42°C) MP media, incubated at 42°C for 16-24 hours and analyzed by PCR. Reference method samples (5 negatives, 20 low, 5 high per method) were enriched and confirmed according to procedures in the USDA MLG Chapter 5C.00 for STEC and Chapter 4.10 for *Salmonella*.

Results: For low inoculum samples, PCR detected 14/20 positives for *E. coli* O157:H7 and 11/20 positives for *Salmonella* using the test method. Additionally, all high inoculum samples (5/5) were detected by PCR, and all results were identical to culture for both organisms. To compare the method performance, the results of unpaired test method samples and reference samples were analyzed using the probability of detection (POD). No significant difference was determined for *E. coli* O157:H7 since the 95% confidence intervals contains 0, but a significant difference was determined for *Salmonella*.

Significance: This study demonstrates the ability of the BAX® System to accurately detect *E. coli* O157:H7 and *Salmonella* from RTE chicken sausage using a single enrichment, statistically equivalent to culture.

P2-45 Analysis of Individual and Pooled Environmental Samples Using the Hygiena™ BAX® System PCR Assays for Genus *Listeria* and *L. monocytogenes*

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Introduction: Environmental sampling sites for *Listeria* should be based on the potential risk of contamination which can become quite extensive. For a more economical approach, some processors will pool multiple enriched sponges to create a single test for analysis, substantially reducing the cost per test in a sampling program.

Purpose: The purpose of this study was to validate the efficacy of a real-time and standard PCR assay for Genus *Listeria* and *L. monocytogenes* from individual and pooled environmental sponges.

Methods: Pre-moistened sponge-sticks were inoculated with *L. monocytogenes* and a competitive microorganism to create 60 low-level samples and 15 high-level samples for a 3-method comparison. Additional samples were included for controls. Thirty sponges per test method were enriched in 90 mL of Actero™ *Listeria* Enrichment media (ALM) or 24 LEB Complete. The remaining 30 sponges were enriched according to the USDA FSIS MLG 8.11. All enrichments were analyzed by PCR as individual test portions and as a 5-sample pool. Results were assessed for sensitivity, specificity and compared using the probability of detection (POD).

Results: Individual and pooled sponges had 100% sensitivity and 100% specificity for both test method enrichments and reference MOPS-BLEB. Sponges enriched in ALM detected 19/20 low spiked and 5/5 high spiked samples. Sponges enriched in 24 LEB detected 15/20 low spiked and 5/5 high spiked samples. Sponges enriched in MOBS-BLEB detected 10/20 low spiked and 5/5 high spiked samples. All presumptive PCR results were identical to culture. POD analysis indicated a significant difference between ALM and MOPS-BLEB with superior recovery for the test method, and no significant difference between 24 LEB and MOPS-BLEB.

Significance: This study demonstrated the capability of the BAX® System to detect low-level *L. monocytogenes* contamination in a single sponge or as a 5-sponge pool with no impact on sensitivity or specificity. Furthermore, equivalent or superior performance was observed when compared to the reference method.

P2-46 Development of a Novel Plating Medium for Selective and Differential Identification of *Escherichia albertii* from *E. coli* and *Salmonella enterica*

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❖ ***Developing Scientist Entrant***

Introduction: *Escherichia albertii* is a foodborne entero-pathogen, identified to cause human diarrheal disease. Recent microbiological surveys in the U.S. have indicated fresh poultry meat may represent a likely transmission vehicle. Culture methods are lacking to facilitate organism isolation for subsequent identification from foods.

Purpose: The purpose of this study was to design a selective and differential plating medium to identify *E. albertii* from the enteric pathogens *E. coli* and *Salmonella enterica* from fresh poultry meat.

Methods: MacConkey agar was selected as basal medium for developing the new medium formulation. Lactose was replaced with 1.0% (w/v) melibiose; the medium was supplemented with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal), a lactose analogue, at 1.0% (w/v). Cocktails of *E. albertii* and *E. coli* from overnight cultures (9.0 ± 0.2 log CFU/mL) were prepared, diluted to approximately ~3.0 log CFU/mL and plated to determine ability to phenotypically differentiate organisms on medium surface. Resulting *E. albertii* and *E. coli* means from three replicates were compared by 2-tailed *t*-test at $P = 0.05$ to determine whether organisms differed from one another following plate incubation for 48 h at 37°C.

Results: In testing, 12 isolates of *E. albertii* all grew well with no detected utilization of melibiose but with strain-specific lactase production (2/12 lactase+). Seven *E. coli* isolates, including members of the generic *E. coli*, O157 and non-O157 STEC, and avian-pathogenic *E. coli* (APEC), demonstrated effective growth, producing bluish-green colonies from X-gal breakdown and melibiose use. *Salmonella* serovars Anatum, Agona, and Enteritidis demonstrated growth with evidence of melibiose use but no breakdown of X-gal. Visual differentiation of *E. albertii* from *E. coli* was achieved with no significant differences in mean counts of organisms and no significant loss of detection.

Significance: This medium may be usefully applied for detection and presumptive identification of *E. albertii* from other human enteric pathogenic bacteria in fresh poultry, aiding food safety protection.

P2-47 Variability in the Detection of *Campylobacter jejuni* in Unpasteurized Dairy Milk By the FDA-BAM Method

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Introduction: *Campylobacter* outbreaks related to the consumption of unpasteurized milk is frequent; additionally, less than 100 cells have been associated with human illness. Milk is a challenging matrix for pathogen detection because of its high lipid and protein content. Currently, the protocol in the FDA Bacteriological Analytical Manual (BAM), chapter 7, is used for pathogen detection in outbreak investigations.

Purpose: To evaluate the impact of the removal of the fat layer on the detection of *C. jejuni* by the FDA-BAM method in artificially contaminated, unpasteurized, non-homogenized, bovine milk.

Methods: The current FDA-BAM method requires centrifugation of 50 mL milk samples for concentration and evaluation for the presence of *C. jejuni*. Locally sourced milk samples were spiked with *C. jejuni* (~80 CFU) and refrigerated for 48 h. Samples (n = 13) were centrifuged, and both the pellet and the fat layer were processed to detect *C. jejuni*. The primary and secondary enrichments were performed as described in the FDA-BAM method with minor modifications.

Results: Storage of spiked milk at 4°C for 48 h resulted in an 80% reduction of culturable *C. jejuni,* of which approximately 96% were partitioned in the fat layer. After enrichment, only 54% of the pellets were positive (7/13) for *C. jejuni* when the samples were processed using the FDA-BAM method. In contrast, *C. jejuni* was detected in the fat layer of all the samples.

Significance: The fat layer of milk harbors *C. jejuni.* Discarding the fat as per the current FDA-BAM, may result in a significantly lower detection rate of *Campylobacter* spp. if the level of contamination in milk samples is less than 1-log. A possible modification to the current FDA-BAM method would be to process both the fat layer and pellet portions of milk samples. Further studies are needed before modifications to the FDA-BAM protocol can be implemented.

P2-48 Advantages of Rapid ATP Sterility Testing Using Hygiena Innovate System for UHT and ESL Alternative Milk-based Products Compared to pH and Standard Plating

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Introduction: The use of ATP sterility systems to rapidly detect contamination in non-dairy milk products has increased recently. The resultant formulations using plant-based protein sources Soy, Oat, Tree Nuts has led to sterility tests requiring to adapt and perform contamination detection in an ever-changing formulation.

Purpose: This poster presents a validation of a plant-based milk product spiked and recovery and sets out some guidelines for users.

Methods: A plant-based milk alternative ESL drink was validated using spike and recovery at 2 levels LOW (<10 CFU 100 mL^{-1}) and HIGH (>1000 CFU 100 mL^{-1}) spiked microbes – *Clostridium sporogenes, Saccharomyces cerevisiae, Staphylococcus aureus, Salmonella* Typhimurium*, Pseudomonas aeruginosa, Bacillus cereus, Aspergillus brasiliensis* and *Geobacillus stearothermophilus.* Packs were incubated at 30°C (55°C for *Geobacillus*) for 10 days and assayed for extractable ATP, pH and plated onto TSA for confirmation.

Results: *Geobacillus, Bacillus, Staphylococcus, Pseudomonas, Salmonella* and *Saccharomyces* were detected at 24 hours mean RLU values of 495211, 356548, 36212, 12861, 139901 and 339, respectively, mean CFU spike 190 CFU 100 mL^{-1} with negative control producing 31 RLUs. The equivalent confirmation growth on TSA plates was 48 hours for bacteria and 72 hours for yeast. pH equivalent was as follows *Pseudomonas* no pH change was observed over 10 days, for *Staphylococcus* after 48 hours and 24 hours for the others. ATP RLUs show that some bacteria can complete growth cycle very rapidly leading to RLU signals decreasing, this is important for determining risk analyses on each product, the *Bacillus cereus* RLUs for each day were as follows Day 1 - 356548, Day 2 - 2641, Day 3 -1312 and Day 4 - 943.

Significance: Using ATP for detection of microbes from milk alternatives can result in rapid detection. The growth of mesophilic and thermophilic bacteria appears to occur very rapidly, compared to plating and pH measurements.

P2-49 Development of a Targeted Real-Time qPCR through the Isolation of the Microbiome of Select Sports Drinks

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Introduction: Ensuring the quality of natural beverage products is complicated via traditional microbial detection methods due to a varied microbiome. Detailed analysis of spoilage organisms allows for a specified assay to rapidly and efficiently monitor for threats to product quality.

Purpose: To assess the microbiome of natural (coconut water based) sports drink and develop a qPCR assay for routine surveillance and quality markers.

Methods: A 3-phase process was used to identify product microbial spoilers, critical contamination thresholds, and to develop a custom qPCR screening assay. In Phase 1, raw materials, environmental swabs, and finished product were enriched in permissive nutrient media. A multiplexed qPCR assay was used to screen purified enrichment DNA, targeting the genera of *Brettanomyces*, *Candida*, *Pichia, Wickerhamomyces*, *Saccharomyces*, *Bacillus*, *Acetobacter*, *Gluconobacter*, *Lactobacillus*, *Pediococcus,* and *Alicyclobacillus*. In Phase 2, product was inoculated with Phase 1 organisms to determine critical quantitative spoilage thresholds and were assessed for gas production, turbidity, and pH. These findings were referenced in Phase 3, where a targeted, threshold-based qPCR assay was developed.

Results: N=287 presumptive positive samples were tested in Phase 1. 60 (20.9%) were positive for the bacteria panel and 50 (17.4%) were positive for the targeted yeast genera. N=190 Environmental samples were tested, and 102 (53.6%) were positive for bacteria, and 71 (37.3%) for yeast. Nine raw material samples yielded 2 (22.2%) positive bacteria results with no yeast detected. Phase 2 results demonstrated critical thresholds for possible spoilage of the yeast and bacterial panels (zero-tolerance for yeast, 5-10 CFU/mL for bacteria). A qPCR assay for zero-tolerance yeast detection and bacteria threshold testing was completed in Phase 3.

Significance: Molecular diagnostic assays for specific spoilage microbes are a powerful tool for quality beverage production. Early detection of deleterious microbes allows producers to take actions to protect flavor, prevent false-positives, and save product and money.

P2-50 Evaluation of the GENE-UP® *Escherichia coli* O157:H7 Method for the Detection of *Escherichia coli* O157:H7: Collaborative Study

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Introduction: The GENE-UP® *E. coli* O157:H7 2 (ECO 2) assay (Performance Tested Method℠ 121805) incorporates Fluorescence Resonance Energy Transfer (FRET) hybridization probes into its proprietary PCR technology for the rapid detection of *E. coli* O157:H7 in select foods.

Purpose: The purpose of this validation was to evaluate the method's interlaboratory performance and submit the result to AOAC INTERNATIONAL for adoption as First Action Official Method℠ for the detection of *E. coli* O157:H7 in select foods.

Methods: The GENE-UP® method was evaluated in a multi-laboratory study as part of the MicroVal validation process using unpaired test portions for one food matrix, raw milk cheese (Comté, 34% fat, 0.8% salt). The candidate method was compared to the ISO 16654:2001 reference method. Fourteen

(14) participants from 13 laboratories throughout the European Union participated. Three levels of contamination were evaluated: a non-inoculated control level (0 CFU/test portion), a low contamination level (~5 CFU/test portion) and a high contamination level (~10 CFU/test portion). Data from that study were analyzed according to the Probability of Detection (POD) statistical model as presented in the AOAC validation guidelines. The difference in laboratory POD ($dLPOD_c$) values with 95% confidence interval across collaborators was calculated for each level between the candidate and reference method results, and between the candidate presumptive and confirmed results.

Results: The $dLPOD_c$ values with 95% confidence interval were; 0.00 (-0.04, 0.04), 0.27 (0.04, 0.49) and 0.17 (0.01, 0.33) for the non-inoculated, low and high contamination levels, respectively.

Significance: The $dLPOD_c$ results indicate a significant difference between the candidate method and the reference method for both the low and high contamination levels, with the candidate method producing higher recovery of the target organism at both levels. The GENE-UP *E. coli* O157:H7 assay provides industry with a rapid, accurate detection method for *E. coli* O157:H7 in a broad range of foods.

P2-51 Development of a Paper-based Test for Discrimination of *Listeria* spp.

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Introduction: The Listeria genus is comprised of 17 species, of which two are pathogenic, *Listeria monocytogenes*, a foodborne pathogen, and *Listeria ivanovii*, an animal pathogen. Biochemical tests used to differentiate *Listeria* spp. include fermentation of sugars and detection of lecithinase activity on agar. Typically, identification by biochemical methods require extensive material preparation or expensive commercial kits. We propose a novel paper-based, inexpensive, and user-friendly platform of miniaturized biochemical tests for discrimination of *Listeria* spp.

Purpose: Our objective was to develop and optimize a paper-based biochemical test for discrimination of *Listeria* spp.

Methods: Individual portions of phenol red broth, agar, and α-methyl D-mannoside, dextrose, maltose, mannitol, rhamnose, or xylose were added to a cellulose acetate membrane and allowed to solidify. Brain Heart Infusion Agar supplemented with NaCl and glutamine was deposited onto Whatman No. 5 filter paper and allowed to solidify. Both *Listeria* spp. profiling tests were inoculated with 10^8 CFU/mL of *L. ivanovii*, *L. monocytogenes*, *Listeria grayi*, *Listeria innocua* and *Listeria welshimeri*, and 10^7 CFU/mL of *Listeria seeligeri*. Tests were placed in Petri dishes, wrapped with Parafilm, and incubated at 37°C for 18 h for sugar agars and 48 h for lecithin agars. Visual confirmation and ImageJ analysis were performed for sugar fermentation tests. Visual presence/absence of lecithin clearing was recorded.

Results: ImageJ analysis and visual confirmation revealed the fermentation of sugars and the clearing of lecithin from miniaturized agar tests. The biochemical profiles exhibited by the *Listeria* spp. allowed for unambiguous discernment, using α-methyl D-mannoside, mannitol, rhamnose, and xylose post 18 hours enrichment (n = 54, P < 0.05). All *Listeria* spp. tested fermented dextrose and maltose. After 48 hours enrichment, 10^8 CFU/mL of *L. monocytogenes* and *L. ivanovii* exhibited clearing of lecithin (n = 18, P < 0.05).

Significance: We developed and optimized a paper-based biochemical test to discriminate and profile pathogenic from non-pathogenic *Listeria* spp.

P2-52 Performance Evaluation of Fluorescence Resonance Energy Transfer-based Real-Time PCR for Detection of *Salmonella* Enteritidis and Typhimurium

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Introduction: In addition to already existing EU regulation, countries such as Brazil, who export poultry meat to Europe, now require raw poultry products that test positive for *Salmonella* spp to also be screened for the presence of *Salmonella* Enteritidis and Typhimurium. GENE-UP® *S.* Enteritidis and Typhimurium duplex assay (SE ST) is a real-time PCR assay that utilizes FRET hybridization chemistry to provide simultaneous multi-target detection.

Purpose: The SE ST method was validated according to the AOAC Microbiological Guidelines in the AOAC Performance Tested Method℠ (PTM) program for the detection of *S.* Enteritidis and Typhimurium in raw poultry products.

Methods: Inclusivity/exclusivity studies and matrix comparison study were conducted for ground chicken and chicken breast. For inclusivity, 100 well characterized *S.* Enteritidis and Typhimurium were grown in BPW for 24 h at 42°C and tested at 100 times the LOD by SE ST assay. For exclusivity, non-target *Salmonella* and non-*Salmonella* strains were grown for 24 h in non-selective broth and tested by the SE ST assay. In the matrix comparison study the alternative method was compared to the ISO 6579-1 reference method for the detection of *S.* Enteritidis and Typhimurium in artificially inoculated ground chicken and raw chicken breast.

Results: Using dDOP analysis the method comparison study demonstrated no significant differences between SE ST method and the ISO 6579-1 reference method. Inclusivity was 100%, and no exclusivity strains were detected.

Significance: GENE-UP SE ST duplex assay is a rapid, real-time PCR method for the simultaneous detection of *S.* Enteritidis and Typhimurium in raw poultry products.

P2-53 Hygiena MicroSnap Total Viable Count Detection System Correlation with Standard Methods from Eighty-Eight Different Food Matrices Covering Dairy, Protein, Produce, Water and Miscellaneous

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Introduction: The MicroSnap system uses biomarkers to measure qualitative and quantitatively bacteria in food matrices using a 7-hour incubation measured in the EnSURE Touch luminometer.

Purpose: This survey demonstrates the correlation using the system referenced to 3M Petrifilm.

Methods: Matrices were purchased, diluted in a decreasing decimal series down to 10^{-5} in buffer. Each dilution was added to each method, each system performed 5 biological and 3 scientific replicates after 7- and 24-hours incubation, respectively, the results were analyzed, log transformed into comparisons of each food type. No spiking of bacteria was used each matrix relied completely on the indigenous flora from each as CFU source.

Results: All matrices comparison (n = 360) demonstrated a R^2 = 0.735 (86% agreement), slope of line of 0.773 the CFU range was from log 1.0 to log 5.0. Dairy matrices (n = 66) the R^2 = 0.802 (89% agreement) slope of 0.824 dynamic range was from log 1.0 to log 5.0, Produce (n = 51) R^2 = 0.729 (85% agreement) slope of 0.870 dynamic range was from log 1.5 to log 4.5, Protein (n = 81) the R^2 = 0.742 (86% agreement) slope of 0.830 the dynamic range was from log 1.5 to log 5.5, Water (n = 125) the R^2 = 0.799 (89% agreement) slope of 0.799 dynamic range was from log 2.0 to log 5.5 and miscellaneous matrices (n = 36) R^2 = 0.814 (90% agreement) slope of 0.876 dynamic range was from log 1.5 to log 4.0. The mean log CFU differences between systems was as follows for ALL food matrices log -0.10, for Dairy produce log 0.06, for Protein produce log -0.32, for Produce log 0.03, for Water log -0.27 and for miscellaneous matrices log -0.38.

Significance: The new Hygiena method and the standard reference method correlate well across the matrices using the incubation time of 7 hours and 24 hours. The delta log values are excellent and within log 0.5.

P2-54 Influence of Surface Morphologies of Fresh Produce on the Detection of *S.* Typhimurium Using a Phage-based Square Planar Inductor System

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❖ ***Developing Scientist Entrant***

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Introduction: Our research group has developed a phage-based square planar inductor system (SPIS) for the direct detection of *Salmonella* on fresh produce. Since fresh produce have diverse surface morphologies, it could affect on the performance of SPIS.

Purpose: The purpose of this study was to investigate the influence of surface morphologies of fresh produce on the direct detection of *S*. Typhimurium using a phage-based SPIS.

Methods: The surface roughness and hydrophobicity, and stomata characteristics of ten fresh produce were investigated using a confocal laser scanning microscopy, a goniometer, and scanning electron microscope, respectively. Principal component analysis and K-mean cluster analysis were conducted to cluster ten fresh produce depending on their morphological characteristics. Finally, four fresh produce were selected from each cluster. *S*. Typhimurium-specific phage isolated from wastewater was immobilized on the sensor surface. The phage-immobilized sensor was placed on the surface of fresh produce inoculated with serially diluted *S*. Typhimurium and the SPIS was performed to measure the resonant frequency shift directly.

Results: Pear and perilla leaf showed the greatest roughness, whereas apple showed the lowest roughness. Most of fresh produce, except for perilla leaf and orange, exhibited the hydrophobic property. The number of stomata on perilla leaf, spinach, and cucumber were significantly greater than others. Ten fresh produce was grouped into 4 clusters and perilla leaf, pear, apple, and spinach were selected. The resonant frequency shifts of the sensors on all fresh produce were increased linearly with an increase of *S*. Typhimurium concentrations. Furthermore, there were no significant differences in the sensitivity and detection limit among the selected fresh produce when performed SPIS.

Significance: This study demonstrated that the phage-based SPIS could be suitable for the direct detection of *S*. Typhimurium on various fresh produce.

P2-55 Comparative Evaluation of Hygiena's BAX® Real-Time PCR Assays for the Detection of Shiga Toxin *E. coli* (STEC) Against the ISO/TS 13136 Reference Method

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Introduction: Detection of Shiga toxin-producing *Escherichia coli* (STEC) in food samples is of increasing importance highlighted by rising numbers/severity of food safety recalls and outbreaks in recent years. This study, performed by ADRIA labs (Quimper, France) aimed to evaluate a real-time PCR method's ability to detect Shiga toxin-producing *Escherichia coli* (STEC) from O26, O103, O111, O145 and O157 serogroups in raw beef, raw dairy products and vegetables.

Purpose: This study assessed real-time PCR assays for screening STEC. Assay sensitivity, relative level of detection, inclusivity and exclusivity were assessed in pure culture and in food enrichments relative to ISO standard 16140-2:2016.

Methods: Fifty target and 30 non-target strains of STEC were tested during inclusivity/exclusivity studies. The inclusivity portion tested 10 cells/225 mL of MP media; the exclusivity portion tested pure cultures at ≥10^5 CFU/mL. Lysates of artificially spiked and naturally contaminated food enrichments from 3 sample categories (n = 267) in MP media or BPW were prepared. Test kit results were compared to independently spiked or naturally contaminated samples, enriched per ISO. The PCR assays were also used as colony confirmation tools.

Results: The assays were 100% inclusive for all species tested; exclusivity was 100% against closely/distantly related genera. Sensitivity studies showed better performance, compared to ISO method, in all 3 sample categories. RLOD were all lower than the fixed AL of 2.5 in all categories. Based on these results and a collaborative study, the assays were granted NF Validation certification (18/11-12/20).

Significance: The real-time PCR assays for STEC exhibited increased assay sensitivity and faster/easier sample preparation and cycling times than the current ISO/TS method. These new assays allow for rapid time-to-results for the testing of food samples, while preserving the ease, accuracy and dependability of the BAX® System.

P2-56 *Listeria* Confirmation with a New Platform of Mass Spectrometry Instruments for Microorganism Identification

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Introduction: Mass spectrometry is an analytic technique and has been used as a rapid microbiological method in recent years. The MALDI Biotyper (MBT) can be applied to identify microorganism and to confirm foodborne pathogens.

Purpose: Our study should show the performance of the new MBT sirius instrument platform with Gram-positive *Listeria* species plus reliable identification of *L. monocytogenes* in comparison to classic MBT platforms.

Methods: The MALDI target plates were used for applying of samples and Bacterial Test Standard (BTS, by Bruker). One part of the instrument is a microflex mass spectrometer equipped with smartbeam laser technology. The mass spectrometer has two purposes: Soft ionization of biological compounds and MS analysis of the respective ions. The prepared MALDI target was introduced into the MBT, measured and identified by using a dedicated reference library. Potential *L. monocytogenes* were analyzed by the MBT Subtyping Module.

Results: We tested different *Listeria* strains with the new sirius platform. Non-selective and selective media were compared plus different sample preparation procedures (DT, eDT and Ext) were applied in parallel. We applied the MBT Subtyping Module for differentiation between the closely related species: *L. monocytogenes, L. innocua, L. ivanovii, L. seeligeri, L. welshimeri.* Results of 5 different instruments were compared. In addition, we calculated 375 different spectra with the new MBT Compass HT software, and compared the data with the standard software.

Significance: Equivalence of MBT sirius to classic mass spectrometry instruments was demonstrated for *Listeria* confirmation. The MBT subtyping module of both software versions, MBT Compass and the new MBT Compass HT, allow to achieve 100% accuracy for confirmation of *L. monocytogenes*. Reliable confirmation starting from colonies is now possible with an easy workflow and fast(er) time-to-result.

P2-57 *Salmonella* Confirmation and Strain Typing with a Two-Step Detection Solution Using Mass Spectrometry and Infrared Spectroscopy in Combination

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Introduction: The MALDI Biotyper sirius (MBT) is a benchtop mass spectrometry instrument that can be used to confirm foodborne pathogens. The IR Biotyper is an infrared spectroscopy solution for source tracking and rapid serogroup differentiation.

Purpose: Part01 of our study should show the application and performance of the new MBT sirius instrument platform for *Salmonella* confirmation compared to classic MBT models. In part02 we evaluated a new two-step approach: Confirmation with the MBT in combination with serogroup differentiation of *Salmonella* starting from colonies using the IR Biotyper.

Methods: Columbia blood agar with 5% sheep blood and XLD Agar, were used for cultivation. For mass spectrometry analysis MALDI target plates were used for applying of colony material. Identification/confirmation took place using the Bruker MBT reference library. For infrared spectroscopy analysis first a sample preparation was performed using the IR Biotyper Kit. Samples and the IR test standard were applied on silicon microtiter plates.

Results: In the first study part we evaluated a high number of mass spectra on the MBT sirius. Log(scores) ≥ 2.0 showed reliable identification. We successfully compared the data with data from previous AOAC validation studies performed at two expert laboratories on classic MBT models. In part02, we analyzed different *Salmonella* strains with importance in the meat & poultry sector. After species confirmation we showed that the IR Biotyper allows differentiation of *Salmonella* O-groups on the same day within 2 hours.

Significance: The MALDI Biotyper sirius system is a mass spectrometry platform useful for confirmation of *Salmonella* spp. Reliable and fast differentiation of foodborne *Salmonella* O-groups is now possible with the IR Biotyper. With the use of two devices the user has a significant lower time-to-result compared to traditional confirmation and subtyping methods.

P2-58 Validation of an Alternative Method for the Detection of *Escherichia coli* O157:H7 in Sprouts in Comparison with FDA Bacteriological Analytical Manual (BAM) Method

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Introduction: Sprouts have been implicated in numerous outbreaks caused by Shiga toxin-producing *E. coli* (STEC). High microbial populations in sprouts create a challenge for the detection of the pathogen from the matrix.
Purpose: This study attempts to validate an alternative method in an official FDA assignment in comparison with the FDA BAM method, for the detection of *E. coli* O157:H7 from sprouts. The alternative method differs from the BAM protocol in enrichment broth and incubation conditions.
Methods: Three produce outbreak associated *E. coli* O157:H7 strains were chosen to be tested with mung bean sprouts in 3 separate trials. In the completed Trial 1, sprouts were inoculated at 1.4 CFU/25 g in 40 test portions for the alternative method and the reference method. There were 10 positive controls inoculated at 13 CFU/25 g sprouts and 10 uninoculated negative controls. All enrichment samples were analyzed by the BAM STEC real-time PCR assay and Atlas® STEC EG2 assay. CHROMAgar O157, Rainbow agar O157 and TC-SMAC were the selective media used in the study.
Results: All 10 uninoculated test portions tested negative and all 10 positive controls were positive for *E. coli* O157:H7 by the correspondent culture, PCR and Atlas® assays. Eleven of 20 test portions were positive for *E. coli* O157:H7 by all 3 assays for the alternative method; 12 of 20 positive for the reference method. There were no statistical differences ($\alpha = 0.05$) between the alternative and reference methods for detecting *E. coli* O157:H7 from mung bean sprouts. In addition, the alternative method recovered 2 logs more target bacteria (CFU/µL IMS beads, $P < 0.0001$) from inoculated sprouts than BAM method.
Significance: The data indicated that the alternative method was equivalent to the reference BAM method in detecting *E. coli* O157:H7 from mung bean sprouts with the advantage of much easier plate reading.

P2-59 Evaluation of Molecular Methods for Detection of *Salmonella enterica* in Green Chile

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Introduction: *Salmonella enterica* has been responsible for foodborne disease outbreaks in a wide range of foods including green chile peppers.
Purpose: The aim of this study is to evaluate three molecular techniques (Neogen ANSR, VIDAS SLM and FDA PRL-SW Salmonella qPCR) for detection of *Salmonella* in artificially contaminated green chile.
Methods: Chopped green chile was inoculated with *Salmonella enterica* at a low level of (~0.04 CFU/g), a high level (~0.4 CFU/g). Uninoculated control samples were also examined. After aging the samples for 72 h, they were enriched in universal broth and incubated at 35°C for 24 h. The enriched samples used for the ANSR test and qPCR. Also, enriched samples were subcultured into Tetrathionate Brilliant Green and Rappaport–Vassiliadis broths and incubated an additional 24 h. Samples were prepared for qPCR, subcultured into M broth for the VIDAS assay and streaked onto selective agar plates (Hektoen agar, Xylose Lysine Desoxycholate agar, Bismuth Sulfite agar and CHROMagar *Salmonella*). After incubation, typical *Salmonella* isolates were confirmed as described in the FDA BAM and by qPCR.
Results: The method comparisons were conducted three times using *S. enterica* strains that were originally isolated from green chile. At the low inoculation level, fractional recovery of the pathogen was observed in the three trials. The Neogen ANSR, VIDAS SLM and qPCR assays performed favorably when compared with the FDA BAM cultural method for all three trials. In trial 3, *Salmonella* was detected in 14/20 samples by culture and VIDAS SLM, while 13/20 were detected by qPCR and the ANSR assay. No significant differences at $P < 0.05$ were between the procedures using McNemar's Chi Square test.
Significance: The Neogen ANSR *Salmonella* assay, the VIDAS SLM procedure and the FDA *Salmonella* qPCR assay were shown to be suitable alternatives to the BAM cultural method for detection of *S. enterica* in green chile. All three methods provided results more rapidly than the cultural procedure.

P2-60 Validation of a Real-Time PCR Assay for Rapid Quantification of *Salmonella* in Commercial Ground Turkey Meat Samples

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Introduction: Rapid *Salmonella* quantification methods that are comparable to the classical most probable number (MPN) method would provide industry with the ability to make decisions about product disposition. The GENE-UP® QUANTPRO *Salmonella*™ (QTS) assay utilizes an enrichment free sample prep paired with a multiplex detection method targeting a highly conserved pan-*Salmonella* genetic signature and an internal amplification control on the GENE-UP® providing *Salmonella* quantification in raw poultry in less than 4 h.
Purpose: The objective of this research was to validate the QTS assay and compare it to the MPN method using commercial samples with naturally occurring levels of *Salmonella*.
Methods: Ground turkey meat was shipped overnight to Auburn University from a commercial processor ($n = 20$). Samples (125 g) were aseptically transferred to sterile filter bags and 5x buffered peptone water was added. Aliquots of each sample were used for QTS assay (40 mL), a modified MPN (mMPN) method (333.3 mL), direct plating in duplicate (0.2 mL), and remaining aliquot was enriched (42°C, 24 h) to verify the presence/absence of *Salmonella* sp. using the *Salmonella* (Slm2) kit on the GENE-UP®. Positive samples (Slm2) with MPN or QTS assay below the detection limit were assigned a MPN value for analysis (0.1 and 0.69, respectively). Differences between testing methods (mMPN-QTS) were tested using a one-sample 2-tailed *t*-test (null = 0).
Results: The levels of *Salmonella* species from the QTS assay for natural samples were 95% in agreement within 1 log of the mMPN method. There were no differences ($P = 0.7621$) between the *QTS* assay (SE = 0.14) or the mMPN method (SE = 0.27).
Significance: Utilizing the GENE-UP® QUANTPRO *Salmonella*™ assay as a rapid method of quantification for *Salmonella* species will allow processors to make risk-based decisions on product disposition.

P2-61 AOAC-PTM Validation of the New Genedisc® Method for the Combo Detection of *Campylobacter* and *Salmonella* in Poultry Plants

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Introduction: *Campylobacter* and *Salmonella* are the two leading sources of foodborne illness with millions of cases worldwide each year. A strong association has been demonstrated between the consumption of poultry products and sporadic outbreaks of bacterial gastrointestinal disease caused by *Campylobacter* spp. and *Salmonella* spp. in humans. In that context, Pall GeneDisc Technologies proposes a new real-time PCR-based method enabling to simultaneously monitor both pathogens, with presumptive results available in less than 24 h.
Purpose: This new method was validated according to AOAC Research Institute (RI) Performance Tested Methods Appendix guidelines by an independent laboratory.
Methods: The scope of the validation included three matrices: raw chicken breast, poultry carcass rinse and poultry carcass sponge. The candidate method was compared with the FSIS MLG 41.04 for the detection of *Campylobacter* and with both the ISO 6579-1:2017 and FSIS MLG4.10 reference methods for *Salmonella* detection. Briefly, the candidate method is based on an enrichment step for 20 - 24 h, then, a one-step DNA extraction before PCR analysis for the specific detection of thermotolerant *Campylobacter* and *Salmonella* spp. The presumptive positive samples are confirmed by plating on chromogenic media.
Results: The specificity study, done with 108 *Salmonella* strains and 50 *Campylobacter* strains for inclusivity and 66 bacterial strains for the exclusivity, gave 100% expected results. The sensitivity study highlighted that the candidate method was equivalent to the reference methods with all sample types,

the sample prep kits, and the PCR kits used. Moreover, the robustness study minimally impacted the candidate method performance.

Significance: This new method offers greater efficiency and flexibility when compared to the reference methods, with a 20-24 h single-step enrichment combined with common sample preparation and common PCR analysis, for monitoring both key pathogens in poultry plants.

P2-62 V Detection of Viable *Listeria monocytogenes* at Low Concentrations Using Whole Genome Amplification and Subsequent Polymerase Chain Reaction

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Introduction: Isolation and detection of some foodborne pathogenic bacteria in food by conventional culture process may be difficult due to factors including stress, competition from non-target bacteria or viable but non-culturable state. Alternative approaches are required for sensitive detection of viable foodborne bacterial pathogens in food.

Purpose: This study aimed to improve molecular detection methods by enriching the genomic DNA of *Listeria monocytogenes* as a model at low concentrations using whole genome amplification (WGA) to the level detectable by polymerase chain reaction (PCR) with a potential for viability determination.

Methods: Four *L. monocytogenes* strains at the concentrations between 10^9 and 10^0 CFU/mL with 10-fold serial dilutions in phosphate-buffered saline (pH 7.4, 0.01 mM) were used. Whole bacterial genomes were amplified using PicoPlex WGA (Rubicon Genomics, USA). The amplification for an individual gene was determined by using PCR detecting a *L. monocytogenes* specific gene. The amplicons of WGA and PCR were analyzed using QIAxcel Smear Analysis and 1.5% agarose gel electrophoresis. The amplification factor was determined by comparing the electrophoresis bands corresponding to various bacterial concentrations before and after WGA and PCR. Propidium monoazide (PMA) treatment of live and heat-killed *L. monocytogenes* was used to determine if PMA could inhibit the WGA of killed bacteria.

Results: The WGA kit amplified the bacterial genome to 10^7 folds, and a *L. monocytogenes* specific gene for 10-100 folds. Preliminary studies showed that PMA treatment inhibited the WGA amplification of dead cells but successfully amplified the DNA from viable cells.

Significance: The results indicated that the combination of WGA and specific PCR could enhance the detection of one or multiple specific target gene(s) with a potential of viability distinction using PMA. The approaches developed would provide alternative means for more sensitive detection of viable foodborne bacterial pathogens.

P2-63 Evaluation of the GENE-UP® *Listeria monocytogenes* Method for the Detection of *Listeria monocytogenes* and the GENE-UP® *Listeria* spp. Method for the Detection of *Listeria* Species: Collaborative Study

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Introduction: The GENE-UP® Listeria monocytogenes 2 (LMO 2) and Listeria spp. 2 (LIS 2) assays use real-time PCR technology and a proprietary detection platform, the GENE-UP® Thermocycler, to detect *Listeria* spp. and *Listeria monocytogenes* in a variety of foods and environmental surfaces.

Purpose: The purpose of this validation was to evaluate the methods' interlaboratory performance and submit the result to AOAC INTERNATIONAL for adoption as First Action Official Method of Analysis℠ (OMA).

Methods: The GENE-UP® methods were evaluated in a multi-laboratory study as part of the AFNOR NF VALIDATION certification process using unpaired test portions for one food matrix, full-cream goat milk cottage cheese (8.4% fat). The candidate methods were compared to the ISO 11290-1/Amd.1:2004 reference method. Sixteen participants from 15 laboratories throughout the European Union participated. Three levels of contamination were evaluated: a non-inoculated control level (0 CFU/test portion), a low inoculum level (~2 CFU/test portion) and a high inoculum level (~10 CFU/test portion). Data from the study were analyzed according to the Probability of Detection (POD) statistical model as presented in the AOAC validation guidelines.

Results: The $dLPOD_c$ values with 95% confidence interval for each comparison were; -0.02 (-0.07, 0.03), -0.08 (-0.31, 0.16) and 0.00 (-0.03, 0.03) for the non-inoculated, low and high contamination levels, respectively.

Significance: The $dLPOD_c$ results demonstrate no difference in performance between the candidate methods and reference method for the matrix evaluated. Highlights: Data from a singular collaborative study was used to achieve both AFNOR NF and AOAC OMA approval.

P2-64 Withdrawn

P2-65 Improvement of DNA Extraction from *Vibrio vulnificus* by Development of Membrane Lysis Buffer

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Introduction: Conventional polymerase chain reaction (PCR) is used to evaluate the presence of foodborne pathogens by detecting DNA. However, commercial kits for DNA extraction usually have many procedures and are expensive.

Purpose: The objective of this study was to improve the detection methods of *Vibrio vulnificus* with conventional PCR by developing the cell membrane lysis buffer.

Methods: Two *V. vulnificus* strains ATCC27562 and NCCP14549 were mixed and diluted to 3.0-3.5 log CFU/mL to prepare an inoculum. Distilled water, buffer solution (phosphate-buffered saline (PBS), Tris-HCl (pH 8.0)), surfactant (1% Triton X-100, 0.05% digitonin, 0.5% 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS)), 0.05 N NaOH, 1 M NaCl, and 0.5 M ethylene diamine tetraacetic acid (EDTA) were applied itself or in combinations to *V. vulnificus*, followed by boiling at 99°C for 15 min and microwaving for 20 s to lysis the cell membrane. To evaluate the efficiency, the presence and intensity of DNA bands in electrophoresis were compared after conventional PCR amplification.

Results: After electrophoresis, the lysis buffer combinations with high definition of the band were priority. Microwaving samples for 20 s in PBS + 0.05% digitonin, PBS + 0.5% CHAPS, Tris-HCl (pH 8.0) + 0.05% digitonin, and 0.05 N NaOH were selected as cell membrane lysis buffers. With these methods, the DNA was extracted even at 1.0 log CFU/mL of *V. vulnificus*. Regarding cost, among these lysis buffers, PBS + 0.5% CHAPS was the most economical.

Significance: These results indicate that the developed lysis buffer can improve the detection for *V. vulnificus* with conventional PCR, especially with PBS + 0.5% CHAPS, considering the cost.

P2-66 Development of Lysis Buffer to Improve DNA Extraction Efficiency for *Escherichia coli*

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Introduction: To identify foodborne pathogens in food with conventional polymerase chain reaction (PCR), DNA extraction is necessary. However, when DNA is extracted with commercial kits, it requires many procedures, time consuming, and expensive cost.

Purpose: The objective of this study was to improve the DNA extraction efficiency from *Escherichia coli* by developing the lysis buffer.

Methods: Two *E. coli* strains KCTC2643 and KCTC2615 were mixed and diluted to 3.0-3.5 log CFU/mL to be inoculum. *E. coli* was treated with distilled water, phosphate-buffered saline (PBS), Tris-HCl (pH 8.0), 1% Triton X-100, 0.05% digitonin, 0.5% 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesul-

fonate (CHAPS), 0.05 N NaOH, 1 M NaCl, or 0.5 M ethylene diamine tetraacetic acid (EDTA) in single treatment or combinations. After adding 1 mL of the lysis buffer to *E. coli,* the DNA was extracted by boiling at 99°C for 15 min or microwaving for 20 s. To evaluate the effect of DNA extraction, the presence and intensity of the bands were observed in electrophoresis after conventional PCR.

Results: Of 48 combinations of lysis buffer, 3 lysis buffer combinations were selected eventually. The DNA extraction efficiency was the most effective, when the lysis buffer with PBS + 0.05% digitonin was boiled or microwaved, or PBS + 0.5% CHAPS + 0.05 N NaOH was microwaved. Especially, DNA was extracted even at 1.0 log CFU/mL of *E. coli* when boiled in the combination of PBS with 0.05% digitonin.

Significance: Lysis buffer with PBS + 0.05% digitonin with boiled can improve the DNA extraction efficiency from *E. coli*, and save time and cost.

P2-67 Real-Time Detection of Norovirus Capsid Protein with an OmpG Nanopore

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❖ ***Developing Scientist Entrant***

Introduction: Human noroviruses are the leading causes of foodborne illness and death globally, and induce a considerable public health burden. The properties of noroviruses make them difficult to control such as low infectious dose and high diversity. Outer membrane protein G (OmpG) based sensing has been demonstrated for these purposes for clinical biomarkers but has not been investigated for foodborne pathogens.

Purpose: The purpose of this study was to develop an OmpG nanopore detection and subtyping method for noroviruses as a real-time and in-field measurement.

Methods: The P domain of norovirus capsid protein was expressed in *E. coli* strain BL21 and purified using glutathione-Sepharose 4B gravity column. The proteins were further purified to consistency with size-exclusion chromatography column Superdex 200. OmpG is engineered in two different ways: using a 12 amino acid peptide sequence and generating an OmpG library. To screen the OmpG using library, P domain was labeled with AFDye™647 and used for flow cytometry.

Results: The recombinant P domain showed homogeneity by size-exclusion chromatography in 1X PBS buffer (pH 7.4), which shows the major peak eluted at 69 kDa. Electrical current recording of the peptide-labeled OmpG exhibited a new open state that 5pA decreased signal, 25pA, at 300mM KCl, 50mM Tris-HCl (pH 7.5). The partially opened current state was observed more frequently for longer period of time when capsid protein were added. The lowest target concentration generating this current signal was 100nM and dose-dependent gating pattern was observed in the range of 0nM to 200nM.

Significance: These data suggest that norovirus capsid protein detection using biological nanopore may help control foodborne outbreaks through rapid detection and subtyping. This work also provides the foundation for further research on virus detection in complex matrices.

P2-68 Matrix Evaluation Level Assessment Tool Case Study: Detection of *Salmonella* in Leaf Tea

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Introduction: We used the "Matrix Evaluation Level Assessment Tool" (MELT) on the Applied Laboratory Methods PDG website to evaluate methods for detecting *Salmonella* in leaf tea. The tool suggested a USP-style suitability test. The standard enrichment protocol (Buffered Peptone Water, BPW) failed suitability. Tea contains antimicrobial polyphenolic compounds, which can inhibit PCR reactions. An alternative enrichment protocol recommended by the kit manufacturer (and FDA BAM) for chocolate, another matrix high in polyphenolics, used enrichment in reconstituted Non-Fat Dried Milk (NFDM) with 0.1 % brilliant green and passed suitability.

Purpose: To trial MELT and verify a method for detection of *Salmonella* in dried leaf tea.

Methods: Black, green and herbal teas were inoculated with *S.* Abaetetuba at 30 CFU/25 g using BioMérieux BioBalls. Single aliquots (25 g) of each matrix, as recommended by MELT, were incubated at 37°C for 21 h in 225 mL of BPW, with a secondary enrichment in Brain Heart Infusion Broth (BHI) at 37°C for 3 h and PCR detection using the BACGene platform. A further inoculated green tea sample was enriched in BPW, as above, and processed by the FDA BAM cultural *Salmonella* method. Finally, seven samples including all tea types were enriched in reconstituted NFDM with 0.1 % brilliant green at 35°C for 24 hours followed by subsampling to BHI for 3 h at 37°C.

Results: Inoculated green tea samples enriched in BPW tested negative for *Salmonella*, by PCR and the FDA BAM method. All seven inoculated samples of three different teas enriched in reconstituted NFDM with 0.1 % brilliant green were detected using the BACGene PCR *Salmonella* method. The enrichment suitable for matrices with high polyphenolic content seems a simple solution to the challenge of detecting *Salmonella* in tea.

Significance: MELT workflow helped to determine the necessary workflow to evaluate a new enrichment for tea.

P2-69 Rapid Detection of Multiple Foodborne Pathogens in Complex Metagenomic Datasets

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Introduction: Comparing to the current foodborne pathogen detection methods, a metagenomics-based approach offers the potential to detect any and all known pathogens present in a complex sample in one assay. However, there are challenges that need to be addressed before pathogen detection from complex metagenomics data becomes practical.

Purpose: In this study we evaluated the influence of bioinformatics pipelines on the detection of foodborne pathogen *Salmonella enterica* in metagenomics data generated from fresh tomato surface wash and optimized the selected bioinformatics pipeline, E- probe Diagnostic Nucleic-acid Analysis (EDNA), for sensitive and multi-target detection of foodborne pathogens in complex metagenomics datasets.

Methods: Metagenomics datasets were generated from DNA extracted from fresh tomato surface washes spiked with *S. enterica* (3 pooled DNA samples for each treatment) and in silico (mock datasets, 10 replications) using MetaSim. Four pipelines, BLAST, DIAMOND, Krake2, and EDNA, were used in bioinformatics analysis and detection.

Results: Among the bioinformatics pipelines evaluated, EDNA offered a rapid (5 min vs up to 500 h) and straight forward process in detection of *S. enterica* in metagenomics data. Further optimization of ENDA (E-probe length, E-value, query coverage, and percentage identity) improved the detection limit significantly ($P < 0.05$), from 1,000 cells/tomato to 10 cells/tomato. Multiple foodborne pathogens, *Campylobacter jejuni*, *Escherichia coli* O157:H7 (STEC), *Listeria monocytogenes,* and *S. enterica* were detected simultaneously in mock metagenomics datasets using the optimized EDNA pipeline.

Significance: EDNA, as a probe-based bioinformatics pipeline, showed the potential to quickly detect any foodborne pathogen present in a single complex sample through metagenomics data mining.

P2-70 Influence of Salt Content in Processed Foods for Next-Day *Listeria monocytogenes* Screening

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Introduction: If proprietary enrichment media for *Listeria monocytogenes* use an elevated level of salt as a selective agent to inhibit less tolerant background, detection of *Listeria* could be compromised in high-salt matrices due to the combined salinity of media and matrix.

Purpose: The purpose of this study was to perform Limit of Detection (LOD_{50}) studies on four matrices: mushroom base (25% salt), beef au jus (30% salt), beef base (35% salt), and chicken powder (40% salt), using two next-day *Listeria* platforms: an ELFA platform (VIDAS® UP) and a PCR platform (iQ-Check® *Listeria monocytogenes* II).

Poster

Methods: Per ISO 161410-2:2016, LOD_{50} determinations were made by weighing 24 x 25 g samples of each matrix. The 24 samples were divided into four spike levels, ranging from ~ 0.5 CFU to 3 CFU. Each matrix was tested on both PCR and ELFA platforms, at both 1:10 and 1:20. The LOD_{50} was calculated using the Wilrich and Wilrich (2009) spreadsheet. An LOD_{50} of ≤ 1 CFU/sample is desirable.

Results: For the matrices containing 30%, 35%, and 40% salt, *Listeria monocytogenes* could not reliably be detected at a level of 1 CFU/25 g on the ELFA platform using a standard 1:10 dilution of the matrix in the proprietary medium, LPT broth. An LOD_{50} of ≤ 1 CFU/25 g could be obtained if the enrichment dilution was raised to 1:20. For the PCR assay, a 1:10 enrichment in proprietary LSB gave acceptable LOD_{50} results for the 25%, 30%, and 35% matrices. A 1:20 dilution was needed only for the matrix containing 40% salt.

Significance: High-salt products tested on next-day *Listeria* screening platforms may need to be enriched above the traditional 1:10 dilution to avoid missing detection of the pathogen.

P2-71 Performance Evaluation of a Loop-Mediated Isothermal Amplification (LAMP)–bioluminescent Assay for Rapid Detection of *Campylobacter* in Poultry from Brazilian Reference Laboratory

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Introduction: *Campylobacter* is a significant problem in the poultry industry throughout the world, and Brazilian poultry industries need safe and accurate methods to detect this important pathogen before its product reaches the consumer's table. *Campylobacter* is a fastidious microaerophilic organism requiring special media and conditions for growth. This has necessitated easy monitoring solutions for laboratory analysis to accurately detect *Campylobacter* and avoid false-negative results.

Purpose: To determine the specificity, sensitivity and accuracy of a Loop-Mediated Isothermal Amplification (LAMP)-Bioluminescent Assay for poultry compared to ISO reference method 10272-1:2017.

Methods: In a paired study 77 poultry samples from meat industries was analyzed. Forty-seven samples was artificially contaminated with *Campylobacter coli* with low (5 CFU/25 g) to high (36 CFU/25) populations and with *E. coli* and *P. mirabilis* (about 10^4 CFU/25 g) like interferents. Thirty samples were naturally contaminated. All samples were enriched 1:10 in 3M™ *Campylobacter Enrichment Broth* (CEB) at 41.5°C for 24-28 h and analyzed with the LAMP-bioluminescent assay and ISO 10272-1:2017 method. Sensitivity, specificity, accuracy and POD were determined.

Results: The alternate LAMP assay was able to detect *Campylobacter* in all samples artificially contaminated and naturally contaminated. Compared to the traditional method, the overall sensitivity, specificity and accuracy of the LAMP Bioluminescent Assay was both 100.00%. The LAMP-Bioluminescent assay had no false positives and no false negatives. The POD analysis between the LAMP method and culture confirmation did not show any significant difference at a 95% confidence interval for all the matrices tested.

Significance: The alternative LAMP-Bioluminescent molecular method enabled reliable, rapid and automated detection of *Campylobacter* in poultry samples. The easy-to-use LAMP-bioluminescent method combined with the *Campylobacter* enrichment broth offers poultry producers and reference laboratories a rapid and safe method to detect this fastidious pathogen.

P2-72 Detection of Inoculated SARS-CoV-2 Virus Analogue from the Surfaces of Raw and Heat-Processed Meat Products

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Introduction: Although food products are not thought to be vehicles for the transmission of SARS-CoV-2, pressure from some countries to certify product SARS-CoV-2 free prior to importation has necessitated the development of methods for screening foods. A method was developed that only tests the surfaces of foods where environmental contamination with SARS-CoV-2 would be most likely to occur. Raw and heat-processed meat products were chosen as representative food commodities and a synthetic SARS-CoV-2 was used as a surrogate for the actual virus. A commercially available qPCR kit with an RNA extraction free lysis protocol was used as the detection method.

Purpose: To determine the sensitivity of an internally developed qPCR-based detection method for synthetic SARS-CoV-2 with an RNA extraction free lysis protocol and from the surfaces of raw and heat processed meat products.

Methods: Detection sensitivity of the modified (w/o RNA extraction) qPCR method was determined by testing dilutions of synthetic SARS-CoV-2 inoculated onto a variety of pork and chicken samples. Meat sample surfaces (2.25 cm^2) were inoculated in triplicate with 10 µL synthetic SARS-CoV-2 at varying levels. Following 1 h drying time, analytical samples were collected by swabbing and then processed by the modified qPCR method.

Results: The modified qPCR method, R^2 around 0.97-0.99 within individual runs, showed good linearity. The sensitivity was similar to the reported detection limits when RNA extraction is used (2.0 copies/reaction with confidence interval: 1.3 - 3.2 copies/reaction). Limits of detection for the method when used to test inoculated product ranged from 3-3,000 copies/2.25 cm^2 depending on the product type

Significance: A simple and sensitive qPCR method without RNA extraction was developed that could be used by food manufacturers to test the surfaces of food for the presence of SARS-CoV-2 if required for importation to a foreign country.

P2-73 Characterization of Stress-Tolerant *Listeria* Species Isolated from Foods

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Introduction: *Listeria* species is an opportunistic foodborne pathogen that can survive in harsh environmental conditions including low temperature, high salinity, and low pH.

Purpose: This study aims to generate and compare growth curve between stress-resistant and -sensitive *Listeria* strains isolated from various foods, humans, and animal farm environmental sources as well as identify stress-related genes prevalence.

Methods: A total of 187 *Listeria* strains from foods, humans, and animal farm environmental sources were isolated in this study (3) and collected from the USDA National Poultry Research Center (55) and other collaborators (129). All *Listeria* strains (187) were tested the stress resistance using a broth model under pH (3 to 5), salinity (3 to 5%), and temperature (1 and 4°C) conditions to identify stress-tolerant and -sensitive strains. The growth kinetics of stress-resistant and -sensitive strains were generated using a response surface methodology (RSM). The prevalence of 11 stress-related genes (*betL*, *cysS*, *fbp, flaA*, *A-lisk*, *lmo2363*, *inlA*, *prfA*, *S-lisk*, *ropB*, and *opuCA*) in *Listeria* species was evaluated using a PCR assay.

Results: A total of 36 out of 187 *Listeria* strains (31 *L. monocytogenes* and 5 *L. innocua*) exhibited the stress resistance to acidity (pH 3), salinity (5%), and temperature (1°C). At pH 3, salinity 5%, and 1°C, a stress-resistant *L. monocytogenes* strain (P00182) showed 4-log reduction after 72 h incubation while a stress-sensitive strain (meat 21) showed 9-log reduction, respectively. Among the 36 stress-resistant *Listeria* strains, 30 strains (27 *L. monocytogenes* and 3 *L. innocua*) possessed all 11 stress-related genes and 5 strains (3 *L. monocytogenes* and 2 *L. innocua*) possessed 6 to 10 stress-related genes. The one stress-sensitive *L. monocytogenes* strain had one gene (*prfA*).

Significance: The results in this study will contribute on ensuring food protection and public health by identification of stress-resistant *Listeria* strains isolated from foods.

Poster

P2-74 Efficiency of Detection of Human Pathogens through Whole Carcass Enrichment and Rinsed Methods in Organic and Conventional Chicken Using Metagenomic Approach

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Introduction: Foodborne pathogens cause human foodborne infections globally. Determination of the microbial community, including foodborne pathogens on poultry, is critical to investigate the control measures of pathogenic bacteria and improve the health of consumers.

Purpose: The study is aimed at evaluating comparative efficiency of human pathogen detection in organic and conventional chicken through metagenomic analysis of whole carcass enrichment and rinsed method.

Methods: Organic and conventional whole broiler carcasses (n = 46) were vigorously shaken with 500 mL buffered peptone water (BPW). For the rinse method, 30 mL aliquot was mixed with 30 mL of BPW. The rest of the sample, including the carcass, was used for the enrichment method. All samples were incubated at 37°C for 24 h. The samples were divided into five groups [Control (only BPW without chicken (n = 5)), Organic-Rinsed (n = 12), -Enriched (n = 13) and Conventional-Rinsed (n = 7), -Enriched (n = 9)] and 50 mL of each sample was subjected to DNA extraction followed by 16S rRNA sequencing to investigate the bacterial community composition.

Results: Proteobacteria and Firmicutes predominate the microbiota of conventional and organic chickens, followed by low abundance of Bacteroidetes and Fusobacterium. While the abundance of Proteobacteria and Firmicutes remains unchanged in organic chicken irrespective of method used, a noticeable shift in Proteobacteria and Firmicutes ratio (59%:39% in rinsed to 38%:60% in enriched) was observed in conventional chicken. Furthermore, choice of method did not yield any differences in species richness among conventional and organic chicken but resulted in a statistically significant difference (P < 0.05) in the diversity index. However, such difference in phylogenetic diversity was not observed in cohorts of *Salmonella*-positive or -negative samples.

Significance: This study demonstrates the impact of the methods in recovery and detection of microbiota in chicken, which can be utilized in the food safety routine testing to improve pathogen detection and by government agencies to improve consumers' health.

P2-75 Detection and Quantitation of Dipicolinic Acid Released from *C. botulinum* Spores Using a Novel, Rapid Liquid Chromatography-Tandem Mass Spectrometry Method

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Introduction: Analysis of dipicolinic acid (DPA) release from *Clostridium botulinum* spores from thermal processing is critical to obtaining a mechanistic understanding of the factors involved in the heat resistance of these spores. Because legacy liquid chromatography methods for DPA analysis typically involve long separations times and use of reagents not compatible with mass spectrometry-based detection, innovations in these methods are needed.

Purpose: This study was performed to develop a rapid (<5 min) ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method for detection of DPA released from *C. botulinum* spores during thermal inactivation.

Methods: Spores of *C. botulinum* (Giorgio-A and 202-F) were suspended in ACES buffer (0.05 M, pH 7.0) and subjected to heat treatments (Giorgio-A at 108°C and 202-F at 83°C) up to 5 min. Treated spore suspensions were filtered through a 0.22 µm membrane, the filtrate heat-treated at 85°C for 10 min, and analyzed for released DPA by UPLC-MS/MS. These data were used to plot a log reduction inactivation curve against DPA release. Experiments were performed in duplicate.

Results: DPA was successfully retained on a mixed-mode C18/anion exchange column and subsequently detected in multiple reaction monitoring (MRM) positive ionization mode. Intraday precision (n = 5 replicate injections) was 3.46% and 1.94% for 10 µg/mL and 50 µg/mL DPA, respectively. Analyte recovery ranged from 90.1-113.9% across 202-F and Giorgio-A *C. botulinum* strains, indicating minimal matrix effects. DPA released from Giorgio-A as a percent of total DPA was 80.3 ± 1.5, 82.9 ± 0.9, and 85.1 ± 2.1 after 1, 3, and 5 min, respectively; this corresponded to a log reduction of -1.4 ± 0.1, -2.7 ± 0.1, and -3.8 ± 0.1 spores at the same time points.

Significance: This method provides a rapid method for detection of DPA release from *C. botulinum* spores with minimal sample preparation. This method is well-suited for use in investigations to further explore mechanistic food safety research questions involving *C. botulinum*.

P2-76 Withdrawn

P2-77 Rapid and Specific Detection of *Staphylococcus aureus* in Milk and Udder Exudate Based on Endolysin-Mediated ATP Release and Bioluminescent Detection

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Introduction: *Staphylococcus aureus* (Sa), a major food poisoning agent, is also a major cause of bovine mastitis leading milk producers to use therapeutic antibiotics that possibly enter the milk stream impacting human health and downstream fermentation processes. Phage lytic enzymes (Ply) are bacteriophage encoded endolysins catalyzing lysis of Gram positive cell walls in a species-specific manner. The Sa-Ply have been studied as biocontrol agents. Here we present a proof-of-concept assay utilizing the same mechanism imparting phage lytic enzyme (Ply) specificity to its target organism, here being Sa.

Purpose: To develop an assay system for Sa which is species-specific, quantitative, rapid and usable on farm and in-plant.

Methods: Endogenous milk ATP is first released with a low strength detergent buffer. Samples are then mixed with *Sa*-Ply which lyses only *Sa* and no other Gram positive or negative bacteria in the sample. ATP from Sa cells is released and reacts with added luciferin/luciferase reagent resulting in ATP-dependent bioluminescence measured as RLUs (relative light units) with a hand-held bioluminometer previously demonstrated to measure total bacterial numbers utilizing a pan-bacterial lysing agent with a high degree of correlation to cultural plate counts. The use of Ply enzymes provides specificity to the assay.

Results: RLUs were plotted against the log of *Sa* concentrations in different buffers and the range of detection determined to be in the linear range from 10^2-10^{5+} CFU/mL. Time to result is ~ 1 minute.

Significance: *Sa* is one of the major bacterial groups involved in bovine mastitis. The others, including *Streptococcus uberis*, have characterized homologous endolysins and amenable to this same assay design concept. Given the quantitative nature of the assay, milk producers can monitor milk quality as impacted by mastitic conditions and make informed decisions on whether antibiotic treatment is warranted based on quantitative results which are specific to the pathogen and obtained in minutes from start to finish.

P2-78 Detection of Aerobic Bacteria from Biofilm on Dried Surfaces Using ATP, Culture, and Selective Microbial Bioluminescent Detection Methods

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Introduction: The food industry uses rapid methods to monitor surface hygiene and pursues correlation to traditional microbial count. Scientific publications indicate ATP RLU do not correlate to microbial CFU when surface biofilms due to variable microbial sizes and differing non-microbial ATP content in foods.

Purpose: This study evaluated the recovery and detection of ATP-based rapid surface hygiene assessment methods compared to aerobic count.

Methods: Three matrices were selected for their inherent ATP levels (high - whole milk, low - UHT soymilk and BPBDW) and inoculated with small-sized bacteria, *Pseudomonas aeruginosa* (ATCC#27853) and larger-sized *Bacillus cereus* (ATCC#33019). Organisms were spiked at 3-log dilutions into each matrix. Inoculated matrices (100 µL) were dried in duplicate on 100 cm^2 aluminum foil squares, swabbed, and tested using four ATP methods, one culture method, and one selective microbial bioluminescent method (SMB): PocketSwab Plus=ATP1; AllerGiene=ATP2; Peel Plate AC (Charm Sciences) with Pur-Blue HiCap Buffer Swab (World BioProducts) for sampling; UltraSnap=ATP3; SuperSnap=ATP4; MicroSnap Total Viable Count (Hygiena).

Results: ATP detection of *Bacillus* in biofilms with soy residue or buffer were: ATP2=Log_{10}(1); ATP1=Log_{10}(2); ATP4=Log_{10}(3); and ATP3=Log_{10}(3) soy only, while *Pseudomonas* detection were: ATP2=Log_{10}(3); ATP1=Log_{10}(5); ATP4=Log_{10}(5) soy only; ATP3=not detected at Log_{10}(5). In the presence of high-ATP food, all ATP methods detected all films. Log_{10}(3 and 4) *P. aeruginosa* biofilms in buffer had significant Log_{10}(1 and 2) lower microbial recovery (<0.1%) compared to with food (1%), whereas *B. cereus* had similar recovery (2.26%) with or without food present. SMB method had erratic positive and negative results with poor correlation to CFU/100 cm^2, R^2 = 0.10 for *Bacillus* and 0.06 for *Pseudomonas*.

Significance: Different ATP systems have varying biofilm detection levels that industry should consider, along with ATP content of processed foods. Data supports literature that ATP RLU does not correlate to microbial CFU. In this study, SMB method did not correlate to microbial concentration in biofilms.

P2-79 Detection of *Enterobacteriaceae* from Biofilm on Dried Surfaces Using ATP Swab, Culture and Selective Microbial Bioluminescence Detection Methods

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Introduction: Surface sampling for Enterobacteriaceae (EB) in food plants is performed to indicate unsanitary conditions. Industry uses a variety of rapid hygiene-monitoring methods at the potential risk of decreased sensitivity.

Purpose: Evaluation of EB microbial detection using culture method compared to ATP swabs and selective microbial bioluminescent method.

Methods: Three matrices were selected for their inherent ATP levels (high - whole milk, low - UHT soymilk and BPBDW) and inoculated with EB organisms, *E. coli (*ATCC#11775) and *Salmonella enterica (*ATCC#53648), and non-EB *Pseudomonas fluorescens* (ATCC#13525). All organisms were individually spiked at 3-log dilutions into each matrix. Inoculated matrices (100 µL) were dried in duplicate on 100 cm^2 aluminum foil squares, swabbed, and tested using ATP swabs (Pocketswab Plus, Charm Sciences), culture method (Peel Plate EB, Charm Sciences) with Pur-Blue HiCap Buffer Swabs (World BioProducts), and selective microbial bioluminescence method (MicroSnap EB, Hygiena).

Results: ATP swab and selective microbial bioluminescence method lacked sensitivity to detect low-level biofilm inoculum (<20 CFU/swab). ATP swab method detected the presence of food (ATP) but not the presence of bacteria. Culture method had a significant, Log = (1) greater recovery of microbes from milk biofilm (*E. coli* 35% and *Salmonella* 100%) compared to biofilm without food residue (3.5% and 10%, respectively). Log (4) higher culture dilutions in soy resulted in ATP swab detection in all samples. Log (6 and 7) *E. coli* and *Salmonella* had insignificant difference in microbial recovery (2.9%) with soy vs. (2.0%) without. *Pseudomonas* Log = (3) surface biofilm was used as an exclusion for both the culture and selective microbial bioluminescence methods, and was not detected by either method. The selective microbial bioluminescence method detected the cultured bacterial biofilms at 20,000 CFU/100 cm^2, and culture method detected at 900 CFU/100 cm^2.

Significance: Biofilm composition plays a significant role in the recovery and detection of Enterobacteriaceae on surfaces. The ATP swab method, though non-specific, was more sensitive than the selective microbial bioluminescent method.

P2-80 Performance Evaluation of Multiplex Real Time PCR for Detection of *Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus* in Nutraceutical and Dietary Supplement Matrices

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Introduction: Rapid methods such as PCR have been making in-roads into the routine nutraceutical and dietary supplements testing, the potential of multiplex PCR for routine detection of multiple pathogens has yet to be ascertained. The diversity and ever-growing list of matrices in these industries further exacerbates the challenges for such methods and thereby the method compatibility should be reviewed with the adoption of new technology.

Purpose: To evaluate the performance of GENE-UP® NutraPlex™ (NP) a triplex real-time PCR assay for detection of *Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus* simultaneously in various nutraceutical and dietary supplement matrices.

Methods: For inclusivity, *E. coli* (14), *S. aureus* (7), *Salmonella* spp. (3) were grown in Nutraceutical Universal Enrichment (NUB) broth at 35 ± 2°C for 24-28 h, while for the exclusivity non-target strains were grown for 24 h in non-selective broth and tested on NP. For analytical sensitivity, *N* = 3 *target* strains were individually grown in NUB for 24 h at 35 ± 2°C then evaluated with NP. For matrix verification samples, eight diverse categories: dehydrated egg powder (5), vitamins (1), fruit drink powders and extracts (5), organics (e.g., Kratom powder) (5), plant extracts (4), probiotics (5), and protein supplements (10) involving total 35 matrices at 10 g size were tested to determine the compatibility with NP. Each matrix was spiked with ≤800 CFU per test portion of each organism and enriched in NUB at 1:10 at 35 ± 2C ° for 24-28 h and tested on NP. All presumptive results were confirmed by culture-based methods.

Results: Inclusivity was 100%, and no exclusivity strains were detected. The analytical sensitivity of target strains was 10^3- 10^4 CFU/mL. The matrix verification trial indicated the NP sensitivity, specificity and overall accuracy to be 100%.

Significance: The challenge studies conducted here demonstrate the efficacy of NP-based pathogen detection for nutraceutical and dietary supplements matrices.

P2-81 High-Resolution Melt Assay for Detection of Virulent Lineages of Shiga Toxin-producing *Escherichia coli* O26 and O111

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Introduction: In 2016, 5,441 culture-confirm cases of Shiga toxin-producing *Escherichia coli* (STEC) infection were reported in the United States. Infection by STEC strains results in bloody diarrhea, hemolytic uremic syndrome, and renal failure. According to the CDC, national enteric disease surveillance report STEC-O26 and -O111 are responsible for 16% and 10.7% of STEC cases, respectively. The presence of avirulent O26 and O111 *E. coli* in red meat can result in product hold up and financial losses. Previously, single nucleotide polymorphisms (SNP) in O-antigen genes were associated with virulent STEC lineages.

Purpose: This study aimed to develop a real-time PCR assay that identified *E. coli* O26 and *E. coli* O111 while determining if they were of a virulent STEC lineage.

Methods: Serogroup-specific primers targeting the O26 *fnl*1 and O111 *wbd*K genes were designed. Two high-resolution melting real-time PCR assays

were developed for specific identification of O26 and O111 serogroups and differentiation of virulent STEC lineages. The assay was validated using DNA from 101 bacterial strains, DNA samples from a federal regulatory surveillance program, and inoculated beef and spinach samples.

Results: Serogroup-specific O26 and O111 primers showed 100% specificity. Virulent STEC-O26 and O111 formed distinct melt profiles in the normalized and differential melting curves plots from those of avirulent *E. coli* O26 and O111 strains. Overall, the O26 and O111 HRM assays showed greater than 90% sensitivity and specificity. The assays were able to accurately detect all inoculated strains following a 15-h enrichment period.

Significance: The assay developed in this study specifically identifies O26 and O111 serogroups and differentiates the positive results into virulent STEC and avirulent *E. coli* lineages. It can be used to reduce the number of potential positive results caused by the presence of mixed avirulent *E. coli* in red meat.

P2-82 Localized Surface Plasmon Resonance Biosensor Based on Polydopamine Molecular Imprinted Polymer for Detection of Multi-Antibiotics in Chicken Meat: Assay Optimization Process and Comparative Study with HPLC

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Introduction: Antibiotics have been widely used in animal food industry since the early 1940s, but have come under consumer criticism due to the potential for antibiotic residues and the development of bacterial resistance. Different countries or organizations have established various maximum residue limits as acceptable levels, and therefore, analytical methods with a low limit of detection (LOD) are critical for identification and quantification of antibiotic residuals for the stewardship of antibiotics.

Purpose: The objective of this project is to develop a localized surface plasmon resonance (LSPR) biosensing system for rapid, sensitive and selective detection of multi-antibiotics in chicken meat, using polydopamine molecular imprinted polymer (PDA-MIP) as the recognition element.

Methods: Detection targets of enrofloxacin, tetracycline and phthalic acid were used as templates, and the PDA-MIP film was fabricated by polymerization of dopamine and purified powder of target antibiotics in Tris buffered saline on a LSPR sensor chip. After removal of templates, the modified LSPR/PDA-MIP biosensor was used for detection of each target analyte at 6 concentrations in the range of 0 to 500 ng/mL. In order to amplify the detection signal, competitors conjugated with bovine serum albumin were injected and adsorbed by the residual binding sites on the PDA-MIP film.

Results: With amplification, the proposed method allowed a detection time of 20 min and LODs of 66.3, 3.7 and 71.7 ng/mL for target analytes, respectively. During the optimization process, parameters including template concentration and polymerization time were adjusted to increase the sensitivity of detection. Three spiked concentrations in chicken meat samples were compared with recoveries obtained by HPLC detection method.

Significance: The developed LSPR/PDA-MIP biosensing method reduced the detection time compared with most reported analytical methods and showed high potential for rapid and sensitive in-field detection of multi-antibiotic residues, which agreed well with the results of HPLC method.

P2-83 Comparison of Two Bacteriophage-based Rapid Assays for the Detection of *Salmonella* spp.

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Introduction: *Salmonella* spp. are a group of Gram-negative foodborne pathogens that cause approximately 87,500 foodborne cases of salmonellosis annually in Canada. Rapid, sensitive, and specific detection methods are required to ensure that foods are produced according to good manufacturing and Hazard Analysis Critical Control Point guidelines. Bacteriophages continue to gain interest as diagnostic reagents due to their specificity for their bacterial hosts.

Purpose: To evaluate and compare the diagnostic specificity and sensitivity of two bacteriophage-based rapid detection assays, the PhageDx™ *Salmonella* Assay (based on luciferase reporter bacteriophages) and the VIDAS® UP *Salmonella* Phage Technology (SPT) assay (based on recombinant phage proteins).

Methods: A total of 233 *Salmonella* isolates from 56 serovars were analyzed to determine sensitivity. A total of 151 non-*Salmonella* bacteria belonging to closely related genera (*Escherichia*, *Citrobacter*, *Klebsiella, Cronobacter*) were used to determine specificity. All isolates were streaked onto the tryptone soya agar (TSA) plates from frozen culture, and after overnight incubation at 37°C, individual colonies were inoculated into tubes containing 10 mL tryptic soy broth (TSB) for 24h ± 1 h at 37°C, with shaking at 200 rpm. The overnight cultures were tested in both assays according to manufacturers' directions. Plate counts on Brilliance™ *Salmonella* Agar were used to determine the analytical sensitivity of the PhageDx™ assay.

Results: The VIDAS® UP SPT assay detected 232/233 (99.6%) *Salmonella* isolates tested. The PhageDx™ assay identified all 233 (100%) of the isolates tested. Of the 233 isolates, the PhageDx™ assay detected 217 (93%) at a direct detection limit of 10^1 to 10^2 CFU/mL. Overnight cultures of the remaining 16 isolates were all detected. The false-positive rate of VIDAS® UP SPT assay is 2.7% (4/151), that of PhageDx™ assay is 3.3% (5/151).

Significance: Both assays demonstrated high accuracy, indicating their usefulness for rapid, specific, and sensitive detection of *Salmonella* spp.

P2-84 Evaluation of a Real-Time PCR Assay for Rapid Detection of *Listeria monocytogenes* in Artificially Contaminated Soft Cheese and Environmental Surface Samples

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Introduction: *Listeria monocytogenes* is an opportunistic foodborne pathogen that can be associated with a variety of foods and environments. Real-time PCR (qPCR) allows rapid and accurate screening of this foodborne pathogen.

Purpose: A previously developed qPCR method was evaluated for a rapid screening of *L. monocytogenes* from cheese and environmental samples and compared with the current Food and Drug Administration's (FDA) Bacteriological Analytical Manual (BAM).

Methods: The evaluation follows FDA and AOAC microbial method validation guidelines. Mexican-style soft cheese and environmental surfaces were artificially inoculated with low levels of *L. monocytogenes* to yield fractional positive results among 20 test portions, in addition to positive controls and uninoculated controls. After a 48-h enrichment described in the FDA BAM, selective agar plates were used for qualitative detection. The 48 h-enriched test portions were also used for DNA extraction on a MagMax/Kingfisher apparatus that is designed to process 96 samples per run. Two manual DNA extraction methods were also evaluated. A real-time PCR that can simultaneously detect *Listeria* spp. and *L. monocytogenes* was performed on the Applied Biosystems 7500 FAST instrument. This qPCR scheme employs an internal positive control.

Results: qPCR screening and BAM cultural scheme performed equivalently by generating 100% consistent results in soft cheeses and environmental surfaces. Fractional positive results were achieved in 6 inoculated environmental and 14 inoculated soft cheese test portions. Positive controls were all positive and uninoculated controls were all negative as well.

Significance: This detection method based on BAM enrichment and qPCR provides highly sensitive screening for *L. monocytogenes* in artificially contaminated soft cheese and environmental samples. Evaluation and further validation of this method help to establish an improved *L. monocytogenes* surveillance strategy with considerably reduced time and costs.

P2-85 Development of Selective Agar Media to Improve *Campylobacter jejuni* Detection in Food

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Introduction: *Campylobacter jejuni* is difficult to isolate from food because of competing with other bacteria and viable but non-culturable *C. jejuni* cells. Thus, selective media are developed by adding antibiotics. However, the number of antibiotic-resistant bacteria is increasing, and thus, the selectivity of the media is decreased.
Purpose: The objective of this study was to develop selective agar media to improve the detection of *C. jejuni* in food.
Methods: *C. jejuni* was inoculated in Bolton broth and chicken tenders. The pathogen was enumerated on modified charcoal-cefoperazone-deoxycholate agar (mCCDA), *Campylobacter* selective agar (CSA) supplemented with 4 µM catalase (CSA-C4), 8 µM catalase (CSA-C8), 20 mM L-serine (CSA-S20) or 50 mM L-serine (CSA-S50), and mCCDA supplemented with 0.5 mM L-cysteine (mCCDA-LC0.5), 1 mM L-cysteine (mCCDA-LC1), 40 µM quercetin (mCCDA-Q40) or 320 µM quercetin (mCCDA-Q320). The detection efficiency was then evaluated by counting colonies on the selective agar media. To evaluate the efficiency of these selective agar media in food, *C. jejuni* in chicken and duck samples were detected with the developed media.
Results: The detection efficiency of *C. jejuni* was high ($P < 0.05$) in the CSA-C4 or CSA-C8 and CSA-S20 or CSA-S50, and the detection efficiency was stable even in the presence of competing bacteria such as *Acinetobacter baumannii.* In the application for chicken and duck samples, CSA-C8 and CSA-S50 demonstrated higher detection efficiencies than mCCDA.
Significance: This result indicates that CSA-C8 or CSA-S50 improve the detection efficiency of *C. jejuni* in poultry carcasses.

P2-86 Intermediate Thermoresistance in Black Yeast Asexual Cells Variably Increases with Culture Age, Promoting Survival and Spoilage in Thermally Processed Shelf-Stable Foods

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❖ ***Developing Scientist Entrant***

Introduction: Black yeasts are characterized by several intrinsic stress resistance traits that promote surface colonization, and have caused spoilage in cold-filled and hot-filled beverages as well as other preserved food products.
Purpose: We established quantitative thermoresistance parameters for the inactivation of 12 total *Aureobasidium* and *Exophiala* isolates through isothermal experiments and a challenge study.
Methods: A collection of black yeast isolates belonging to the genera *Aureobasidium* ($n = 9$) and *Exophiala* ($n = 3$) were assembled. Fungal inocula were heat treated in a temperature-controlled water bath with an independent temperature indicator for treatment, then immediately removed and transferred to an ice-chilled ethanol bath to halt the treatment. A three-strain cocktail of *Exophiala* isolates was used to inoculate hot-filled food products to assess survival under food processing-relevant conditions for the challenge study.
Results: Culture age (2-day vs. 28-day) variably affected the thermoresistance among the black yeast strains. Variation in thermoresistance exists within each genera, but the two most resistant strains were *Exophiala* isolates. While these thermoresistance levels were, in some cases, greater than those for vegetative cells from other common food spoilage fungi, they were more sensitive than ascospores of heat resistant molds (HRM) most associated with spoilage of hot-filled products. A hot-fill challenge study was performed in an apple cider and maple syrup. When apple cider was hot-filled at 82°C, black yeast counts were reduced by 4.1 log CFU/mL 24 h after the heat treatment, but the survivors increased up to 6.7 log CFU/mL after two weeks. In comparison, the counts were below the detection limit after both 24 h and 14 days of shelf life in both apple cider and maple syrup when filled at their boiling points.
Significance: This intermediate degree of thermoresistance may support survival following introduction after hot-fill during active cooling before package seals have completely formed. Ensuring the microbial quality of water in cooling tunnels may be essential in mitigating the introduction of these fungi.

P2-87 Kerry's Citrapure® Citrus Extract Technology is Effective at Inhibiting Microbial Spoilage

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Introduction: Increased popularity of cleaner labels have resulted in food/beverage manufacturers looking for potassium sorbate alternatives to inhibit spoilage.
Purpose: This study investigated the effectiveness of a natural citrus extract technology at inhibiting yeast growth (*Zygosaccharomyces bailii, Zygosaccharomyces rouxii, Candida tropicalis, Debaryomyces hansenii, Pichia membranifaciens, Pichia orentalis, Saccharomyces cerevisiae*) in six refreshing beverages (four still, two carbonated) (pH 2.70-4.71, 0.45-8.30°Brix) and one sauce (pH 6.20, 70°Brix).
Methods: Control and test samples of beverages and sauce were inoculated with 3-log CFU/g yeast cocktail. Control samples contained no preservative, while test samples contained sensory acceptable levels of citrus extract (refreshing beverages: 0.02-0.10%, sauce: 0.14%). An additional 0.10% sorbate-positive control was prepared for the sauce samples. Samples were aseptically portioned in sterile tubes and stored at 25°C. The still beverages were assayed daily for changes in populations of yeast by pour-plating on acidified PDA for 4 days, carbonated beverages were assayed weekly for 28 days, and the sauce was assayed biweekly for 96 days. Samples were tested in triplicate.
Results: The addition of citrus extract significantly inhibited yeast growth in both the refreshing beverages and sauces compared to the control ($P < 0.05$). Yeast outgrowth (> 2 log CFU/g) was observed for control refreshing beverages by the end of testing, while test samples showed a cidal impact against yeast by the second testing timepoint (still – day 1, carbonated – day 7). The sauce's no preservative and sorbate samples supported >1-log CFU/g yeast growth after 14 days, while the test samples had a >2-log CFU/g yeast reduction after 14 days and remained below detection limit for 96 days (detection limit: <1.48 log CFU/g).
Significance: The findings demonstrate the effectiveness of this citrus extract against yeast growth in different food/beverage matrices. The cidal impact of yeast highlights this solution could also be a practical hurdle to implement against microbial contamination in open shelf life.

P2-88 Spoilage Potential of Biofilms and Planktonic Cells of *Bacillus subtilis* and *Bacillus velezensis* in Extended Shelf Life Milk

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❖ ***Developing Scientist Entrant***

Introduction: Thermophilic bacilli pose a threat in the dairy industry because of their inherent ability to survive pasteurization either as planktonic cells or process biofilms. This contamination results in the spoilage of extended shelf life (ESL) milk due to the production of proteolytic and lipolytic enzymes by the bacteria.
Purpose: In this study, we aimed to quantify and compare biofilm-induced proteolysis and lipolysis of *B. subtilis* and *B. velezensis* with that of the planktonic cells.
Methods: To grow the cells, 0.5 mL and 0.1 mL of pre-incubated bacterial inocula were added to a centrifuge tube containing 4.5 mL of UHT for the biofilms and planktonic cells, respectively. A stainless-steel coupon was placed in each of the centrifuge tubes containing the inoculated UHT milk as a substratum for biofilm formation except for planktonic cells. All the tubes were incubated for 24 h at 30°C. Both planktonic and biofilm cells were stained and

enumerated in a flow cytometer after incubation. The concentrations of proteolytic and lipolytic enzymes produced by the submerged biofilm and planktonic cells of the isolates were quantified using azocasein and p-nitrophenol palmitate (p-NPP) assays, respectively. All experiments were done in triplicate.

Results: In the planktonic cells, sample B48 (B. subtilis NCIB 3610) has the highest proteolysis with 1033.6 ρL/CFU while B50 (B. subtilis ATCC 11774) has the highest lipolysis of 34.5 ρL/CFU. For the biofilms, B168 (B. subtilis ATCC 168) has the highest proteolysis and lipolysis per cell with a mean of 3706 ρL and 179.9 ρL. The result of this study indicated that the spoilage potential (proteolysis and lipolysis) both of biofilms and planktonic culture are strain dependent, and that there seems to be a relationship between the strength or complexity of the biofilms and spoilage potential of the isolates.

Significance: The implication to the industry is that weak biofilm formers have better spoilage potential than strong biofilm formers in spore-forming bacilli.

P2-89 *Listeria* Control in Plant Protein-based Foods

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Introduction: Plant-based meat was one of the trends in food that went skyrocket in 2020. This food type is, like normal meat, exposed to a microflora. When stored refrigerated this food type will generally show the same safety issues as other cold stored foods. The refrigerated environment will select for *Listeria*, an opportunistic pathogen which is associated with over 1,000 illnesses with a relative high mortality rate, each year in the US (ref. CDC). In this report it is shown that *Listeria* can be controlled in plant protein-based products using a vinegar-based preservative.

Purpose: Show the inhibitory properties of ProNiaturèl Plus, a sodium-free, dry blend based on calcium and potassium neutralized vinegar.

Methods: Three treatments of a plant protein model application (82% cooked chickpeas, 0.1% Garlic powder, 1% NaCl, 3% lemon juice, water 13.25-13.9% and pH 6.2-6.4; a_w 0.977-0.982) included a control without antimicrobials and different concentrations of ProNiaturèl Plus (0.5% and 0.75%). The fresh application was prepared by mashing and mixing and inoculated with 3-4 log CFU/g *Listeria inoccua* (NCCB 100510). The inoculated product was vacuum-packed in portions of approximately 30 g in sterile plastic bags and stored at 4 and 7°C up to 45 days. At each time of sampling during storage *Listeria* were enumerated in triplicate by surface plating on PALCAM agar. $N = 3$ was done for all treatments and all samplings and ANOVA was used to determine statistical significance.

Results: Growth of *L*isteria to 7-8 log CFU/g was observed during 45 days on the control treatment at both 4°C and 7°C, with an average increase of, respectively, 1 and 1.5 log CFU/g per week. Other treatments (both, 0.75% and 0.5% ProNiaturèl) showed significant ($P < 0.01$) reduced growth of *Listeria.* 0.75% ProNiaturèl Plus reduced growth to less than a 2 log CFU/g increase in concentrations during 45 days at 4°C and 7°C.

Significance: This research demonstrated the possibility to increase safety of Plant protein-based food using preservatives that meet current food trends, like sodium reduction and natural origin.

P2-90 Effect of Lactic Acid on Shelf Life of Fresh Crawfish Tail Meat

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✦ ***Undergraduate Student Award Entrant***

Introduction: Louisiana is the main crawfish producer in the US. In recent years, the crawfish market has been growing from a local to regional and national level. Under current processing conditions, fresh crawfish tail meat has a shelf life of six days. Lactic acid has been recognized for its antimicrobial properties on a wide spectrum of microorganisms and is currently approved by the FDA to use on crustaceans.

Purpose: The purpose of this study was to determine the effect of lactic acid on the shelf life of fresh crawfish tail meat.

Methods: Crawfish was steamed and treated using 0, 1, and 2% lactic acid at the chilling step, right before peeling. Samples were collected and analyzed for microbiological and physical/chemical changes for a period of 14 days.

Results: The application of 1% lactic acid on crawfish tail meat had a significant effect on the microbial quality of the tail meal ($P < 0.5$). A threshold of Aerobic Plate Count (APC) of 6 log CFU/g was set for acceptance. Control group reached 6.40 ± 0.26 log CFU/g at day 12, while 2% lactic acid reached 6.31 ± 0.12 log CFU/g at day 14. The 1% lactic acid group only reached 5.70 ± 0.45 log CFU/g at day 14. No significant difference was found in texture, lipid oxidation, color, pH or moisture content ($P > 0.5$).

Significance: The intervention with lactic acid during the chilling step extends shelf life of fresh crawfish tail meat allowing for processors to reach larger markets at the regional and national level.

P2-91 Impact of Polysaccharide Incorporated Ice on Microbial Load of Catfish Fillets during Chilled Storage

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❖ ***Developing Scientist Entrant***

Introduction: Channel catfish (*Ictalurus punctatus*) is a Southern classic found on menus across the nation. Due to COVID-19, the catfish industry has seen a change in supply-demand from frozen catfish sold to restaurants to fresh catfish sold to retail grocery stores. Therefore, catfish shelf life is expected to last longer post-processing (from processor, to grocery store, to consumer preparation at home). To decrease post-processing food waste and increase the shelf life of catfish, a natural preservative possessing antimicrobial properties should be applied. Chitosan, a natural polysaccharide, from the exoskeletons of crustaceans is nontoxic and has antimicrobial, antifungal, and film-forming properties.

Purpose: The objective of this study was to investigate the efficacy of chitosan ice (CHI) and electrostatic sprayed chitosan ice (ESI) on the microbial load of catfish fillets and runoff during chilled storage.

Methods: Catfish was purchased from a local supermarket and randomly separated into different ice treatments. The fillets were placed on CHI, ESI, or a Control (non-treated) ice. The catfish was refrigerated on ice for 7 days at 4°C. The fillets and runoff, from melted ice, were sampled intermittently for microbial analysis.

Results: Catfish was considered spoiled at approximately 6 log CFU/g. On Day 3, Control samples had counts of 5.68 log/CFU, considered spoiled. At Day 7, the end of the study, the CHI and ESI treated fillets had counts of 3.35 log/CFU and 2.67 log/CFU, respectively, compared to the control counts of 7.81 log/CFU. The use of the CHI significantly ($P < 0.05$) reduced the bacterial load on the fillets over time, thereby extending the shelf life by at least 3 days. ESI runoff was significantly ($P < 0.05$) more effective at inhibiting microbial growth.

Significance: The application of natural preservatives, specifically chitosan incorporated into ice on fresh fish storage has the potential to extend shelf life and would be applicable to the catfish industry.

P2-92 Withdrawn

P2-93 The Cantaloupe Farm Environment Has a Diverse Genetic Pool of Antibiotic-Resistance Genes

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Introduction: Intra (inARG) and extracellular antibiotic-resistance (exARG) genes in the cantaloupe farm environment may spread throughout the food chain.

Purpose: The goal of this study was to assess the distribution of intra and extracellular antibiotic-resistance, and mobile genetic element genes in the cantaloupe farm environment.

Methods: A total of 200 samples from cantaloupe (*N* = 99), farm workers' hands (*N* = 66), and production water (*N* = 35) were collected in México in 2017. Each sample was assayed for the presence of 13 antibiotic-resistance genes (ß-lactams: *blaCARB-4* and *blaSHV*; tetracyclines: *tetA* and *tetB*; macrolides: *ermB* and *ermF*; sulfonamides: *sul1*; quinolones: *qnrA*; polymyxins: *mcr1*; and glycopeptides: *vanB*), and 3 mobile genetic elements (integrons: *intI* and *intII*; and a plasmid incompatibility group: *oriV*) by PCR, in the intra and/or extracellular matrix. The relationship between gene presence and sample type was evaluated using logistic regression.

Results: ARG of tetracyclines (18% in cantaloupe and 45% in workers' hands), and sulfonamide (30% in cantaloupe and 71% in workers' hands) were the most frequently found. A similar trend was detected when exARGs were analyzed. ARGs of tetracyclines were in 25% of cantaloupe samples; sulfonamides were in 21.2% of workers' hands. Furthermore, the colistin resistance gene (*mcr1*) was detected in 10% of cantaloupe and 23% of workers' hands in the inARGs, and 2% of cantaloupes and 13.8% of workers' hands in the exARGs samples. There was a significantly higher likelihood ($P < 0.05$) of detecting antibiotic-resistance genes on workers' hands, but not cantaloupe or water samples, in both inARG and exARG.

Significance: These results indicate that inARG and exARGs were detected in the cantaloupe farm environment. Because these genes could transfer antibiotic-resistance among the bacteriome, it is important to reduce this risk by pursuing better agricultural practices.

P2-94 Prevalence and Antibiotic Resistance of Pathogenic *E. coli* in Dairy Farm

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❖ ***Developing Scientist Entrant***

Introduction: Pathogenic *Escherichia coli* is a constant public health concern and frequently reported to be associated with outbreaks. The environment is the largest reservoir for all types of *E. coli* including the pathogenic ones which can potentially be transmitted to humans via food chain.

Purpose: Estimating the prevalence and antibiotic-resistant pathogenic *E. coli* strains in conventional dairy farm environments.

Methods: A total of 554 samples were collected between June-August 2019 from 3 dairy farms in Maryland, USA: including water, feed, feces, bedding, soil, and compost. Each sample was processed and enriched by incubating at 37°C for 24 h in LB broth supplemented with 5% sheep blood. About 10 µL of the enriched culture was streaked on sorbitol MacConkey (sMac) agar to selectively isolate pathogenic *E. coli* strains which were further confirmed by PCR. Later, the antibiotic resistance of pathogenic *E. coli* isolates was evaluated to a selected pool of antibiotics using standard agar dilution method recommended by Clinical and Laboratory Standards Institute (CLSI). Fisher's exact test and Cochran-Mantel-Haenszel (CMH) test were used to analyze statistical significance.

Results: The overall prevalence of pathogenic *E. coli* in the dairy farm was 7.76% (43/554) ($P < 0.001$). A total of 63 pathogenic serotypes of *E. coli* including EIEC, STEC, EAEC, and EPEC was confirmed. The highest prevalence of pathogenic *E. coli* was found to be in soil (18.34%); feed and feces (11.35% and 11.95%), bedding (10%), and water (6.25%). The overall prevalence of multiple antibiotic resistance in the confirmed serotypes was estimated 88.89% whereas only 4.76% of serotypes were sensitive to all tested antibiotics. Among the isolated pathogenic serotypes, observed antibiotic resistance pattern was EIEC (47.62%), STEC (25.40%), EAEC (12.70%), and EPEC (3.17%), respectively ($P < 0.001$).

Significance: Pathogenic and antibiotic-resistant *E. coli* serotypes are present in conventional dairy farms and warrant proper steps to control its transmission.

P2-95 Current and Aggregative Pre-Harvest Sampling Comparison in Commercial Fields

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❖ ***Developing Scientist Entrant***

Introduction: Current pre-harvest composite grab sampling practices yield few positive results because contamination typically occurs at low levels. Novel aggregative and non-destructive sampling methods have been developed for food safety testing in the meat industry.

Purpose: This study compares current sampling strategies to aggregative swab sampling by evaluating their efficacy in detecting organisms of interest in experimental trials in commercial plots.

Methods: Two trials were performed in one-acre fields in California. The fields were planted with Romaine lettuce, which was artificially inoculated with a cocktail of *E. coli.* The fields were divided into plots with inoculated (via spraying 7 log CFU/mL) and non-inoculated sections. Both sections were subdivided to perform (1) stratified random (STRS) and systematic (SS) composite tissue sampling, and (2) aggregative swabs (MT) within and across sections in each plot. Sampling was performed 0, 2 and 5 days following inoculation. The samples collected for the first trial consisted of 7 MT, 2 STRS, 12 SS, and 12 MT, 6 STRS, and 8 SS for the second. They were subject to culture-based testing for *E. coli* presence/absence, Coliforms (CC) and Aerobic Plate Counts (APC) and compared regarding inoculum detection and concentration recovered.

Results: In the first trial, the inoculum was detected in 2 SS and 4 MT samples. MT recovered 8.39 log CFU/g in APC, and 5.51 log CFU/g in CC, while tissue samples recovered 7.84 log CFU/g and 4.14 log CFU/g in APC and CC, respectively. In the second trial, the inoculum was detected in 1 STRS, 2 SS, and 8 MT samples. Both swabs and tissue samples recovered concentrations above countable ranges for APC while recovering 2.65 log CFU/g and 2.38 log CFU/g of CC for swabs and tissue samples, respectively.

Significance: This study suggests that aggregative swab sampling has similar efficacy in detecting hazards as conventional tissue sampling in fresh produce production.

Poster

P2-96 Prevalence of *Salmonella* in Integrated Crop-Livestock and Dairy Farms in Maryland–Washington D.C.

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❖ ***Developing Scientist Entrant***

Introduction: Increasing popularity of antibiotic-free organic farming has slowed antibiotic resistance patterns in bacteria but has placed them at risk of increased prevalence of zoonotic pathogens. Farms in Maryland-Washington DC area are commonly organic pasture dairy farms and integrated crop-livestock farms (ICLF). *Salmonella* is one of the most prevalent zoonotic pathogens in the US because of its ubiquitous nature, making it an important etiological agent found in multiple niches within farms and food products at pre-harvest.

Purpose: Determine the prevalence of *Salmonella* in different farm components and products from dairy farms and ICLFs at pre-harvest.

Methods: Multiple components from dairy farms and ICLFs, including water, feed, animal feces, bedding, soil, manure and compost, as well as pre-harvest produce from of ICLFs were collected (total sample *n* = 1,152) and used to isolate *Salmonella* using LB broth enriched with 10% sheep blood and sub-cultured on XLD agar. Presumptive positive colonies were confirmed through PCR with genus-specific primers for confirmation.

Results: Presumptive positive *Salmonella* isolates were found in both dairy farms (70/359) and ICLFs (114/783). Multiplex colony PCR was used to amplify genus specific *acek* and/or *oriC* in presumptive positive *Salmonella* isolates. Positive isolates were confirmed in both dairy farms (21/359) and ICLFs (43/783), though resulting in a lower count than initially calculated and no statistically significant difference between farm models. Confirmed isolates from dairy farms were predominantly distributed between bedding, feces and feed, accounting for 9.52%, 38.10% and 52.38% of confirmed samples, respectively, and was the farm model with slightly higher confirmation to sample ratio. From ICLFs confirmed samples were found primarily distributed across bedding (2.32%), water (2.32%), feed (6.98%), compost (13.95%), soil (23.26%), feces (23.26%) and produce (27.9%).

Significance: Monitoring *Salmonella* in organic farms at the pre-harvest level can improve farming practices that ultimately result in safer products and reduced foodborne infections.

P2-97 *Salmonella* in Environmental Samples Differed between Virginia Produce Growing Regions and Was Associated with Land-Use and Weather Factors

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne pathogen contamination risk is not uniform within produce growing environments; thus, mitigating contamination risks of preharvest produce is challenging.

Purpose: The goal was to identify geospatial and meteorological factors associated with *Salmonella* isolation in soil, drag swab, and water samples from produce farms.

Methods: Soil (*N* = 400), drag swab (*N* = 400), and water (*N* = 120) samples were longitudinally collected from ten produce farms in Virginia. Samples were tested for *Salmonella* using a modified FDA-BAM method. Presumptive positives were confirmed by *inv*A PCR, and one isolate per sample was serotyped. For each sample, metadata on weather and land use factors were also collected. Conditional forests and Bayesian mixed models were used to characterize the relationship between geospatial (e.g., region, land use) and weather (e.g., rainfall, humidity), and *Salmonella* isolation. Surrogate trees were used to visualize forest results.

Results: *Salmonella* was isolated from 5.3% (49/920) of samples, with the majority coming from the Eastern Shore Virginia (46/49). Summer (18/230) yielded the most *Salmonella* positive samples, followed by spring (14/230), fall (12/230), and winter (5/230). There were 14 different *Salmonella* serovars with 33% yielding serovar Newport. Based on conditional forest analysis, sample type and region were most strongly associated with *Salmonella* isolation. Specifically, based on the mixed models, likelihood of detection was significantly higher in water compared to terrestrial samples (Odds Ratio = 6.5, 89% Credibility Interval = 3.3, 14.9). According to the surrogate tree the likelihood of isolating *Salmonella* was highest from water samples ($P < 0.001$) collected from the Eastern Shore ($P < 0.001$) when dewpoint was above 9.4°C ($P < 0.001$).

Significance: These findings identify factors that increased the likelihood of isolating *Salmonella* in preharvest environments, and are helpful in development of control strategies within small scale growing regions for specific scenarios to minimize produce contamination.

P2-98 Seasonality Drives the Likelihood of Isolating *Listeria monocytogenes* from Field and Water Samples Collected from Virginia Produce Farms

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❖ ***Developing Scientist Entrant***

Introduction: The heterogeneity of farm environments complicates the development of strategies for managing preharvest produce safety risks. Thus, understanding pathogen ecology in farm environments is key to the development of effective mitigation strategies.

Purpose: To identify spatio-temporal factors associated with *Listeria monocytogenes* and nonpathogenic *Listeria* isolation from soil, drag swab, and water samples collected in produce preharvest environments.

Methods: Soil (*N* = 400), drag swab (*N* = 400), and water (*N* = 120) samples were collected from ten Virginia farms. All samples were tested for *Listeria,* with presumptive isolates being confirmed to species-level by *sig*B gene partial sequencing. Conditional forest analysis and Bayesian mixed models were used to identify associations between spatial (e.g., growing region) and temporal (e.g., weather) factors and likelihood of *L. monocytogenes*, and nonpathogenic *Listeria* isolation. Surrogate trees were used to visualize hierarchical associations identified in the forests.

Results: According to the mixed models and surrogate trees, the likelihood of isolating *L. monocytogenes* and nonpathogenic *Listeria* was significantly higher in samples collected in winter, compared to fall, spring or summer ($P < 0.001$*). L. monocytogenes* was most likely to be isolated from water samples, regardless of source, ($P < 0.001$) collected in winter ($P < 0.001$) from sites where <36% of land was within 122 m of forest or wetland cover ($P < 0.001$). Conversely, *L. monocytogenes* was least likely to be detected in soil samples, and water samples collected from streams (compared to ponds; $P < 0.001$) in fall, spring, or summer ($P < 0.001$) when air temperature 0-3 d before sampling was >6°C ($P < 0.001$). Nonpathogenic *Listeria* were most likely to be isolated from drag swabs samples ($P < 0.001$) collected in winter ($P < 0.001$) when no rainfall occurred 1-2 d before sample collection ($P < 0.001$).

Significance: These findings suggest there is a low likelihood of *Listeria* contamination in soil and water on VA farms during the growing season but that likelihood of contamination can be increased depending on environmental conditions.

Poster

P2-99 Prevalence and Characterization of *Salmonella* spp. Isolated from Fresh Produce and Agricultural Environment in Korea

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❖ ***Developing Scientist Entrant***

Introduction: The prevalence of antibiotic-resistant *Salmonella* associated with fresh produces has recently increased due to the interaction of agricultural soil and manure. Although previous studies have already documented the prevalence of *Salmonella* in fresh produce, the studies on the association of antibiotic-resistant *Salmonella* with fresh produce are underestimated.

Purpose: Hence, the purpose of this study was to isolate *Salmonella* from fresh produce and environment samples and to investigate their characteristics in the aspect of the pathogenicity and antibiotic resistance.

Methods: Two hundred fresh produce and environmental samples homogenized in PBS buffer were spread on MacConkey and XLD agar plates for *Salmonella* isolation. IMViC test was then performed for the confirmation of presumable *Salmonella* prior to 16S rRNA sequencing. Presence of virulence genes (*inv*A, *stn*, *fim*A, *spv*R, and *spv*C genes) of *Salmonella* confirmed by 16S rRNA sequencing was investigated using conventional PCR. Antibiotic resistance of the confirmed *Salmonella* was investigated by a disc diffusion assay against 22 antibiotics. A minimum inhibitory concentration for each confirmed *Salmonella* was determined by an E-test method.

Results: From the selective media, thirty colonies were isolated. Ten out of thirty isolated colonies were presumed as *Salmonella*. Among ten presumable *Salmonella*, 5 strains of *S*. Typhimurium (>99.72% identity), three strains of *S*. Montevideo (>99.72% identity) and two strains of *S*. Saintpaul (99.93% identity) were confirmed. The *inv*A and *fim*A gene were detected in ten confirmed *Salmonella* strains. Among them, five *Salmonella* strains contained *stn* gene also. Furthermore, eight strains of *Salmonella* were resistant to at least one antibiotic. Among them, four *Salmonella* strains were resistant to ampicillin, streptomycin, sulfisoxazole, and tetracycline. Overall, eight *Salmonella* strains were confirmed to be pathogenic and antibiotic resistant.

Significance: This study gives an insight to the need for continuous monitoring the prevalence of antibiotic-resistant *Salmonella* in fresh produce and environment for ensuring the safety of fresh produces.

P2-100 Prevalence of *Listeria* Species in Environmental Samples Collected from Urban Farms Along a North–South Gradient in the Eastern USA

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Introduction: While urban agriculture has grown ~30% in the last three decades, there are limited data on the prevalence of foodborne pathogens in urban farm environments.

Purpose: A cross-sectional study was performed to characterize the (i) prevalence of *Listeria* species in urban farm environments, and (ii) food safety and horticultural practices used by urban growers.

Methods: Between July and November 2020, 38 urban farms in six states along the US East coast were sampled. At each sampling, a semi-structured interview was conducted to characterize management practices. Subsurface soil (25 g; *N* = 1,016) and drag swabs (*N* = 132) were collected from each farm, with the number being proportional to acreage. All samples were analyzed for *Listeria* presence, and presumptive *Listeria*-positive samples were confirmed by *sig*B PCR. Univariable analyses were conducted to compare the prevalence of farm management practices, and *Listeria* spp. between farms and growing regions (i.e., Northeast, Mid-Atlantic, and Southeast).

Results: *Listeria* prevalence was significantly lower in soil (18%), compared to drag samples (29.5%; *P = 0.*007). While *Listeria* prevalence was comparable in the Northeast (26%; 54/406) and Southeast (24%; 105/435), *Listeria* prevalence in the Mid-Atlantic was substantially lower (13%; 22/175). Furthermore, *Listeria* prevalence varied substantially by farm. Practices with food safety implications showed strong regional patterns. For example, the use of poultry litter and manure were largely restricted to Southeastern and Northeastern growers, respectively. More Northeastern growers (77%) reported receiving food safety training, compared to Southeastern (50%) and Mid-Atlantic (36%) growers. Stray cats were reported as an issue by almost all growers, regardless of region.

Significance: To our knowledge, this is one of the first surveys to investigate and compare the food safety management practices and *Listeria* prevalence across urban farms in multiple growing regions. Findings will facilitate the development of food safety best practices on urban farms.

P2-101 Organic Farming Practices Versus Conventional Production: Associated Pathogens and Food Safety Concerns in a Sustainable Development

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Introduction: Organic agriculture has been strongly enhanced in the last years, as a consequence of a great consumer demand, a great concern for environmental health and a constant effort for a sustainable development based on green economy.

Purpose: The aim of this work was to investigate the urgent food risk notifications and product recalls from the market associated with organic production in the last nine years in Europe.

Methods: We carried out a literature review of original research articles and systematic reviews that examined the incidence of foodborne pathogens in organic production, compared to conventional production. We used the RASFF portal for an interactive searchable online database to identify all official notifications (food recalls in EU countries) associated to organic food in the last nine years.

Results: Between 2012 and Mars 2021 a total of 118 notifications associated with organic food and pathogenic microorganisms were communicated in the European RASFF Portal. Ninety of them (76.3%) were classified as serious risk decision. *Salmonella enterica* was the predominant pathogen in organic food (92%, 83 out of 90 notifications), and nut products and seeds the main products concerned (50%, 45 out of 90 notifications). Other minor pathogens implicated were *Bacillus cereus* (3.3%), *Clostridium sulphite reducer*, and *Norovirus* (both of them with 2.2% of cases), in fruits and vegetables (3.3%), meat and meat products (other than poultry) (2.2%), and herbs and spices (1.1%). Regarding antimicrobial resistances, the published literature reported higher resistances in conventional production when pathogens like *Campylobacter*, *Salmonella* and *E. coli* are investigated in poultry, swine and dairy products.

Significance: In this work we highlight the potential risk of pathogenic microorganisms in organic production and we detected the most common implicated organic foods in product recalls in EU, all in the context of a global evolution towards the green economy.

P2-102 Development of Sanitizing Methods to Reduce *Listeria monocytogenes* Contamination in Radish, Melon and Carrots during Post-Harvest Washing

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Introduction: Contamination of soil-borne pathogen *Listeria monocytogenes* in packaged produce is a major concern. Post-harvest washing step removes field-acquired contamination such as dirt, chemicals, cell-exudates that support bacterial growth and reduce microbial load up to 1-2 logs. However, inadequate quality of washing water can potentially be a major source of cross-contamination.
Purpose: The purpose of this study was to investigate the efficiency of organic acid, ethanol and UV to eradicate *L. monocytogenes* contamination in agricultural products.
Methods: Organic acids (acetic acid, ascorbic acid, citric acid, malic acid, lactic acid and tartaric acid at concentrations of 1% and 3%), ultraviolet (UV at 15, 30 and 45 cm distance for 1, 5, 15 and 30 minutes) and ethanol (5%, 10%, 20%, 50% and 70%) were analyzed either alone or in combination to reduce *L. monocytogenes* population in radish, melon and carrot samples.
Results: In radish samples, 3% malic acid combined with UV at 30 cm for 1 minute significantly reduced (>4 log CFU/g) the population of *L. monocytogenes* (1.44 ± 0.5) compared to control sample (5.14 ± 0.09) without treatment. In the case of melon samples, exposure of UV at 30 cm for 1 minute combined with 3% malic acid (2.30 ± 0.01) or 3% lactic acid (2.73 ± 0.75) or 50% ethanol (2.30 ± 0.01) were effective against *L. monocytogenes* compared to control (5.10 ± 0.19). In carrot samples, 3% lactic acid combined with UV at 30 cm for 1 minute reduced *L. monocytogenes* population (4.48 ± 0.25) than in control sample (5.85 ± 0.08).
Significance: These results reveal that prevention method (sanitizer) which is effective for one crop is less effective for another crop indicating that effective prevention methods should be customized for each agricultural product to prevent pathogen cross contamination during the washing step in the production process.

P2-103 Pre-Harvest Biocontrol of *Listeria* and *Escherichia coli* O157 on Lettuce by Lactic Acid Bacteria

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Introduction: Recent outbreaks linked to the contaminated lettuce highlight the need for identifying effective natural approaches to improve produce safety at pre-harvest level.
Purpose: Efficacy of lactic acid bacteria (LAB) including *Lactococcus lactis, Lactobacillus plantarum, Lactobacillus johnsonii*, and *Lactobacillus acidophilus,* as pre-harvest biocontrol against *Listeria* and *E. coli* O157 on lettuce grown in field and growth chamber was investigated.
Methods: Field grown lettuce cultivars "Green Star" and "New Red Fire" were spray-inoculated with *Listeria innocua* or *E. coli* O157:H12 (5 log CFU/g) as non-pathogenic surrogates for *Listeria monocytogenes* and *E. coli* O157:H7, respectively, a week prior to harvest. Inoculated lettuce plants were sprayed with water (control) or a mixture of LAB (8 log CFU/g). On 0, 3, and 5 days-post-inoculation (dpi), four samples from each group (20 g/sample; $N = 144$) were collected and bacterial populations were determined by spiral plating on selective agars. The experiment was repeated in a growth chamber with "Green Star" lettuce using pathogenic *L. monocytogenes* and *E. coli* O157:H7.
Results: In field study, LAB significantly reduced *E. coli* O157:H12 by up to 2.5 log CFU/g on lettuce cultivars on 0 dpi as compared to the control. On 5 dpi, ~1 log CFU/g of *E. coli* O157:H12 were recovered from both LAB-treated lettuce cultivars compared to ~2 log CFU/g recovered from the control ($P < 0.05$). Similarly, LAB reduced ~1 log CFU/g of *L. innocua* populations on "Green Star" lettuce on 3 dpi ($P < 0.05$). Further, 1.5 and 1.0 log CFU/g reductions in *L. monocytogenes* populations were observed on lettuce grown in growth chamber following LAB treatment on 3 and 5 dpi, respectively ($P < 0.05$). LAB significantly reduced *E. coli* O157:H7 by 2.2 log CFU/g on 0 dpi.
Significance: LAB can be potentially used as pre-harvest biocontrol agents against *Listeria* and *E. coli* on lettuce.

P2-104 V. Application of Cinnamon Oil Nano-Emulsion in Inhibiting *Salmonella* spp. on Mungbean Seeds and Sprouts

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Introduction: Legumes and fresh produce are an increasingly important cause of outbreaks of foodborne diseases, such as salmonellosis, listeriosis, and others. These outbreaks have usually been linked to contamination of fresh produce during postharvest handling, shipping, or processing in circumstances that permitted bacterial multiplication. In the last decade, multiple outbreaks related to consumption of mungbean have been encountered throughout the world. Several antimicrobial treatments have been done to seeds and sprouts and chlorination is most popular among them.
Purpose: The purpose of the study is to evaluate the antimicrobial potential of cinnamon-oil nano-emulsion on mungbean seeds and sprouts against *Salmonella*.
Methods: Cinnamon-oil nanoemulsion was prepared by using Tween 80 as a surfactant. The antimicrobial activity of cinnamon-oil nanoemulsion was assessed by using three different strains of *Salmonella* using a micro broth dilution assay. Seeds and sprouts were artificially inoculated with *a Salmonella* cocktail and dried overnight. Following artificial inoculation, seeds and sprouts were treated with DI water as a control and 0.5% and 0.75% cinnamon oil-nanoemulsion for 60 seconds. Samples were collected at 0 h, 24 h, and 48 h and plated on XLD agar for *Salmonella* enumeration.
Results: The average diameter of nanoemulsion was 9.63 ± 0.3nm. The minimum inhibitory concentration (MIC) of cinnamon oil nanoemulsion for *Salmonella* strains was 0.039% v/v, and the minimum bactericidal concentration was 0.078% v/v. Compared to control, 0.50% and 0.75% nanoemulsion resulted in 2.00-log CFU/g and 2.25-log CFU/g reduction in mungbeans seeds, respectively. The sprouts did not show a significant amount of reduction compared to seeds; Future studies will be tested on sprouts with higher concentrations. Based on the results of the present study, cinnamon oil nanoemulsion can be utilized as an effective natural antimicrobial agent against *Salmonella* pathogens.
Significance: These data suggest that nanoemulsions of cinnamon oil may be effective as an antimicrobial treatment for mung beans and sprouts.

P2-105 Development of Plasma-based Decontamination Treatment for Hydroponic Nutrient Solution

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❖ ***Developing Scientist Entrant***

Introduction: Plasma is the fourth state of matter, achieved by adding energy to the gas phase. When air plasma is exposed to water it generates plasma-activated water (PAW), and when exposed to nutrient solution it generates plasma-activated nutrient solution (PANS). Controlled environment agriculture (CEA) farming may be susceptible to the spread of foodborne pathogens through its nutrient solution irrigation. PAW can successfully inactivate microorganisms in planktonic solutions, but limited research is available for PANS.
Purpose: We aim to validate the use of plasma treatment to maintain sanitization of nutrient solutions and compare the antimicrobial benefits of PANS to PAW.
Methods: Inactivation of *E. coli* DH5α in planktonic solution was performed in triplicate against six agents: water, chlorine (Cl, control), PAW (activation time: 5, 10, or 15 min), nutrient solution (NS), PANS (activation time: 5, 10, or 15 min), and chlorine-treated nutrient solution (NS+Cl), for two incubation times: 5 or 10 min. Enumerated bacterial counts were compared using Kruskal-Wallis nonparametric test. PAW and PANS were chemically characterized for pH, oxidation-reduction potential (ORP), and the concentration of NO_3^-, NO_2^-, and plant growth nutrients.

Results: Treatments of Cl, PAW-10, PAW-15, PANS-10, and PANS-15 significantly ($P < 0.05$) inactivated *E. coli* by >8.5 ± 0.1 log CFU/mL. Chemical characterization of PAW and PANS showed similar trends as a function of activation time: pH decreases, ORP increases, and increases in NO_3^- (up to 2308 ppm ± 918.2 ppm) and NO_2^- (up to 89.0 ppm ± 12.0 ppm), long-lived RNS. In addition, nutrient analysis of PAW and PANS revealed that the concentration of plant nutrients were not harmful for the growth of basil when compared to recommended values.

Significance: PANS is a promising alternative to chemical treatments of nutrient solutions to improve the food safety in CEA.

P2-106 Impact of Chlorine and PAA on Inactivation of S*almonella* in Agricultural Water

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Introduction: Preharvest agricultural water has been recognized as one of the major routes of contamination for foodborne pathogens during fruit and vegetable production. Additionally, *Salmonella* is commonly associated with produce related outbreaks and can be regularly isolated from surface water sources. Several strategies have been proposed to reduce the risk of pathogens, including preharvest water chemigation but literature is lacking with regards to microbiological inactivation.

Purpose: To determine the survival of *Salmonella* in surface irrigation water after exposure to chlorine and peracetic acid (PAA).

Methods: Surface water fed by the Sunnyside Valley Irrigation District was collected over the summer of 2019. Water was autoclaved, divided into 100 mL samples and inoculated with a cocktail of five *Salmonella* serovars (Newport, Poona, Montevideo, Michigan, and Saintpaul) at a concentration of 10^6 CFU/mL. Samples were then treated with chlorine and PAA to obtain free chlorine or PAA concentrations of 3, 5 and 7 ppm (6 replicates for every concentration). Aliquots were taken over time and neutralized using sodium thiosulphate, serially diluted and plated on XLT-4 agar, and incubated at 37°C for 24 h prior to enumeration.

Results: *Salmonella* count was significantly ($P < 0.05$) reduced after treatment with chlorine and PAA at concentrations of 3, 5 and 7 ppm. A 6-log reduction of *Salmonella* was observed within 10, 2.5, and 2 min of treatment with PAA at 3, 5 and 7 ppm, respectively. Longer treatment times were required at 3 and 5 ppm free chlorine, up to 60 min for a 6-log reduction; however, at 7 ppm 2 min was sufficient. PAA was shown to be significantly better ($P < 0.05$) at inactivation of *Salmonella* when compared to chlorine.

Significance: These data will provide validation for efficacy of chemical sanitizers like chlorine and PAA for inactivation of *Salmonella*. Thus, benefitting the growers in selection of an appropriate method for in-field treatment of irrigation water.

P2-107 Validating Agricultural Water Treatment on Farms

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❖ ***Developing Scientist Entrant***

Introduction: Market and regulatory pressures are driving growers towards treating surface agricultural water that contacts the harvestable portion of the crop. Very little information exists for growers to validate water treatment systems work on their farms.

Purpose: To validate the in-field effectiveness of agricultural water treatment technologies.

Methods: Surface water was treated with injection systems (diaphragm, aqueous chlorine (NaOCl); peristaltic, peroxyacetic acid (PAA)) to achieve concentrations of 2-4 ppm free Cl and 5-10 ppm PAA. The contact time was 31 s. Water samples (300 mL) were collected at 0, 20, 40, and 60 min (reactions were neutralized with 0.12% w/v sodium thiosulphate or sodium metabisulphite for NaOCl and PAA, respectively), and evaluated for physicochemical attributes. Populations of generic *E. coli,* total coliforms were enumerated using IDEXX Quanti-Trays. *Salmonella* presence was determined following filtration, cultural methods.

Results: In 8 trials in 8 ponds, untreated water at 18.8-31.8°C contained coliform and *E. coli* populations between 2.64 - 5.00 and <0 - 1.88 log MPN/100 mL, respectively. Post-treatment coliforms populations ranged from <0 - 2 log MPN/100 mL and *E. coli* ranged from <0 - 1.80 log MPN/100 mL. Reduction during chlorine treatment was 1.99- >4.87 log MPN/100 mL for coliforms and *E. coli* was below the limit of detection. Reductions during PAA treatment were >2.64 - >5.00 and -0.18 - >1.75 log MPN/100 mL for coliforms and *E. coli*, respectively. Turbidity, TDS, TSS, COD, pH, free chlorine, and PAA concentration of the treated water ranged between 8-104 FAU, 40-274 ppm, 0.01- 0.25 ppm, 10-472 ppm, 6.46-10.70, 2-4.5 ppm, and 3-11 ppm, respectively. Breakpoint to 4 ppm free chlorine was between 4.8-12 ppm.

Significance: Significant reduction in microbial populations, once systems stabilized occurred with both NaOCl and PAA treatments. Both technologies have the potential to effectively reduce microbial populations in surface waters and can be used to help mitigate risks associated with agricultural water used during produce production.

P2-108 Decrease in Disinfection Efficacy of Peracetic Acid (PAA) and Sodium Hypochlorite in the Presence of Nitrogen-based Fertilizers Used on Leafy Greens

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❖ ***Developing Scientist Entrant***

Introduction: Recent *E. coli* outbreaks linked to contaminated irrigation water have elicited new auditing schemes that mandate the use of water treatment to minimize risk. However, little information is currently known with respect to potential interactions of commonly used crop-products such as fertilizers and water treatment chemicals such as Peracetic Acid (PAA) or Sodium Hypochlorite (Chlorine).

Purpose: The purpose of this study was to assess the decrease in disinfection efficacy of two commonly used sanitizers in the presence of nitrogen-based fertilizers.

Methods: Agricultural irrigation water was collected from irrigation canals in Maricopa, AZ. Chlorine or PAA were applied in doses ranging from 2 ppm to 8 ppm in 1 L samples of collected water in the presences of either UAN32 or CAN17. Trials were done in triplicate for each sanitizer and fertilizer pair ($n = 96$). Five minutes of contact time was allowed prior to neutralization. Pre- and post-treatment samples (100 mL) along with appropriate controls were assessed for Total Coliform bacteria (TC) and *Escherichia coli* (EC) using the IDEXX Colilert Quanti-tray method.

Results: Overall, Chlorine was more impacted by the presence of fertilizer than PAA. At low doses, Chlorine was roughly half as effective at disinfection in the presence of fertilizer. At the highest dose, Chlorine's disinfection efficacy decreased by 1.36 log when fertilizer was introduced. In contrast, PAA's disinfection efficacy ranged from 1.69 to 2.78 and indicated a 0.4 log increase in efficacy for both doses with UAN32.

Significance: When introduced to fertilizer, PAA showed little to no interaction, while Chlorine indicated a dramatic decrease in disinfection efficacy. This research demonstrates the importance for industry to monitor their fertilizers' impact on disinfection efficacy. Possible solutions include the use of PAA when fertilizer is applied, restricted fertilizer use during water treatment, or use of an alternate method to reduce risk when using fertilizer and sanitizer together.

P2-109 Effects of Abiotic and Biotic Factors on Survival of Enterohemorrhagic *Escherichia coli*, *Salmonella enterica*, and *Listeria monocytogenes* in Soil Extracts

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Introduction: Foodborne pathogens can be transmitted to crops by agricultural soil and water. *Salmonella*, *L. monocytogenes* and Enterohemorrhagic *E. coli* (EHEC) have been implicated in numerous outbreaks attributed to fresh produce and can survive in agricultural soils and water. Nutrients from soil can leach into standing water following heavy rains or flooding and impact pathogen behavior in water.

Purpose: Quantify survival of EHEC, *Salmonella*, and *L. monocytogenes* in soil extracts and determine associations among soil extract microbiome, chemical profiles and pathogen survival.

Methods: Soil was collected from two agricultural sites and 25-g portions were soaked in 50 mL sterile water for 24 h to create soil extracts. Initial chemical assessment of nitrogen, carbon, and phosphate was used to classify the extracts as either low nutrient (LN) or high nutrient (HN). A portion of each extract was used without sterilization, while a separate portion was filter sterilized. Soil extracts were inoculated with two strains each of EHEC, *Salmonella* and *L. monocytogenes* and incubated at 15°C for 14 d. Pathogens were enumerated by plating at 8 points over the 14 d. Chemical composition of soil extracts was quantified, and microbiome analyses are in progress.

Results: A significant reduction in pathogen numbers occurred in all the extracts, except for sterilized HN extract, where cell density increased from 0.65-2.4 log CFU/mL among the strains. In non-sterilized HN extract, log reductions of 0.37-0.62 log CFU/mL were observed among strains. In both sterilized and non-sterilized LN extract, log reduction of *Salmonella* was 2-3 times greater than for *L. monocytogenes*. Data so far indicate that chemical composition and presence of native microbes impacts survival of pathogens in soil extracts.

Significance: Chemical composition and microbiome profiling can aid in identifying factors that affect pathogen survival in soil extracts. Soil microbes that are associated with pathogen decline in soil extracts can be studied as potential biocontrol agents.

P2-110 Survival and Transfer of *E. coli* to Fresh Produce from Organically Managed Soils Amended with Poultry Litter

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Introduction: Untreated poultry litter used as organic fertilizer for growing fresh produce may harbor fecal pathogens. The National Organic Program (NOP) stipulates a 90-120 day wait time between application of raw manure and produce harvest.

Purpose: Compare survival and transfer of generic *E. coli* from NOP-certified soils amended with poultry litter products to cucurbits, table radish, and spinach.

Methods: A replicated (n = 4) randomized complete block field study with 3 biological soil amendments (6-month-aged (APL), composted (CPL), or heated-pelletized poultry litter (HPPL)), incorporated by tillage or subsurface trenching, was conducted (2018-2019) by spray-inoculating plots with a three-strain cocktail of environmental, generic *E. coli* (gEc$^{rif\text{-}R}$), thereafter tillage or subsurface-incorporated into soil (day-0 amendment). Survival and transfer of g*Ec*$^{rif\text{-}R}$ to four crops was determined quantitatively at periodic intervals throughout 0-120 days post-inoculation (dpi).

Results: All amended, inoculated soils were positive for g*Ec*$^{rif\text{-}R}$ (6 log MPN/g) after inoculation. At 90 dpi, g*Ec*$^{rif\text{-}R}$ was present in all plot soils; by 120 dpi g*Ec*$^{rif\text{-}R}$ concentrations declined significantly (P < 0.05) in 2018 and 2019, during periods coinciding with temperature declines (~16°C). No pathogens (*Salmonella, Listeria monocytogenes, Staphylococcus aureus*) were detected at 0 or 90 dpi in soils. Generic *Ec*$^{rif\text{-}R}$ was detected from four of 16 cantaloupes, and four of 16 cucumbers from APL plots. For table radish (n = 20), four bulbs from till-incorporated and four from subsurface-applied APL tested positive for g*Ec*$^{rif\text{-}R}$ at 90 dpi. At 120 dpi, only two spinach plants (n = 20) were positive from subsurface applied APL plots. At 120 dpi, no pathogens or g*Ec*$^{rif\text{-}R}$ were recovered for all other crops in 2018 and 2019.

Significance: Results will assist organic growers and decision makers in evaluating food safety risks associated with application-to-harvest wait times for aged, non-heat-treated poultry litter soil amendments.

P2-111 Prevalence and Characterization of *Salmonella* Isolated from Goat Feces in the NAHMS Goat 2019 Study

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* is among the top five foodborne pathogens causing illness to humans as reported by the Centers for Disease Control and Prevention. Contaminated food, water, and animal contact are major sources of these pathogens for humans and are responsible for both sporadic cases and outbreaks worldwide.

Purpose: The goat industry is rapidly expanding in the United States, but no study has been conducted to surveil presence of *Salmonella* within this population. The aim of this study was to investigate fecal prevalence, antimicrobial resistance (AMR), biofilm potential, and virulence characteristics of *Salmonella ssp.* isolated from goat fecal samples as part of the 2019 National Animal Health Monitoring System Goat 2019 Study.

Methods: A total of 5,050 fecal samples were collected from 342 operations over 3 months in the Fall of 2019. *Salmonella* was isolated using traditional culture methods and antimicrobial susceptibility was assessed using microbroth dilution. Biofilm potential was determined using a crystal violet assay and normalized to a positive control strain.

Results: Very low prevalence (0.69%) of *Salmonella* was detected overall. A broad range of serotypes were detected (Newport, Bareilly, Give, Poona, Rough O:k:1;6, Manhattan, Oranienburg, Uganda, Muenchen, Braenderup, Anatum, Rubislaw, Infantis, and Sharon), 34% of which were *S.* Bareilly, and included the very rare *S.* Sharon (2.86%, 1/35). All isolates were phenotypically pan-susceptible to a panel of 13 antimicrobials with the exception of only one isolate resistant to tetracycline (MIC ≥ 32µg/mL). All but 3 out of 35 isolates were found to possess 90% (n = 8/9) of virulence determinants screened for by PCR and 46% (n = 16/35) were able to form weak, moderate, or strong biofilm on 96 nunc plates under laboratory conditions.

Significance: This is the first study of this size on the presence and characteristics of *Salmonella* on goat operations in the United States.

P2-112 Persistence of *Salmonella* Typhimurium in Poultry Litter as a Function of Water Activity and Total Ammonia Nitrogen

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❖ ***Developing Scientist Entrant***

Introduction: The persistence of foodborne pathogens in biological soil amendments of animal origin (BSAAOs) is a major pre-harvest food safety concern and understanding the factors that influence their survival will address this concern.
Purpose: This study was designed to evaluate the influence of water activity (a_w) and total ammonia nitrogen (TAN) on the persistence of *Salmonella* Typhimurium in poultry litter.
Methods: Rifampicin-resistant *Salmonella* Typhimurium was inoculated (5 log CFU/g) into 200 g of sterile poultry litter adjusted to a pH of 9.0, a_w of 0.84, 0.92, or 0.96, and with or without ammonium sulfate added to increase the level of TAN. Treatments were performed in triplicate, incubated at 30°C, and sampled on days 0, 1, 2, 3, 4, and 5. Litter samples (10 g) were diluted and plated onto xylose lysine deoxycholate (XLD) agar supplemented with 80 ppm rifampicin, and pH, a_w, and TAN were measured. Samples were enriched in Rappaport Vassiliadis (RV) and tetrathionate (TT) broths when they fell below the limit of detection (<1 log CFU/g) for plating. Significant differences ($P < 0.05$) between treatments were determined by Tukey's honest significance test.
Results: After 5 days of incubation, the *Salmonella* populations significantly decreased ($P < 0.05$) in all treatments, except for the 0.96 a_w litter without ammonium sulfate added. On day 1, litter treatments at 0.84 a_w had significantly lower ($P < 0.05$) populations compared to all other treatments. The counts in these litters with and without ammonium sulfate were 1.49 and 1.30 log CFU/g, respectively. On day 5, populations in the litter had fallen below the limit of detection (<1 log CFU/g) for all treatments, except for both 0.96 a_w treatments.
Significance: This study demonstrates the effects of a_w and TAN on the survival of *Salmonella* in poultry litter, which will aid in understanding the persistence of foodborne pathogens in BSAAOs.

P2-113 Woodchips Increase the Inhibitory Abilities of White-Rot Fungi, *Pleurotus Ostreatus,* in Manure Inoculated with *Escherichia coli*

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❖ ***Developing Scientist Entrant***

Introduction: Lignin-degrading white-rot fungi species can enhance preharvest safety by reducing the risks of untreated dairy manure and inhibiting pathogenic bacteria.
Purpose: The effects of *Pleurotus ostreatus* (PO) on *Escherichia coli* within the presence of different matrices were evaluated.
Methods: Controlled batch tests with PO-treated woodchips (WC), spent mushroom compost (SMC) and reticulated polyurethane foam (RPF) were performed. Bioreactors (n = 4-6) were maintained on benchtop with ambient temperatures of 16–26°C. For all, matrices (300 g) were inoculated with PO millet-spawn (at 60%) and incubated at 22°C for 2 weeks. Inoculated matrices were then added to sterile 1 L bioreactors, to which 1 L inoculated manure was added (6.11 log-CFU/mL). Manure effluent (3 mL) was sampled on 0, 1, 3, 5, 7, and 10 d post-inoculation (dpi) and matrices (30 g) on 0, 5, 10 dpi. *E. coli* TVS355 was enumerated on MacConkey agar with rifampicin. Controls included bioreactors without fungi and/or bacteria. Chemical analysis included pH, moisture and ergosterol which quantifies the fungal presence within each bioreactor. Data were analyzed using one-way ANOVA and Student's *t*-test across 7 trials, with n = 4-6 per treatment.
Results: Between 0 and 10 dpi all bioreactors showed similar decline of *E. coli* in the absence of PO ($P = 0.0472$). In PO-treated SMC and RPF, *E. coli* at 10 dpi was significantly different from WC ($P < 0.0001$). There was a greater bacterial reduction in WC compared to SMC in PO-treated bioreactors ($p<0.0001$); likewise, a bacterial reduction was observed in WC compared to RPF ($P < 0.0001$). Bacteria were undetectable in appropriate controls throughout the study. *E. coli* detection was corroborated with metrics used for ligninolytic activity, as determined through ergosterol analysis, indicative of fungal integrity and concentration. There was greater activity of ergosterol in WC compared to other matrices ($P = 0.0231$).
Significance: *Pleurotus ostreatus* inhibited *E. coli* TVS355 in woodchips compared to other matrices, indicative of the ligninolytic activity and overall fungal activity.

P2-114 Differential Interactions of *Pleurotus Ostreatus* with *Escherichia coli* TVS355 and *Escherichia coli* O157:H7

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❖ ***Developing Scientist Entrant***

Introduction: *Pleurotus ostreatus* (PO), is a lignin-degrading white-rot fungus that may enhance preharvest food safety to reduce risks associated with untreated manure and within different matrices can provide key nutrients which allow PO to enter ligninolytic phase to inhibit bacteria.
Purpose: The effects of PO grown within varying matrices were observed on *E. coli* TVS355 and O157:H7 over 50-days to determine differences in matrix utilization and inhibition variation by strain.
Methods: PO (100 mL/300 g substrate) was inoculated onto treated woodchips (WC), spent mushroom compost (SMC) and reticulated polyurethane foam (RPF). Inoculated materials were maintained in an incubator at 22°C for 2 weeks. Inoculated matrices were distributed among 198 sterile 50 mL conical tubes (3 g/tube), and 18 tubes (30 mL) were sampled every 5 d for 50 d. *E. coli* TVS355 and O157:H7 were enumerated on MacConkey agar with rifampicin or nalidixic acid. Controls included samples without fungi and bacteria. Data were analyzed using one-way ANOVA and Student's *t*-test across 2 trials, with n = 7 per treatment.
Results: SMC, WC, and RPF provide variable growth and nutrients for PO over 50 d in all samples. Across all studies, initial effluent concentrations were 4 log CFU/mL. Over the course of the trial, bacterial counts in SMC were similar to RPF and both were statistically significant from WC for both organisms ($P < 0.005$). Observed differences were noted in TVS355 and O157:H7 in all matrices over 50 d ($P < .0001$), indicating potential for differences in interaction with PO. Regardless of matrix, TVS355 increased to a significantly higher level at 15 dpi and remained significantly ($P < 0.001$) higher throughout the study. A reduction of O157:H7 was observed in the RPF at 50 dpi (0.85 log CFU/mL) with a difference of 3.12 log- CFU/mL.
Significance: White-rot fungi did not affect *E. coli* strains equally and inhibition was observed against the pathogenic strain. Inhibition was greater in a foam matrix (RPF), which induces ligninolytic activity.

P2-115 Differential Attachment of Wild Type *Salmonella enterica* serotype Tennessee and Its Mutant Cells to Peanut Seeds

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❖ ***Developing Scientist Entrant***

Introduction: The *Salmonella enterica* serotype Tennessee strain involved in the 2007 peanut butter-associated outbreak was subjected to mini-Tn*10*

mutagenesis in an early study of our laboratory. Five knock-off mutants (L7, L17, L32, S29, and S32) were acquired, which had a defective gene encoding for the bacterial cell membrane lipoprotein, DNA topoisomerase III, attachment invasive locus protein, bacteriocin immunity protein, and cell division protein, respectively.

Purpose: This study was conducted to compare the ability of the wild type *S.* Tennessee and its mutant cells to attach to peanut seeds.

Methods: Sterilized sandy soil (20 g) inoculated with 10^6 or 10^7 CFU/g of lyophilized cells of wild type and mutant *Salmonella* were mixed with sterilized peanut seeds with or without the skin (2 g) at room temperature for 1 h with vigorous mixing. Peanut seeds with attached *Salmonella* cells were soaked in 5 mL phosphate buffered saline (PBS) at 4°C for 0 or 24 h. The number of *Salmonella* cells in PBS was enumerated on tryptic soy agar before and after the soaking process. The percentage of cells in contaminated sandy soil attached to the peanut seeds were subsequently calculated.

Results: Type III error analysis (n = 48) shows that *Salmonella* strain is a significant factor and seed soaking time is an insignificant factor influencing the attachment of *Salmonella* cells to peanut seeds. Cells of the *Salmonella* mutants had a significantly lower percentage of attachment than those of the wild-type parent. On average, more *Salmonella* cells attached to peanuts without the skin than those with the skin, especially at the 10^6 inoculation level. Seed soaking time did not significantly affect the release of *Salmonella* cells from peanut seeds to PBS buffer.

Significance: The genes that were interrupted by mini-Tn*10* in *Salmonella* mutants could be promising targets for developing effective interventions to control *Salmonella* attachment to peanut seeds.

P2-116 Evaluation of the Hygiena™ BAX® System Real-Time PCR Assay for *Salmonella* in Poultry Primary Production Boot Swabs

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Introduction: *Salmonella* commonly infects poultry and is extensively found in primary production facilities throughout the United States and UK. Since the organism is shed in feces, boot swab sampling provides an easy collection system to assess the prevalence of *Salmonella* in flocks. If found positive, the appropriate control measures and sanitation procedures can be applied to decrease and prevent contamination during processing. PCR screening methods are a fast and effective option for detecting *Salmonella* in such sample types.

Purpose: The objective of this study was to evaluate the performance of the BAX® real-time PCR assay for *Salmonella* in poultry primary production boot swab using 2 different enrichment volumes.

Methods: Boot swabs acquired from an industry partner over two weeks were tested for the presence of *Salmonella*. Samples from week 1 (n = 26) were enriched in 225 mL of pre-warmed BPW while samples from week 2 (n = 20) were enriched in 100 mL of pre-warmed BPW. After incubation at 37°C, all sample enrichments were screened by PCR with and without a BHI secondary enrichment and culture confirmed according to the isolation procedures in the NPIP standards.

Results: Boot swabs with and without a BHI secondary enrichment returned positive results for *Salmonella* in 11/26 (week 1) and 6/20 (week 2) samples. All PCR results were confirmed correct by culture demonstrating 100% sensitivity and 100% specificity.

Significance: These results demonstrate the ability of a rapid real-time PCR assay to be used as a reliable indicator for the status of *Salmonella* in flocks statistically equivalent to culture.

P2-117 *Salmonella* Quantification (SalQuant™) with the Hygiena™ BAX® System for Turkey Feet Swabs and Cloacal Swabs

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Introduction: *Salmonella* control programs are important for both on the farm and in the plant for poultry processors to control and reduce levels of contamination. Since Salmonellosis is concentration dependent, rapid methods for quantification are desired to prevent more foodborne illnesses.

Purpose: The purpose of this study was to develop and verify a Real-Time PCR assay for *Salmonella* quantification (SalQuant™) in turkey feet swabs and cloacal swabs.

Methods: Turkey feet swabs and cloacal swabs (composites of 10) provided by an industry partner were pre-screened by adding 100 mL of BPW. A 10 mL aliquot was incubated for 18-24 hours, while the remaining 90 mL was stored at 4°C. Samples that were negative were pooled together to create a bulk negative slurry. Thirty milliliter aliquots from each slurry were inoculated with *Salmonella* Typhimurium ATCC 14028 across 5 levels (1, 10, 1,000, and 10,000 CFU/mL). Samples were combined with 30 mL of pre-warmed MP media with antibiotics, incubated at 42°C for 8-12 hours and tested by real-time PCR in quintuplet. The best linear fit equation was determined with R-squared and Log RMSE using JMP 15.

Results: The 10-hour enrichment produced the best linear fit equation for both matrices. The turkey feet swab curve has a R-squared of 0.90 and Log RMSE of 0.38. The cloacal swabs curve has a R-squared of 0.89 and Log RMSE of 0.40. When compared to MPN, PCR results displayed higher accuracy and closeness to the actual log CFU/mL input.

Significance: These studies demonstrate accurate and rapid quantification of *Salmonella* from turkey feet swabs and cloacal swabs using the BAX® System. Processors that adopt these protocols use SalQuant as a tool to measure and improve the efficacy of various antimicrobial interventions on the farm and in the plant, leading to better identification and management of products posing a high public health risk.

P2-118 Understanding the Environmental Prevalence of *Salmonella* spp. in Finishing Pigs at Commercial Swine Farms

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Introduction: Salmonellosis remains a major foodborne disease threat to public health worldwide. Swine are considered a reservoir for many *Salmonella* serotypes that can infect humans. However, not all serotypes of concern in food animal products cause clinical signs of infection in swine.

Purpose: Evaluate prevalence and distribution of *Salmonella* spp. in finishing pigs at commercial swine farms across Kansas.

Methods: Five commercial farms were selected and sampled within four weeks of the first load out for slaughter. A total of 31 samples per farm were collected: 5 swabs from 6 individual pens on the front gate, floor, back wall, feeder, waterer, feces (~ 20 g) and one ventilation exhaust fan. Samples were collected using a pre-moistened sponge and transported at refrigerated conditions to the lab for processing following USDA-FSIS guidelines. *Salmonella* presumptive positive samples were confirmed by PCR.

Results: A total of 186 samples were collected among the five farms. A total of 100 samples resulted positive following *Salmonella* culture tests, and 14 were confirmed by PCR. No differences, for proportion of culture positive samples, were observed for either farm or sampling site within pen ($P > 0.05$). Conversely, a significant interaction was detected between farm and sampling site within pen for proportion of PCR positive samples ($P < 0.05$). Feces or site in contact with fecal matter tested positive more often at one farm than other locations on any other farms.

Significance: These results suggest that *Salmonella* prevalence in farm might depend on farm site, cohort-level and several risk factors, such as contact with *Salmonella*-positive fecal matter. A low level of *Salmonella* spp. in the environment was detected.

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P2-119 Prevalence of Biofilm Formation Among E. coli Isolated from Goat Feces

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❖ ***Developing Scientist Entrant***

Introduction: Biofilm formation in farm environments such as water bowls and fences enables persistence of commensal and pathogenic bacteria; persistence may increase the likelihood of transmission among animals and humans.

Purpose: This study characterized the extent of biomass accumulation by generic *Escherichia coli* and Shiga toxin-producing *E. coli* (STEC) isolated from goat feces.

Methods: Biomass accumulation of a random selection (n = 683) of generic *E. coli* and all STEC (n = 9) isolates obtained from fecal samples collected as part of the 2019 National Animal Health Monitoring System Goat study was determined using a crystal violet colorimetric assay. Briefly, 0.5 McF of each culture was added to triplicate wells of a 96-well plate of M9 media. After incubation (37°C, 24 h), plates were stained with 0.3% crystal violet, rinsed, then solubilized with ethanol prior to measuring absorbance at 570 nm. Three biological replicates were averaged. Absorbance values exceeding twice the mean of the positive control (ATCC 25922) plus three standard deviations were designated as moderate to strong biofilm-forming. Isolates were also plated onto Yeast Extract Congo Red Agar (YESCA) to screen for formation of curli, an amyloid associated with biofilm production. Following incubation (22°C, 48 h), the color of growth was scored as white (W) or pigmented (P). Absorbance values of each group were compared using a two-sample t-test.

Results: Absorbance values of all isolates ranged from 0.126 to 2.535 (Mean: 0.309, Median: 0.227). None of the STEC isolates and 6.6% of the generic isolates exceeded 0.687, the internal value indicating moderate-to-strong biomass accumulation. A total of 59% of the generic and 33% (3 of 9) of the STEC isolates had "P" scores on the YESCA, suggesting a capacity for curli formation, however measured biomass absorbance of these isolates was not significantly different (P = 0.52) from the "W" phenotype.

Significance: These data show that generic and pathogenic *E. coli* isolated from goats infrequently form biofilms, suggesting a low risk of persistence on farm surfaces.

P2-120 Effect of Type of Mulch on Microbial Food Safety Risk on Cucumbers Irrigated with Contaminated Water

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Introduction: Mulch is used to block light and retain soil moisture which may affect the survival of bacterial pathogens on soil.

Purpose: This study examined the effectiveness of different mulches to minimize microbial risk from contaminated water used for irrigation of cucumbers.

Methods: A production plot of 120 ft^2 with 18 beds (30 ft long) covered with five different types of mulch (paper, paper with fertilizer incorporated (PF), maize-based mulch, biodegradable plastic covering and conventional plastic) including three beds with no cover were planted with Dasher 2 Variety cucumber. Soil samples (200 g) were collected from each bed for five weeks to examine natural *E. coli* and coliforms. Well water contaminated with or without nalidixic acid-resistant *E. coli* (8 log CFU/mL) was used for drip irrigation 7 days before harvesting cucumbers of approximately 250 g.

Results: Prior to irrigation of contaminated water, naturally present *E. coli* and coliform in the soil samples with or without mulch were in the range of 3.45-3.78 log CFU/g and 4.18 – 5.31 log CFU/g, respectively. *E. coli* levels on cucumbers harvested from mulched plots except PF and irrigated with contaminated water had significantly higher (P < 0.05) levels of *E. coli* as compared with samples from plots irrigated with non-contaminated water. Samples collected from plots irrigated with non-contaminated water also resulted in *E. coli* levels ranging from at or below the detectable limit (10 CFU) (Maize) to 2.28 log CFU/g (PF). Cucumber harvested from plots with paper mulch had the highest levels of *E. coli* (3.76 log CFU/g) while samples from maize mulch had the lowest levels (1.68 log CFU/g). A significant die-off of inoculated *E. coli* was observed on cucumber within 3 days (>1.17 log CFU/g) and >1.38 log CFU/g after 4 days.

Significance: Plots with maize mulch were effective on minimizing *E. coli* contamination on cucumber from contaminated irrigation water.

P2-121 V. Comparison of the Microbiological Quality and Safety of South African Grown Cucumbers

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Introduction: Cucumber safety may be affected by the microbial quality of irrigation water. In South Africa (SA), cucumbers are grown vertically and drip-irrigated with groundwater or surface-water. Cucumbers are sprayed overhead for cooling and receive pesticide applications using the same water as for irrigation.

Purpose: To determine the microbial safety and quality of SA-grown cucumbers and irrigation water using comparative systems i.e., borehole (groundwater) and canal (surface) water.

Methods: Cucumber cultivars 'Larino' and 'Fero' were collected from Farm A at harvest, with corresponding borehole and reservoir water. On Farm B English cucumbers and canal and reservoir water were collected. In total, 46 cucumber and 30 water samples were collected. Total coliforms (TC) and *Escherichia coli* (EC) were enumerated using Colilert-18 and 3M Petrifilm EC/TC. The presence of EC and *Salmonella* was determined after enrichment and plating onto RAPID-*E.coli*2 and XLD.

Results: On Farm A, EC was detected following enrichment from 'Larino' and 'Fero' cucumbers and from borehole water. However, no EC was enumerated from cucumbers nor from water. No TC were enumerated from borehole water. TC levels in reservoir water ranged from 0.93-2.15 log MPN/100mL. The TC levels on 'Fero' cucumbers were higher than that of 'Larino' cucumbers (P = 0.016) and exceeded the SA guideline for ready-to-eat fresh produce. On Farm B, *Salmonella* was detected in reservoir water and EC in canal and reservoir water, but not from cucumber samples. On Farm B, no TC nor EC were enumerated from cucumbers. TC levels in water ranged from 3.97-4.79 log MPN/100mL and EC levels in water ranged from 0.30-3.19 log MPN/100 mL, with 80% of water samples containing enumerable EC levels.

Significance: Although Farm B's water was contaminated with TC and EC, cucumbers were categorized safe for consumption. In contrast, cucumbers from Farm A were categorized as "not fit for human consumption."

P2-122 Melon Phytocompounds May Impact Foodborne Pathogen Persistence in Melon Juice

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Introduction: Melon-*Listeria* and melon-*Salmonella* associations have caused several foodborne illnesses and product recalls. Various melons types differ in sweetness, acidity and phytonutrient levels. Whether these traits impact enteropathogen-melon associations is not clear.

Purpose: Evaluate *Listeria monocytogenes* (*Lm*) and *Salmonella enterica* growth in melon juices and analyze data in relation to melon biochemical profiles.

Methods: Store-bought cantaloupe, honeydew and watermelon were cut in half for excision of 200 g of flesh. This was mashed in Whirl-pak bags using

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a stomacher. Three *Lm* and three *Salmonella* strains were separately inoculated into 1 mL of sterile juice, incubated at 35-37 for 24 hours, serially diluted and quantified. Total sugars, total phenolics, vitamin C and antioxidant capacity were measured, and data analyzed in JMP 14.

Results: The survival of *Lm* ATCC19115 was 0.31 log CFU/mL higher in cantaloupe juice than in honeydew juice ($P = 0.06$). *Lm* FS2025 grew better in honeydew than in watermelon juice ($P = 0.06$). The survival of *S.* Javiana and *S.* Enteritidis was 0.44 and 0.3 log CFU/mL higher in cantaloupe than in honeydew juice, respectively ($P < 0.05$). *S.* Enteritidis counts were 0.28 log CFU/mL higher in watermelon juice compared to honeydew ($P < 0.05$). The survival of *S.* Javiana in cantaloupe juice was 0.32 log CFU/mL higher than *S.* Newport ($P < 0.05$). Ascorbic acid concentration was higher in watermelon juice than in cantaloupe ($P < 0.05$). By contrast, more total phenolics were detected in honeydew juice than in watermelon ($P < 0.05$). Cantaloupe juice had higher antioxidant capacity than honeydew juice ($P < 0.05$). Sugars were highest in honeydew but differences were not statistically significant.

Significance: Differences in melon juices' ability to support Lm and *Salmonella* were not pronounced, albeit significant. Watermelon was the least favorable juice for all strains and also had the highest ascorbic acid levels. The phytochemical composition of various melons may affect the food safety risk of these fruit.

P2-123 Survival of Planktonic and Biofilm-Grown Listeria monocytogenes on Apples as Affected by Waxing and Storage Conditions

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❖ ***Developing Scientist Entrant***

Introduction: Waxed apples have been recently linked to multiple recalls involving *Listeria*. Therefore, information is needed on the ability of wax coatings to influence the survival of pathogens on the apple surface.

Purpose: The objective of this study was to assess the fate of *Listeria monocytogenes* (*Lm*) on apples before and after waxing.

Methods: Unwaxed Gala (G), Granny Smith (GS), and Honeycrisp (HC) apples from a Michigan grower were dip-inoculated in an 8-strain (~6.5 log CFU/mL) *Lm* cocktail of planktonic- or biofilm-grown cells. Apples were then stored for 7 months under controlled atmosphere (1.5% O_2, 1.5% CO_2) at 2°C. Additional non-inoculated apples were similarly stored and later inoculated after 7 months. All apples were then waxed with Shield-Bright AP-40 shellac (Pace International, Rochester, MN) and stored aerobically at 2°C. Two different samples, i.e., the combined calyx and stem portion or the remaining skin, were stomached in sterile PBS (42°C) with 1% Tween 20 added for the waxed samples, serially diluted, and plated on Modified Oxford Agar for *Lm* enumeration after incubation (37°C/48 h).

Results: *Lm* survived similarly on waxed and unwaxed apples ($P > 0.05$). Previous growth of *Lm* as a biofilm significantly decreased survival ($P < 0.05$) on apples regardless of waxing. After 14 days of storage post waxing, *Lm* populations decreased -0.4-1.0 log on G, GS, and HC apples that were previously inoculated with biofilm-grown cells and waxed after 7 months. After 14 days storage, *Lm* populations decreased 1.6-0.4 log on G, GS and HC apples that were inoculated with planktonic cells and waxed after 7 months compared to reductions of 1.3-1.7 log for similarly waxed apples that had been inoculated with biofilm-grown cells.

Significance: The physiological state of *Lm* (planktonic vs biofilm) can impact overall survival of *Lm* on apples, but waxing does not impact survival.

P2-124 Survival of *Listeria Monocytogenes* in Romaine Lettuce Juice and Isolation of Antilisterial Bacteria

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Introduction: Multiple studies indicated that *L. monocytogenes* could grow on produce, like lettuce, especially at abused temperature. However, the survival of *L. monocytogenes* in lettuce juice as affected by phytobiota has not been well studied.

Purpose: To investigate the survival of *L. monocytogenes* in romaine lettuce juice and isolate antilisterial bacteria from lettuce leaves.

Methods: Raw juice was extracted from three brands of romaine lettuce (P, G, W) using a juice extractor. Sterile juice was prepared by centrifugation of raw juice at 4,000 g for 30 min and filtration using 0.22 µm membranes. *L. monocytogenes* was inoculated in the three different raw juices at concentrations ranging from 3-100% and incubated at 30°C for two days. Growth and reduction of *L. monocytogenes* in 10% sterile juice with/without cut lettuce leaf pieces were also tested. Bacterial populations and isolates were enumerated by plate count and identified by 16S rDNA sequencing.

Results: *L. monocytogenes* levels decreased from 4 log CFU/mL to undetectable in all three types of raw juices at 100%. Suppression was also detected in 13% raw juice of P lettuce. In contrast, *L. monocytogenes* could grow from 4 log CFU/mL to about 8 log CFU/mL in 10% sterile lettuce juice of all three brands after two days at 30°C. At least 2-log reduction was observed in 17% P, 10% G, and 4% W sterile juice samples when 0.1 g cut lettuce pieces were added. Antilisterial bacteria were isolated and identified from samples causing over 4-log reduction of *L. monocytogenes* compared to control, including *Lactococcus*, *Leuconostoc*, and *Weissella* spp. One isolated *Lactococcus lactis* strain inhibited *L. monocytogenes* growth at the ratio < 1:1,000.

Significance: Antilisterial bacteria isolated in this study could be applied as potential biocontrol agents to mitigate the contamination of *L. monocytogenes* on fresh produce and in produce juice.

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P2-125 Comparison of Existing and Novel Produce Intervention Chemistry Systems to Reduce Bioburden and Spoilage/Pathogenic Bacterial Load on Raw Leafy Produce Products

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Introduction: Chemical intervention strategies are used to reduce loads of spoilage/pathogenic bacteria upon RTE raw produce products ascertain surfactant-biocide combinations for produce processors, maintaining quality.

Purpose: Compare food grade surfactant's microbial reduction performances, with and without a novel biocide alone, or with approved intervention biocide chemistry Peroxyacetic acid at use concentrations.

Methods: Retail raw kale [1 kg] mixed in shaker @ 10 deg. C 1 hour. Enumeration: TPC, Coliforms [TSAF, MacConkey agar ISO GRID® membrane filtration. Cut kale into 2 x 2 in. portions, 100 grams, 1 liter sterile DI water. agitating @ 10 C for 15 minutes @ 125 rpm. Diluents onto TSAF & MacConkey incubated at 36 C for 48 hours/24 hours for Baseline Negative controls.

One-step / a two-step procedure: Sir Fact® @ 0.5 & 2 oz/ gal [RMC produce wash]. Veg-Aphex® [electrolyzed produce wash : Aphex Bio-cleanse .] ; Sir Fact® in Veg-Aphex® 1X at both levels; all 3 permutations plus Peroxyacetic acetic {PAA} at 100 ppm [Enviro Guard® -Envirotech Corp.] PAA alone @ 100 ppm, 300 ppm. 2 step permutation: Sir Fact® @ 0.5 & 2 oz/gal, then PAA 100ppm, 300ppm.

Results: Baseline TC / Coliform were 5.14 Log10 [1.37 x 10^5 CFU/ml]. Show Sir Fact® attains Mean Log10 at 0.5 oz/gal [0.4%] of -1.4 Log10 for TPC and -1.3 Log10 Coliform At 2 oz/gal [1.6% v/v] Sir Fact® -1.94 Log10 TPC with -2 Log10 Coliform.

Veg-Aphex® 1X a -1.69 Log 10 TC and -1.74 Log10 Coliforms.

Combinations ranged -1.45 Log10 to -5.15 Log10 TC, [96% - 99.999%]. Effective blend of SirFact@0.5oz/gal, VegAphex, PAA 100ppm gave -3.44Log10 TC. 2 step permutation performed better, SF @0.5oz/gal THEN PAA 300ppm gave -4.44 Log10 TC.

Significance Bench top protocol & field trials creates best practices for a produce multi hurdle intervention strategy. This will reduce spoilage microbes, enhance product shelf life with pathogen reductions on high risk RTE produce products.

P2-126 Leaf Phytochemical Profiles Differ by Lettuce Variety and Shift in Response to Water Stress, Impacting the Association with *Salmonella enterica*

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❖ ***Developing Scientist Entrant***

Introduction: Abiotic stresses such as drought affect plant physiology. The impact abiotic stresses have on surface-associated microbiota of various lettuce varieties, including *Salmonella enterica*, merit investigation.

Purpose: Evaluate the effect of cultivar and drought on the lettuce leaf metabolome and the subsequent impact on epiphytic *Salmonella*.

Methods: Red loose-leaf lettuce cultivar 'Mascara' and Romaine lettuce cultivar 'Parris Island Cos' were grown for 4 weeks in a greenhouse (23, 16h L: 8h D) then subjected to drought for 6 days or watered regularly (control). About 10^6 *Salmonella* Newport or *Salmonella* Typhimurium were inoculated onto the abaxial surface of the third true leaf of plants. Inoculated leaves were enumerated for *Salmonella* 24 hours post-inoculation. Leaf samples were flash-frozen, ground and mixed in methanol/formic acid. Solutions were used for biochemical analyses. Data were analyzed using JMP 14.

Results: For *S*. Newport and *S*. Typhimurium, retrieval from drought-subjected 'Mascara' leaves was 0.68 and 0.89 log CFU/plant lower, respectively, than from controls ($P < 0.05$). *S.* Newport counts were 0.70 log CFU/plant lower on drought-subjected Romaine than from controls ($P = 0.05$), but no difference was found with *S*. Typhimurium. The recovery of *S*. Newport was 1.33 log CFU/plant and 1.32 log CFU/plant lower from regularly-watered and water-stressed 'Mascara' lettuce, respectively, than from Romaine ($P < 0.05$). For the drought-exposed group, *S*. Typhimurium counts were 1.53 log CFU/plant lower on 'Mascara' lettuce than Romaine ($P < 0.05$). Drought-subjected 'Mascara' had higher total phenolics, flavonoids and anthocyanin levels compared to controls ($P < 0.05$). No such differences were detected between control and drought-subjected Romaine lettuce. All 'Mascara' lettuce had a higher antioxidant capacity and accumulated more flavonoids than Romaine ($P < 0.05$). Water-stressed 'Mascara' also had higher total phenolics than Romaine ($P < 0.05$).

Significance: Lettuce favorability to *Salmonella* may be related to total flavonoids, total phenolics and antioxidant capacity of lettuce variety, or as a result of plant response to drought.

P2-127 Evidence of Microbial Transfer from Furrow Water to Leafy Greens during Irrigation

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Introduction: Agricultural surface water used for irrigation can become contaminated and has the potential to transfer pathogens onto crops through furrow irrigation. Harvested crops can then transfer that contamination onto equipment causing cross contamination and risk to public health.

Purpose: The purpose of this study was to evaluate commercial scale produce production practices in order to provide evidence of microbial transfer from contaminated furrow irrigation water to crops and then into the harvesting chain.

Methods: Irrigation water spiked with *E. coli* TVS353 was applied to axial leaves of the first three heads of romaine on each line of a one-acre plot at a concentration of 1 x 10^5 CFU/100 mL. Standard "S" and "Z" pattern pre-harvest sampling of $n = 60$ was conducted 7 days after contamination. Four 100-g sub-samples were enriched then 100 µL of enrichment spread plated onto ChromAgar ECC + 80 µg/mL Rifampicin. On harvest day, five sampling teams positioned in-front of a harvest crew, swabbed the following locations: workers gloves, knifes, cutting table, conveyer belt, elevator/down spout. Crews stopped every 15 minutes (5 stops per acre). During each "stop," sampling teams swabbed the aforementioned surfaces.

Results: Results indicate "Z" pattern samples 4/4 (100%) positive and "S" pattern 0/4 (0%) positive. Commercial Harvest Stop #2 show *E. coli* TVS353 contamination on gloves, knife and table indicating furrow contaminated romaine can transfer *E. coli*. Stop #3 had no contamination on gloves or table and Stop #4 & #5 no *E. coli* TVS353 was detected. Post-harvest bin sampling of furrow contaminated fields resulted in zero detectable bacteria.

Significance: Contamination simulations were detectable through harvest and point to the ability of furrow contaminated water to transfer to harvestable romaine lettuce. Resulting best management practices include raised bed height, omission of harvest at the head of the field, as well as improved or increased frequency in knife cleaning practices.

P2-128 Colonization of Cantaloupe Fruit with *Escherichia coli* O157:H7 through Blossom Inoculation

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Introduction: Fresh produce have been involved in numerous foodborne outbreaks involving O157:H7 and other Shiga toxin-producing *Escherichia coli* (STEC). While cantaloupes are typically implicated as vehicles of outbreaks involving *Salmonella* and *Listeria*, it is important to examine the role female blossoms may contribute to colonization with other enteric pathogens, including *E. coli* O157:H7.

Purpose: To examine any pre-harvest risks of cantaloupe when plant blossoms are exposed to a leafy green-associated outbreak strain of *E. coli* O157:H7.

Methods: Cantaloupe plants (*Cucumis melo* 'reticulatus') from a single cultivar 'Primo' (Western variety) were grown from commercial seed and maintained in the NCSU BSL-3P phytotron greenhouse. *E. coli* O157:H7 contamination was introduced via blossoms at ca. 6.1 log CFU/blossom. Surface and internal samples of mature fruits were processed and enriched for *E. coli* O157:H7 in accordance with modified FDA BAM methods. Data were analyzed for prevalence of contamination [surface and inside (blossom versus stem side)].

Results: Of the cantaloupe fruit harvested from *E. coli* O157:H7-inoculated blossoms, 97.8% (44/45) were found colonized and 84.4% (38/45) had evidence of internalization into the fruit. Of those fruits affected, the majority, 68.9% (31/38), were found to harbor O157:H7 within the entire fruit. The remaining fruits were observed to be contaminated with the inoculated strain only within the stem side, 15.8% (6/38), or blossom side, 2.6% (1/38), of the fruit pericarp.

Significance: These results demonstrated blossoms as a route by which a leafy green outbreak-associated *E. coli* O157:H7 can colonize and internalize cantaloupe fruit at a high percentage. Blossoms may serve as an important route for pathogen contamination in atypical commodity-pathogen pairs.

P2-129 Effects of Short-Term Temperature Abuse during Storage of Fresh-Cut Cantaloupe on *Listeria monocytogenes* Growth

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Introduction: *Listeria monocytogenes* can persist on fresh produce at typical storage temperatures, and cantaloupe, in particular, is of significant concern, as the fresh-cut melon promotes the growth of the bacteria. Short durations of temperature abuse during the transport and retail storage of fresh-cut cantaloupe could cause more rapid growth, potentially increasing foodborne infection risk.

Purpose: We aimed to determine the growth potential of *L. monocytogenes* on fresh-cut cantaloupe as a function of abuse temperature and duration over a range of relatively short exposure times to create a scheme for predicting conditions that increase the growth rate of the pathogen.

Methods: Whole cantaloupes were cut and packaged in the laboratory to simulate retail processing of the fresh-cut melon. Uniform pieces of cantaloupe (20 g each) were placed in plastic food-grade cups with snap-on lids and chilled to 4°C before inoculation with a three-strain *L. monocytogenes* cocktail at $5x10^5$ CFU per 20-gram sample. The samples were then subjected to a range of abuse conditions (time and temperature), and the final populations on each were enumerated to determine the sets of conditions that significantly increase the growth rate of the pathogen.

Results: Isothermal temperature abuse for a duration of 24 hours resulted in increases of ~0.9 log CFU/sample and ~4.5 log CFU/sample at 12°C and 24°C, respectively (n = 4, each; $P \leq 0.05$), data which was then used to inform predictions for shorter-term abuse at a wider range of temperatures.

Significance: Our results demonstrate that extrinsic, environmental factors like storage temperature and duration impact *L. monocytogenes* growth on fresh-cut cantaloupe.

P2-130 Nationwide Survey of Microgreens Consumers' Food Safety Handling Practices and Perceptions

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Introduction: Microgreens are a specialty food which have gained popularity due to their sensory attributes as well as bioactive components. They are distinguished from other fresh produce by the age and size at which they are harvested, being 10 to 14 days from seeding at 1 to 3 inches in height. Though there have yet to be outbreaks of foodborne illness attributed to microgreens, the continued growth in production and consumer demand has also resulted in an increased interest in food safety.

Purpose: To characterize microgreen consumers and determine their knowledge, attitude, and self-reported practices related to microgreens and food safety.

Methods: The 37-item survey was launched via Qualtrics™ to collect data for 24 hours on April 13th 2021. Preliminary data were collected pertaining to consent, eligibility, consumption, purchase, storage, washing, food safety perception, and demographics. Statistical analysis was performed using SPSS Version 26. Descriptive statistics were performed on all variables to determine frequencies and distribution.

Results: The pilot survey collected data from n=69 individuals across 25 states with an average 4-minute completion time. Participants were mostly aged 30-39, with a bachelor's degree, and annual income of $75,000-149,999. Most respondents (58.0%) consumed microgreens 1-2 times per week and stored them 6 days or less (85.0%). Consumers were confident they could store and handle microgreens safely 37.0 and 40.0% of the time, respectively, and more than half (65.2%) of all consumers washed microgreens. Consumers were mostly ambiguous on whether eating microgreens could result in food-borne illness (30.0%) and felt that microgreens were as safe as fresh produce (37.0%).

Significance: The data suggest that most microgreens consumers exhibit safe practices when it comes to the storage and handling of microgreens. However, there was a disconnect between the reported practices and confidence in related knowledge. As most respondents also did not know the difference between a microgreen and a sprout, outreach materials may be best served to build the knowledge base of consumers on the different aspects of microgreens as compared to other produce as well as their risk associated with food safety.

P2-131 Persistence of Foodborne Pathogens during Microgreen Production in Soil-Free Cultivation Matrix and Subsequent Transfer to Mature Microgreens

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❖ ***Developing Scientist Entrant***

Introduction: Microgreens are an emerging salad crop similar to sprouted seeds and lettuce. Several microgreen products have been recalled due to possible contamination with bacterial pathogens including *Salmonella* and *Listeria monocytogenes.*

Purpose: To investigate persistence of bacterial pathogens in microgreen cultivation systems and transfer of bacterial pathogens from soil-free cultivation matrix (SFCM) to mature microgreens.

Methods: Sunflower (SF) and pea shoot (PS) seeds were planted on SFCM (Biostrate and peat) inoculated with approximately 10^8 CFU of *Salmonella* Javiana and *L. monocytogenes.* Following germination, microgreens grew to maturity under a 16-h photoperiod. Soil-free cultivation matrices were sampled in unplanted, PS, and SF areas at day 0 and 10. Bacteria were recovered from SFCM in 10 mL phosphate-buffered saline (PBS) by vortexing. Microgreens were harvested on day 10, and bacteria were recovered in 10 mL PBS by stomaching and manually crushing. *Salmonella* and *L. monocytogenes* were quantified from samples on selective media using spread plate method.

Results: Pathogen levels on day 10 varied across SFCM and cultivar. Overall, *Salmonella* increased by 1.3 to 2.13 log CFU/g in Biostrate with minimal changes in peat. *L. monocytogenes* remained unchanged in Biostrate planted with microgreens but decreased by 2.9 and 2.63 log CFU/g in unplanted peat and peat with PS, respectively. Microgreens cultivated in Biostrate contained higher bacteria levels compared to peat. Lower bacteria concentrations were detected in PS versus SF grown in both SFCM types. For PS, higher concentrations were recovered on average from the microgreens grown in Biostrate (1.83 log CFU/g) compared to peat (0.5 log CFU/g). For SF, 5.29 and 2.77 mean log CFU/g bacteria were detected when cultivated in Biostrate and peat, respectively.

Significance: Enhanced understanding related to the transfer of bacterial pathogens from SCFM to mature microgreens will inform more effective risk management practices to reduce the risk of foodborne disease transmission.

P2-132 Growth Kinetics of *Salmonella* in Fresh-Cut Papaya as Effected by the Storage Temperature and Relative Humidity

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* has been a recurring issue in the papaya industry. The post-harvest factors that are responsible for the growth and persistence of *Salmonella* in papayas is not well understood.

Purpose: To study the effect of storage temperature and relative humidity on the growth kinetics *Salmonella spp*., on fresh-cut papaya

Methods: Fresh papayas were cut into 3 cm x 3 cm x 5 mm size samples and spot inoculated with 25 µL of nalidixic acid (50 µg/mL) adopted *Salmonella* spp (4-strain) to achieve a 4.0 log CFU/sample. The inoculated samples and appropriate controls were then stored in a Forma™ Environmental chamber at different temperatures (4, 12 and 21°C) and RH (55 and 90%) conditions for up to 14 days. At each sampling time (0, 1, 3, 5, 7, 9, 11, and 14) the levels of *Salmonella* on both selective (XLDN) and non-selective (TSAN) media and the changes in physico-chemical properties (pH, TSS, color, and water activity) were determined. Statistical analysis of the data was performed using SPSS, and the data were tested to determine the predictive power of different models.

Results: Temperature, RH and water activity has showed a significant effect ($P \pounds 0.05$) on the survival of *Salmonella.* Increasing the storage temperature from 4 to 21°C and RH from 55 to 90% increased the log survival. At 21°C, *Salmonella* increased from 4 to 6.75 log CFU/sample at 90% RH while the levels were decreased to 2.11 log CFU/sample at 55% RH after 7 days storage. However, no significant effect ($P > 0.05$) of RH was observed when the samples were stored either at 12 or 4°C for 14 days. The levels were decreased to around 1.5 (for 12°C at 55 or 90% RH) and 2.5 (4°C at 55 or 90% RH) log CFU/sample.

Significance: The findings of this study help to develop better storage and distribution practices to mitigate the risk of *Salmonella* in papaya industry.

Poster

P2-133 Comparison of the Behavior of *Salmonella* Typhimurium and *Listeria monocytogenes* on Papaya and Avocado during Storage and Chlorine Dioxide Treatment

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❖ ***Developing Scientist Entrant***

Introduction: Tropical fruits have distinct surface properties that may affect the growth potential and sanitizer susceptibility of foodborne pathogenic bacteria.
Purpose: This study aimed to compare the fate of *Salmonella* Typhimurium and *Listeria monocytogenes* on whole papaya and avocado stored at different temperatures and treated by aqueous chlorine dioxide (ClO_2).
Methods: Fresh papayas and avocados were washed with tap water and air dried at room temperature. The fruits were inoculated with about 7 log CFU tested bacteria on the marked surfaces and dried in a biosafety hood. Inoculated papayas and avocados were then stored at 7 or 21°C for two weeks (10 days for avocado at 21°C due to decay). Samples were collected at selected days, and bacteria were recovered for counting. Additionally, papayas and avocados were inoculated with about 8 log CFU tested bacteria and dried for two and three hours, respectively. The fruits were immersed in ClO_2 solutions made with $NaClO_2$ and HCl or malic acid for 5 min, then enumerated for the residual bacteria on the marked surfaces.
Results: *S.* Typhimurium and *L. monocytogenes* grew by 1.88 and 1.51 log CFU, respectively, on papayas stored at 21°C. Both bacteria maintained a stable population on papayas stored at 7°C and avocados stored at 21 and 7°C. On both fruits, ClO_2 generated with malic acid showed stronger antimicrobial effects than the solution generated with HCl. 10 and 15 ppm ClO_2 deactivated all detectable bacteria on papaya and avocado, respectively.
Significance: This study showed *S.* Typhimurium and *L. monocytogenes* behaved differently on whole papaya and avocado, with papaya being more susceptible to bacteria proliferation at 21°C and avocado requiring higher concentration of ClO_2 during sanitation.

P2-134 Evaluation of the Hygiena™ BAX® System PCR Assays for the Detection of *Salmonella* from 375 g Frozen Potatoes

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Introduction: Potatoes are the world's fourth-largest food crop, following rice, wheat and maize. Food safety hazards associated with potatoes are not common since this vegetable is often cooked before its consumed, but prepared dishes, such as deli-made potato salad, have been a frequent source in foodborne outbreaks.
Purpose: This study was designed to validate the performance of a PCR-based method compared to the U. S. FDA BAM reference method for the detection of *Salmonella* in frozen potatoes.
Methods: Frozen potatoes were divided into representative test portions for the test method (375 g) and the reference method (25 g) in an unpaired validation. Samples were inoculated with *Salmonella* at a low fractional level to obtain a target of 1 CFU and high level of 11 CFU and then held at -20°C for 14 days. Test method samples were enriched 18-24 hours in BPW and tested by PCR while reference method samples were enriched and confirmed according to the procedures in the FDA BAM Chapter 5.
Results: Test method samples analyzed by standard and real-time PCR returned fractional positive results for 8/20 low-spiked samples and all positives results for 5/5 high-spiked samples. All PCR results were in 100% agreement to culture. Test method results were compared to the reference method results using the probability of detection (POD). This analysis indicated no significant statistical difference since the 95% confidence interval contains zero (-0.10, CI -0.37, 0.19).
Significance: This study demonstrates the ability of the BAX® System to accurately detect *Salmonella* in 375 g frozen potatoes statistically equivalent to the reference method.

P2-135 Evaluation of the Hygiena™ BAX® System Real-Time PCR Assays for the Detection of *E. coli* O157:H7 from Mixed Sprouts

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Introduction: Pathogen contamination in sprouts is a major cause of foodborne illness worldwide. In the United States alone, 46 outbreaks occurring between 1996 and 2016 were attributed to the consumption of various types of sprouts. Contamination is challenging to control and eliminate since the same warm and moist conditions required for seed germination also support the growth of pathogens.
Purpose: A rapid commercial PCR method for the detection of *E. coli* O157:H7 was evaluated in artificially inoculated sprouts and compared to the U.S. FDA BAM reference method.
Methods: Mixed sprouts (clover, radish, and cabbage) were inoculated with a low (1 CFU/25 g) and high (10 CFU/25 g) level of *E. coli* O157:H7 and held at 4°C for 48 hours. Test method samples (n = 30) were enriched in pre-warmed (42°C) BPW with novobiocin, incubated at 42°C for 12-24 hours and tested by real-time PCR with and without a BHI secondary regrowth. Reference method samples (n = 30) were enriched and confirmed according to the procedures in the FDA BAM Chapter 4A.
Results: Real-time PCR returned 9/20 positives for low-spiked test method samples after a BHI regrowth and all 5/5 high spiked samples. All PCR results were in 100% agreement to culture. The reference method samples returned culture positive results for 6/20 low-spiked samples and 5/5 high spiked samples. Using the probability of detection to compare the method performance, no significant difference was observed between the test method and the reference method.
Significance: The results of this study demonstrated a sensitivity rate of 100% and a specificity rate of 100% for the BAX® System detection of *E. coli* O157:H7 in mixed sprouts with statistical equivalence to the reference method.

P2-136 V. Differential Ability of Various Conventional and Heirloom Tomato Fruit to Support *Salmonella* Association

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Introduction: Tomatoes with higher proportions of fatty acids are less favorable for *Salmonella enterica* association. Several commercial and heirloom tomato varieties are available but their fatty acid profiles have not been investigated in relation to *Salmonella* association.
Purpose: Screen tomato cultivars for *Salmonella* association and compare fatty acid levels in conventional and heirloom varieties.
Methods: Fourteen tomato cultivars (8 commercial, 6 heirloom) were grown in high tunnels or greenhouse (26°C day and 18°C night; 1 h L, 8h D). At peak ripeness, fruit surface metabolites exudates were collected by placing fruit in 5% methanol/water solution for 3 h. Around 10^3 *Salmonella* Newport cells were inoculated into 900 µL of fruit exudate solution and incubated at 35°C with shaking at 200 rpm for 20 h. *S.* Newport was enumerated by serial dilution and plate counting. For fatty acid analysis, the surface washes were lyophilized, derivatized to esterify fatty acid chains and analyzed with gas chromatography mass spectrometry (GC-MS). Microbial data was analyzed with JMP Pro 14.1.0. Fatty acid data were evaluated for differential fatty acid profiles among cultivars.
Results: All tomato varieties supported higher *Salmonella* levels after 20 h of incubation in fruit exudates (P < 0.05). Counts in exudates of cv. 'Emerald

Evergreen' (EE) were higher than in cv. Purple Bumble Bee' (PBB) (0.63 and 0.16 log CFU/100 mL/cm^2; $P < 0.05$). Cvs. 'Amish Paste', 'White Tomasil' and 'Dixie Red' all supported more growth than 'Purple BumbleBee' and 'Green Zebra' ($P < 0.05$). No difference in *S.* Newport levels was found when fruit were grouped as conventional or heirloom. Fatty acid analysis identified several fatty acids such as palmitic, stearic and linoleic acid.

Significance: Fatty acid profiles of tomato fruit should be investigated to assess their role in the differential ability of *Salmonella* to associate with various tomato cultivars.

P2-137 Survival of Inoculated Generic *Escherichia coli* on Walnuts at Different Phases of Fruit Maturity

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Introduction: The impact of using contaminated water for application of agricultural chemical to developing walnuts is unknown.

Purpose: To determine the survival of generic *Escherichia coli* on walnuts at different maturity.

Methods: Over two growing seasons, Chandler walnuts were spray inoculated in June as walnut fruits developed, and before (August) and during (late September) husk split. The inoculum consisted of three non-pathogenic rifampicin-resistant *E. coli* (commonly used environmental surrogates for *Salmonella* and Shiga toxin-producing *E. coli*) at high (~6 log CFU/mL) or low (~3-4 log CFU/mL) levels. Walnuts (10–40 per timepoint) were aseptically harvested for up to 5 weeks after inoculation. Walnuts were rinsed in 0.1% peptone and the diluent plated onto tryptic soy (TS) agar + rifampicin (+rif) and then filtered and plated onto CHROMagar ECC+rif. The remaining walnut was enriched in TS broth+rif and then streaked onto CHROMagar ECC+rif.

Results: Immediately after inoculation, *E. coli* populations were 5.80 ± 0.26 (June), 5.88 ± 0.31 or 2.64 ± 0.16 (August), and 3.77 ± 0.32 (September) log CFU/walnut. In June and August, *E. coli* populations declined by >3.5 log CFU/nut or were undetectable (0/10) after 24 h for high and low inoculum levels, respectively. Populations for the high inoculum level continued to decline with small numbers of positive samples at week 4 (6/40). During husk split, *E. coli* populations declined by 2.08 ± 1.40 log CFU/nut at 24 h and changed < 0.05 log CFU/nut in the next week. Thereafter, populations of *E. coli* ranged from undetectable to >5 log CFU/nut, which were observed on day 2 (2/20), and weeks 1 (5/40), 2 (2/40), 3 (1/40), 4 (3/40), and 5 (2/40).

Significance: Walnut maturity impacted the survival of inoculated *E. coli* and should be considered when assessing the quality of water used for agricultural chemical application.

P2-138 The Effect of Inoculation Method on Growth of *Listeria monocytogenes* on the Surface of Ten Different Types of Whole Uncut Fresh Produce

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❖ ***Developing Scientist Entrant***

Introduction: Dry inoculation methods can influence microbial behavior in low moisture foods, but few studies have investigated dry inoculation methods for fresh produce.

Purpose: This study elucidates whether the growth observed on the surface of raw whole produce is influenced by inoculation method.

Methods: Ten different produce types (blueberry, broccoli, carrot, cauliflower, cherry, mandarin orange, lemon, raspberry, tomato) were investigated. A cocktail of 5 rifampicin resistant *L. monocytogenes* outbreak strains suspended in 0.1% peptone water was used as the wet inoculum. The dry inoculum was prepared by mixing the wet inoculum with sterile sand and drying at 40°C for 24 h before use. Six replicates of each produce type were inoculated (~3.5 log CFU/sample), incubated at 2, 12, 22, 30 and 35°C and enumerated over time. Growth rates were estimated with DMFit and growth was defined as an increase of >1 log CFU for at least two time points. The average maximum population increase was also calculated.

Results: Inoculation method had a significant effect on the average maximum increase of *L. monocytogene*s on all commodities and at all temperatures ($P < 0.05$). This effect varied by commodity and was significantly enhanced as temperature increased, specifically at 22 and 35°C, where wet inoculation always led to higher growth (0.75 – 2.5 log CFU/g) depending upon temperature and commodity. Wet inoculation also resulted in higher *L. monocytogenes* increases at 12°C, except for tomatoes, where no difference was found. The effect of inoculation method at 2° was highly dependent on food commodity. Carrots did not support growth of *L. monocytogenes* at any temperature regardless of inoculation method.

Significance: Inoculation method is a key variable and its effect should be considered in studies on fresh produce. Use of wet inoculation methods may significantly overestimate *L. monocytogenes* growth potential in fresh produce.

P2-139 Lauric Arginate Enhancing the Efficacy of Peroxyacetic Acid Against *Listeria* on Fresh Apples

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❖ ***Developing Scientist Entrant***

Introduction: Peroxyacetic acid (PAA) is the most commonly used antimicrobial sanitizer to decontaminate *L. monocytogenes* on fresh apples. Lauric arginate (LAE) is a cationic surfactant with antimicrobial efficacy to control *Listeria monocytogenes*.

Purpose: To investigate the antimicrobial and the wettability effects of LAE on enhancing anti-*L. monocytogenes* efficacy of PAA during fresh apple processes.

Methods: *L. monocytogenes* inoculated apples were treated with PAA solution in the combination of different concentrations of LAE at 22°C or 46°C via submerge intervention or spray treatment. Survived *Listeria* on apples post-treatments was detached and enumerated.

Results: Adding LAE significantly increased the antimicrobial effectiveness of PAA at 22°C. A 30-120 s wash by 80 ppm PAA with 0.01% and 0.05% LAE at 22°C reduced *L. monocytogenes* on apples by 2.1-2.3 and 2.5-2.6 log CFU/apple, respectively. The effectiveness of PAA with LAE solutions in decontaminating *L. monocytogenes* significantly increased when the treatment temperature increased from 22°C to 46°C, which caused an additional 0.4-log reduction of *L. monocytogenes* in fresh apples. A 30-s spray application of 80 ppm PAA with 0.05% LAE at 22°C and 46°C reduced *L. innocua* by 1.7 and 2.1 log CFU/apple on fresh apples.

Significance: This study provides an improved PAA process/preventive strategy for ensuring microbial food safety of fresh apples that is applicable to commercial apple packing lines. (Supported by WTFRC)

P2-140 Low-Dose Continuous Gaseous Ozone in Controlling *Listeria innocua* on Red Delicious Apples during Commercial Cold Storage

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❖ ***Developing Scientist Entrant***

Introduction: Recent listeriosis outbreaks linked to apples highlight the importance of controlling *L. monocytogenes* on apples. Ozone is a strong oxidant and potent antimicrobial agent, which does not leave any residue on the treated produce.

Purpose: To investigate the effects of different storage regimes in conjunction with low-dose continuous gaseous ozone on controlling *Listeria innocua* on Red Delicious apples and quality attributes of fruit during long-term commercial cold storage.

Methods: Apples were dip inoculated with *L. innocua* at 6.2 log CFU/apple and then subjected to refrigerated air storage (RA, ~0.6°C) or controlled atmosphere storage (CA, ~0.6°C, 2% O_2, 1% CO_2) with 60.2-78.7 ppb of continuous gaseous ozone in a commercial facility, and sampled at 3, 6, 12, 18, 24, 30, and 36 weeks of storage for bacterial enumeration. Concurrently, a separated set of non-inoculated apples were included in the above storage conditions for resident microflora enumeration and apple quality evaluation.

Results: There was 2.2 log CFU/apple reduction of *Listeria* on Red Delicious apples over 36 weeks of cold storage under a commercial RA and CA storage environment. The application of ozone gas, regardless of dose, significantly enhanced *L. innocua* reduction on Red Delicious apples. It caused an additional 3 log log CFU/apple reduction of *L. innocua* compared to RA or CA treatment alone after 36-week of storage. Low dose gaseous ozone application also retarded the growth of decay microorganisms and resident bacteria on fresh apples. Gaseous ozone application during 9-month storage improved the visual appearance of apples, did not cause ozone burn, and had no impact on the superficial scale, lenticel decay, russet, CO_2 damage on apples compared to CA alone fruits.

Significance: Low-dose ozone gas application during storage could be a promising strategy for the apple industry to control *Listeria* spp. and microbial decay on Red Delicious apples. (WTFRC#AP-18-104A)

P2-141 Evaluation of an Enzymatic Treatment to Control Listerial Biofilm on Produce

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❖ ***Developing Scientist Entrant***

Introduction: Listeriosis outbreaks linked to consumption of contaminated produce such as cantaloupe, apples, and celery have recently been on the rise, highlighting the need for intervention strategies to reduce incidence of *L. monocytogenes* on produce. We observed that *L. monocytogenes* growing on plant materials form exopolysaccharide (EPS)-based biofilms, which confer enhanced resistance to desiccation and antimicrobials.

Purpose: The purpose of this work is to assess the effect of an EPS hydrolase, the enzyme PssZ, on dispersion of listerial biofilm on fresh produce.

Methods: The *L. monocytogenes* strains producing natural (strain EGD-e) levels of EPS, high levels or no EPS, were grown in minimal liquid medium at 30°C in the presence of wooden coupons or pieces of fresh produce. Bacteria were scrapped from wooden disks and enumerated by plating. The PssZ protein, purified from an *E. coli* overexpression strain, was added to growth media, and biofilm dispersion was then evaluated by the sedimentation assay via spectrophotometry at an optical density (OD) of 600 nm. Further, listerial suspensions were spotted on the rinds of whole cantaloupes, dried, and sampled daily to test for listerial survival with or without PssZ treatment via direct plating.

Results: The ability to synthesize EPS greatly increases the number of listeria attached to wooden coupons, confirming the role of EPS in listerial attachment to plant matter. The number of attached bacteria from the high-EPS strain, compared to no-EPS strain, differed by > 100-fold. Treatment with PssZ drastically decreases bacterial attachment to wood and various kinds of fresh produce ($P < 0.05$, $n = 12$).

Significance: Treatments of plant materials, including fresh produce, with the EPS-degrading enzyme, PssZ, is an effective way to disperse biofilm. Ongoing work is evaluating suitability of PssZ as a natural antimicrobial to improve microbial safety of various types of fresh produce under different storage conditions.

P2-142 Autoaggregation and Biofilm Formation of *Listeria monocytogenes* in Cantaloupe Juice Extract and on Food Contact Surfaces with Cantaloupe Juice

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Introduction: Cantaloupe juice supports *Listeria monocytogenes* (Lm) growth and displays an increased risk for Lm contamination during cantaloupe processing. Biofilm formation of pathogens on food contact surfaces increase the persistence of bacteria and the risk for food contamination.

Purpose: This study is to identify conditions that promote biofilm formation of Lm in cantaloupe juice and characterize Lm biofilms on food contact surfaces with cantaloupe juice.

Methods: Dilutions of cantaloupe juice extract in water were distributed in polystyrene microtiter plates with/without stainless steel coupons and inoculated with 4 log CFU/mL Lm culture. Plates were incubated at 37°C for 48 h, and at 24°C and 4°C for 3 days. Unbound cells were removed by washing wells with water and attached biofilms were stained with 0.1% crystal violet. Biofilm mass was quantified by solubilizing crystal violet with 30% acetic acid and reading the OD at 595 nm. The autoaggregation of Lm in cantaloupe juice was measured by OD of the supernatant of static cultures and aggregates were visualized by live/dead staining and fluorescence microscopy.

Results: Lm biofilm formation in cantaloupe juice extract was positively associated with incubation temperature and juice concentration. Lm biofilms formed faster at 37°C vs 24°C, and no biofilm formation was detected at 4°C after 3 days. Additionally, Lm biofilms were significantly denser in 50% than 10% cantaloupe juice on polystyrene surfaces and stainless steel coupons ($P < 0.05$). Lm autoaggregation led to a decrease in OD in the supernatant of static cultures and cell clumps at the bottom of wells. Lm cell aggregates formed in 50% and 10% cantaloupe juice at 37°C and 24°C and were visualized by fluorescence microscopy.

Significance: The information can benefit the fresh produce industry in assessing risks of Lm biofilm formation when processing cantaloupe, and in developing specific control practices for Lm biofilms.

P2-143 Efficacy of the Overhead Washing and Waxing System in Improving the Microbiological Quality of Fresh Peaches

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❖ ***Developing Scientist Entrant***

Introduction: Overhead spray washing and waxing systems (WWS) are used commercially aiming to reduce the risk of microbial contamination and improve the quality of fresh produce during packing.

Purpose: This study evaluated the microbiological quality of fresh peaches before and after they pass the WWS on fresh peach packing lines.

Methods: Eight independent samples, each with 3 pre- or post-washed/waxed peaches were collected after 0, 2, 4, and 6 h into the packing process in three fresh peach packing facilities in Georgia in 2019. The samples ($n = 192$) were maintained at 4°C after sample collection till microbiological analysis on the following day. Each sample was rinsed with 200 mL phosphate buffer saline on a platform shaker at 225 rpm for 30 min, subsequently massaged by hands for 1 min. The levels of total aerobes (TA), total yeasts and molds (YM), and total coliforms (TC) in each sample rinsate were fitted in a Proc Mixed model and evaluated by the Kenward-Roger approximation. The incidence of thermo-tolerant coliforms (TTC) and enterococci (EC) at different sampling sites and times in different facilities were also determined.

Results: Samples collected before the WWS had significantly higher ($P < 0.05$) average TA and TC counts but a similar YM count ($P > 0.05$) compared to the samples collected after the WWS. The mean TA, YM, and TC counts varied significantly in samples collected from different packing facilities. Only mean TA and TC counts were affected by sampling times. The incidence of TTC and EC in peach samples collected after the WWS was 67.71 and 10.42%, respectively, compared to a TTC incidence of 23.96% and non-detectable EC in samples collected before the WWS.

Significance: The results suggest that the WWS used in the visited fresh peach packing facilities had a limited effect in improving the microbiological quality of fresh peaches during packing.

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P2-144 Effect of Oregano Oil Nano-Emulsion in Inhibiting *Salmonella* spp. on Mungbean Seeds and Sprouts

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Introduction: There is an increase in consumption of sprouted seeds due to health benefits during recent years. Mung bean seeds and sprouts have been associated with deadly outbreaks of *Salmonella.* During past few years, there has been radical research in the development of natural antimicrobials that are safe and do not generate any hazardous chemicals during processing of food. Oregano oil exhibits antimicrobial properties but is of limited use as food treatment due to its hydrophobicity. Nanoemulsions of oregano oil made through sonication offer a stable, homogenous solution that may provide an effective treatment for mung bean sprouts.

Purpose: This study examined the antimicrobial properties of oregano oil nanoemulsion on mungbeans (pre-harvest) and mungbeans sprouts (post-harvest) against *Salmonella.*

Methods: Oregano oil nanoemulsion was prepared using Tween 80 as food grade surfactant and high intensity sonication. Dynamic light scattering technique was used to determine the particle size. Antimicrobial efficacy of oregano oil nanoemulsion was evaluated against three different strains of *Salmonella* using microbroth dilution assay. Mungbeans and mungbeans sprouts were inoculated artificially with *Salmonella* cocktail, dried for 24 h followed by a 1 min dip treatment with control (DI water) and oregano oil nanoemulsion (0.5% and 0.75%). Samples were collected at 0 h, 24 h and 48 h and plated on XLD agar for *Salmonella* enumeration.

Results: Minimum inhibitory concentration was found as 0.039% (v/v) while minimum bactericidal concentration was 0.078% (v/v) for *Salmonella.* Compared to control, 0.75% nanoemulsion resulted in 2.59 log CFU/g in mungbeans and 2.95 log CFU/g in mungbeans sprouts, respectively. Based on results of the present study, oregano oil nanoemulsion has potential to be used as effective natural antimicrobial strategy against *Salmonella*.

Significance: These data suggest that nanoemulsions of oregano oil may be effective as an antimicrobial treatment for mung beans and sprouts.

P2-145 Effectiveness of Chlorine and Chlorine Dioxide Against *Listeria monocytogenes* in Lettuce Seeds Used for Hydroponic System

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❖ ***Developing Scientist Entrant***

Introduction: Contaminated seeds present a food safety risk in a hydroponic system where water-soluble nutrients are used for crop production. Research indicated that heat treatment is not an option for seed because of its adverse effect on the viability of the seeds.

Purpose: This study examined the effectiveness of chlorine and chlorine dioxide against *Listeria monocytogenes* in lettuce seeds.

Methods: Green salad bowl lettuce seeds were inoculated with a cocktail of *Listeria monocytogenes* strains (101M, Scoot A, LCDC (81-861, 4b) and V7 ½a) (4 log CFU/g). Contaminated seeds (10 g) were treated with chlorine (1,000, 10,000, and 20,000 ppm) or Chlorine dioxide solutions (100, 200, and 300 ppm) solutions for 15, 30 and 60 minutes. After treatment sanitizers were drained and seeds were placed in a stomacher bag with 90 mL of DE broth. *Listeria monocytogenes* counts on the seeds were enumerated using oxford plates. The viability of the seeds was examined by sprouting the seeds for 7 days.

Results: *Listeria monocytogenes* levels were significantly reduced after treatment with chlorine and chlorine dioxide solution. Chlorine solution at 1,000, 10,000 and 20,000 ppm significantly reduced *Listeria* levels on the seeds from 4 log CFU/g to 1.38 to 1.74 log CFU/g, 2.12 – 2.23 log CFU/g, and 2.13 – 2.2 log CFU/g, respectively. Similarly, chlorine dioxide at 100, 200 and 300 ppm significantly reduced *Listeria* levels on the seeds to 2.34 – 2.86 log CFU/g, 1.87 – 2.25 log CFU/g and 1.73 – 1.86 CFU/g, respectively. Treatment time (15, 30 and 60 min) for both chlorine and chlorine dioxide was not a significant factor for reduction of *Listeria monocytogenes.* For all treatments, the viability of the seeds was around 95% for chlorine and 92% for chlorine dioxide.

Significance: Seeds treated with chlorine or chlorine dioxide will reduce the food safety risk associated with *Listeria monocytogenes* in a hydroponic system.

P2-146 Biocontrol of *Salmonella* on Alfalfa Seeds and Sprouts Using a Multi-Hurdle Approach

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❖ ***Developing Scientist Entrant***

Introduction: The increased consumption of fresh produce including raw sprouts has led to a concomitant increase in produce-related outbreaks. *Salmonella* Enteritidis (SE) has been linked to multistate outbreaks associated with contaminated Alfalfa sprouts. Contaminated seeds were identified as the primary source of the pathogen in these outbreaks. Further, sprouting provides an ideal warm and humid environment for pathogen survival. Hence there is a need to develop effective interventions to control SE on seeds and sprouts.

Purpose: This study evaluated the efficacy of a natural, multi-hurdle approach to control SE on alfalfa seeds and sprouts using protective cultures (PC) and organic acids.

Methods: Alfalfa seeds were rinsed with 80 ppm peracetic acid (PAA), inoculated with SE cocktail (~4 log CFU/g) and sprayed with PC. PC used were *Lactococcus lactis* B-23802, *L. lactis* B-23804, *Lactobacillus acidophilus* La5 and *Hafnia Alvei* B16 at ~9 log CFU/g. The seeds were then dried and stored under ambient condition. For the sprouting experiments, seeds inoculated with SE were rinsed with PAA and PC were applied as a pre-germination soak. The seeds were set for sprouting in commercial sprouters for 7 days. On day 7, sprouts were washed in 2% lactic acid as post-harvest washing. Surviving SE and PC populations on seeds and sprouts were enumerated at different intervals.

Results: The multi-hurdle approach significantly reduced SE population on seeds and sprouts comparing to untreated control at each sampling point ($P < 0.05$). Seeds SE populations were enrichment negative by 31-day of storage. However, 1.04 log CFU/g of SE was detected in the untreated controls. Additionally, our method reduced SE population on sprouts by 2.9-4.1 log CFU/g after harvest, while 7.2 log CFU/g of SE was still recovered from the sprouts in the control group.

Significance: The multi-hurdle approach could potentially be used to control SE on Alfalfa seeds and spouts, thereby promoting its microbial safety.

P2-147 Evaluating the Potential of 25 PPM Sodium Hypochlorite in Preventing Cross-Contamination of Tomatoes in a Laboratory Model Flume

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Introduction: In 2007, the Florida T-GAPs regulation mandated flume tanks maintain 150 ppm free chlorine (FC) to prevent cross-contamination. Recently, the tomato industry has questioned if this high level is necessary and wanted to explore lower FC concentrations, with and without organic load. This research examined 25 ppm FC levels at pH 7.0 as a possible future critical limit. FC was challenged with organic load levels of 0, 100 and 300 ppm chemical oxygen demand (COD).

Purpose: This research evaluated the efficacy of 25 ppm FC with and without organic load, to prevent cross-contamination of *Salmonella* on the tomato surfaces in a simulated flume.

Methods: COD of 0, 100 and 300 ppm was added to the laboratory model flume. Tomatoes were inoculated with a five serovar cocktail of rifampin-resistant *Salmonella enterica*. Experiments utilized inoculated (10^4, 10^6 and 10^8 CFU/tomato) and uninoculated tomatoes exposed to 0 and 25 ppm HOCl, with 0, 100 (10^4 CFU/tomato inoculum only) and 300 ppm of COD. Statistical analysis was performed using Microsoft-Excel (version 1808; Microsoft Office 365

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ProPlus).

Results: In experiments using the inoculum level of 10^4 CFU/tomato with 100 and 300 ppm COD in water, 25 ppm HOCl prevented cross-contamination of tomatoes. In experiments using 10^6 and 10^8 CFU/tomato, exposed to 25 ppm HOCl at 0 and 300 ppm COD, four out of 144 uninoculated tomatoes tested positive for *Salmonella*.

Significance: This study showed that 25 ppm HOCl prevented cross-contamination at 10^4 CFU/tomato but failed at higher inoculation levels. These findings may call for re-evaluation of the new recommendation of using 25 ppm HOCl for preventing cross-contamination of tomatoes in flume tanks.

P2-148 Evaluate the Efficacy of a Mixture of Peroxyacetic Acid and H2O2 Against the Survival and Cross-Contamination of the *Salmonella* Surrogate *Enterococcus faecium* on Tomatoes during Triple-Wash

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❖ ***Developing Scientist Entrant***

Introduction: Triple-wash with a mixture of peroxyacetic acid and H_2O_2 (SaniDate-5.0) during post-harvest processing of fresh produce has been recommended by West Virginia Small Farm Center to improve microbial safety. It has been well recognized that the washing of produce is more important for preventing cross-contamination than reducing foodborne pathogens.

Purpose: To determine the efficacy of SaniDate-5.0 for reducing the survival and preventing cross-contamination of the *Salmonella* surrogate *Enterococcus faecium* on tomatoes during triple-wash.

Methods: *E. faecium* ATCC-8459 (resistant to 100 ppm nalidixic acid) was dip-inoculated onto 2 tomatoes and triple-washed with 4 un-inoculated tomatoes following the procedure of water dip, water dip, and SaniDate-5.0 dip (0, 0.0064, 0.25, and 0.50%) with 45 s of each step. Each tomato was placed into sample bags with 150 mL of sterile tryptic soy broth for 2 min in a stomacher blender. The inoculated surrogate bacteria on tomatoes or in wash-waters were enumerated using a modified MPN method in 8×6 deep-well micro-plates. The turbidity of each well after incubation (35°C, 24 h) was confirmed by adding 3 µL droplets of the incubated liquid arrayed onto bile esculin agars plus 100 ppm nalidixic acid. The final MPN values of each treatment were determined by an online MPN calculator followed by analyzed using SAS (n = 6-12, One-Way ANOVA, P = 0.05).

Results: Initial population of *E. faecium* recovered on tomatoes was 5.39 log MPN/g. Triple-wash with water reduced (P < 0.05) the surrogate bacteria by 1.94 log MPN/g. Reductions increased (P < 0.05) to 2.46, 3.85, and 3.79 log MPN/g after applying 0.0064, 0.25, and 0.50% of SaniDate-5.0, respectively. *E. faecium* cross-contaminated onto the fresh tomatoes by 1.84 and 0.21 log MPN/g with water and 0.0064% of SaniDate-5.0, respectively. Cross-contamination was prevented when 0.25 and 0.50% of SaniDate-5.0 was applied. No *E. faecium* cells were detected in the third step wash-waters containing 0.25 and 0.50% of SaniDate-5.0.

Significance: SaniDate-5.0 is an effective antimicrobial agent that could be used by locally small produce growers in triple-wash process to improve microbial safety of locally grown tomatoes.

P2-149 Evaluation of Temperature, Concentration, and Contact Time on Bacterial Reduction in Surface Waters by Peroxyaetic Acid (PAA)

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Introduction: Agricultural water may carry human pathogens, and when it directly contacts the harvestible portion of the crop during irrigation or protective sprays it could pose a human health risk.

Purpose: Our objective was to assess the effectiveness of PAA against *Salmonella* (SAL), Shiga-toxigenic *Escherichia coli* (STEC), *Listeria monocytogenes* (LM), and naturally occurring *E. coli* and coliform populations in ground and two agricultural pond waters.

Methods: Water was inoculated at 6 log CFU/mL with a rifampicin-resistant 5-strain cocktail of either SAL, STEC, or LM and held at 4, 10, or 15°C (n = 6). PAA concentrations (0, 5, 20, 50, 80 and 100 ppm) were tested at six contact times (0, 30, 60, 120, and 300 s). Sodium metabisulfite (SMBS) was added to quench the reaction (1 ppm PAA: 1.25 ppm SMBS). Water samples were diluted and plated onto non-selective (TSA-SAL, STEC; or BHI-LM), and selective (XLT4, SAL; SMAC, STEC; MOX, LM) media supplemented with rifampicin (80 µg/mL). Uninoculated water sources were treated in the same manner to enumerate generic *E. coli* and coliform populations (log MPN/100 mL) using colilert Quantitrays.

Results: For all water sources, temperatures, and organisms, the longer the contact time and the higher the concentration the greater the microbial reduction. SAL, STEC and LM populations declined below the limit of detection (0 log CFU/mL) when treated at 100 ppm PAA for 300 s. Population declines ranged from 0 log CFU/mL (SAL 4, 10, and 15°C, 5 ppm PAA, 10 s) to > 6.5 log CFU/mL (LM 15°C, 100 ppm PAA, 5 min). Indigenous *E. coli* and coliform populations declined to below the limit of detection (<0 log MPN/100 mL), 80 ppm PAA, 5 minutes. In general, reductions of bacterial populations were greater as the temperature of surface water increased (i.e., STEC: 2.5, 4.8, and 5.0 log CFU/mL reduction at 4, 10, and 15°C, respectively).

Significance: PAA used to treat agricultural water is impacted by concentration, contact time, and temperature.

P2-150 Sanitizer Selection is Critical for the Mitigation of Foodborne Pathogen Cross Transfer during Hydrocooling

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Introduction: Produce hydrocooling and washing require contact with water which can, if improperly managed, serve as a medium for microbial cross transfer. Hence, sanitizer use is important to mitigate contamination during these practices.

Purpose: The efficacy of chlorine, ozone, and chlorine dioxide in preventing cross contamination of *Escherichia coli* O157:H7 from a contaminated batch of iceberg lettuce to subsequent iceberg lettuce heads being cooled was evaluated. The potential for pathogen survival and regrowth on stored lettuce was also evaluated.

Methods: Five batches of lettuce (6 heads per batch) were cooled consecutively in a tank with 15 L of 4°C sterile deionized water (SDW), SDW with chlorine (200 ppm), chlorine dioxide (5 ppm), or ozone (6 ppm) for a duration of 30 min each. The first batch was inoculated with approximately 6 log CFU/lettuce of green fluorescent protein-labeled *E. coli* O157:H7 (H1730) prior to cooling. All subsequent batches were not inoculated prior to cooling. After hydrocooling, three heads of lettuce from each batch were stored at 4°C for one week. The outermost lettuce leaves (25 g) were used for enumeration of transferred/surviving bacteria by plating on TSA with 100 µg of ampicillin and streptomycin.

Results: After hydrocooling, the population of *E. coli* O157: H7 on contaminated lettuce was 5.62 ± 0.1 log CFU/g. The use of chlorine and ozone in water resulted in a reduction of *E. coli* O157:H7 population to 3.71 ± 0.24 log CFU/g and 4.37 log CFU/g, respectively. Chlorine dioxide did not result in significant reductions on inoculated leaves. No cross-contamination was detected among the cooled batches of lettuce only when chlorine was used. The *E. coli* O157:H7 population decreased during storage on lettuce cooled in chlorine or ozone-containing water.

Significance: Sanitizer selection for lettuce hydrocooling impacts cross-contamination control.

P2-151 Effect of Ultraviolet Light on Microbial Reduction and Antioxidants of Fresh Strawberries during Storage

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Introduction: Strawberries are rich in antioxidants, vitamins, and minerals, but have limited shelf life and can be contaminated with bacteria, molds, or yeast throughout the supply chain/storage due to minimal processing. Ultraviolet (UV-C) is an effective non-thermal treatment to sanitize and disinfect food and surfaces to improve food safety and quality.

Purpose: The objective of this research was to understand the effect of UV-C on microbial reduction and antioxidants of strawberries during storage.

Methods: For microbial analysis, fresh strawberries 'Chandler' were inoculated with *Escherichia coli*. Then strawberries were stored at room temperature for 4 h and treated with UV light (4 lamps) at intensity of 318 μW/cm^2 for 60, 90, and 120 s. After treatments, strawberries were stored at 4°C for 15 d. For total anthocyanin (TAC) and total phenolic content (TPC), fresh strawberries were treated with UV-C and stored as mentioned above. The data on TPC and TAC were collected every 5 d over the 15-d storage.

Results: The mean population of *E. coli* in the inoculum and inoculated samples before UV treatment were 8.95 log CFU/ml and 6.48 log CFU/g, respectively. After 60-, 90- and 120-s UV-C treatment, the *E. coli* reduction was 1.78 ± 0.16, 1.83 ± 0.14, and 2.13 ± 0.08 log CFU/g, respectively. There was no significant change in *E. coli* population during storage. Both TAC and TPC of UV-C treated strawberries were higher than untreated strawberries (control) and increased with an increase in UV-C dose (treatment-time). TAC of control strawberries decreased during storage whereas TAC of treated strawberries increased on day 5, decreased on day 10, and increased on day 15. TPC of both treated and untreated strawberries decreased during storage.

Significance: UV-C treatment could be a productive post-harvest treatment for improving microbial safety and quality of fresh strawberries.

P2-152 Prevalence of Colistin-resistant Gram-Negative Bacteria in Fresh Vegetables

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Introduction: Colistin is a last resort antibiotic used to treat human infections caused by multi-drug resistant bacteria. Plasmid-mediated colistin-resistant bacteria are recovered more frequently in the environment and from livestock and represent potential challenges for public health.

Purpose: This study examined the prevalence of colistin-resistant bacteria in fresh vegetables produced in the US.

Methods: Thirty-two fresh vegetable samples were collected from different local markets, including organic and conventional grown produce. Presence of colistin-resistant bacteria was determined using MacConkey agar supplemented with 2 μg/mL colistin. A total of 5-10 colonies were selected based on morphology, size, and pigmentation from MacConkey agar; checked for purity and stored. Disc diffusion assay and broth micro-dilution assay were conducted to evaluate antibiotic susceptibility and determine MIC for colistin. The 12 antibiotics evaluated include 7 classes: penicillin, monobactams, cephalosporins, carbapenems, aminoglycoside, tetracycline, and quinolones.

Results: The positive rate of colistin-R bacteria in organic lettuce (70%, 7/10) was higher than that in non-organic lettuce (37.5%, 6/16). For sprouts, all samples evaluated has colistin-R gram-negative bacteria. In total, 99 colistin-R isolates were obtained from fresh vegetables evaluated. Approximately, 71.7% (71/99) of the colistin-R isolates were multidrug resistant, 68.7% (68/99) of isolates were resistant to more than two antibiotics, and 8.1% (8/99) of isolates were resistant to up to 5 antibiotics. One isolate, from alfalfa sprouts, was resistant to 10 antibiotics including all carbapenem antibiotics tested. The MIC to colistin ranged from 2 and 512 μg/mL for all isolates evaluated.

Significance: This study demonstrates the association of colistin-resistant gram-negative bacteria in fresh vegetables grown in the US, highlighting a potential transmission of antibiotic resistant spoilage and pathogenic bacteria to humans.

P2-153 Initial Microbial Evaluation of a Commercial Coupled Aquaponics Farm

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Introduction: Aquaponics is a sustainable method of integrating aquaculture and hydroponic farming by harnessing the byproducts of one industry for use in the other. However, little is known regarding food safety hazards present within these systems at commercial scale and the potential for produce contamination by foodborne pathogens.

Purpose: The purpose of this study was to perform an initial evaluation of a commercial aquaponics farm to determine potential food safety hazards present within the system.

Methods: Over a 6-month period, a microbial evaluation was performed on three indoor coupled recirculating aquaponic systems using Nile tilapia and several varieties of lettuce. Samples were collected (n = 300) bimonthly at multiple locations in triplicate from each system, including lettuce, roots, fingerlings (0-3 mo), fish (>3 mo), water, and sponge samples from the tank interior. Total plate count (TPC) was enumerated on Tryptic Soy Agar, while total coliform and generic *Escherichia coli* most probable numbers (MPN) were determined using IDEXX Colilert Quanti-Tray. Direct plating was used to detect Shiga-toxigenic *E. coli* (STEC), *Salmonella enterica*, *Listeria monocytogenes*, *Aeromonas hydrophilia*, and *Pseudomonas aeruginosa*.

Results: The average TPC and coliform counts were 5.08 ± 0.82 log CFU/mL and 3.64 ± 0.61 log MPN/mL. Generic *E. coli* counts analyzed were below the detectable limit, 1 organism/100 mL. There were no presumptive positives of foodborne pathogens on the edible portion of the lettuce, but water, fish, and root samples were presumptively positive for *S. enterica*, STEC, *L. monocytogenes, A. hydrophilia*, and *P. aeruginosa*.

Significance: While prior studies have surveyed foodborne pathogens present in small or hobby-scale aquaponics systems, this study quantified biological hazards within a large-scale, commercial aquaponic farm from initial start-up. Preliminary data indicate that pathogens are present in the system, but to-date have not been isolated from the edible portion of lettuce.

P2-154 Microbial Hazards Associated with Fresh Produce Distribution Centers

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❖ ***Developing Scientist Entrant***

Introduction: Little is known about microbial hazards present in distribution centers (DCs) that handle fresh produce for retail or food service.

Purpose: This study investigated *Listeria* prevalence, sanitation regime, facility design, and location in DCs to probe factors associated with risk of produce contamination.

Methods: Environmental samples, including surface swabs (n = 982) and air impaction samples (n = 170), were collected in 18 DCs throughout the US. Sampled sites (ca. 55 per DC) included surfaces at receiving/loading docks, refrigerated and ambient storage, conveyor belts, forklifts, cleaning equipment, among others. Each sample was tested for *Listeria* spp. using the FDA BAM and confirmed by *sig*B PCR. Adenosine triphosphate (ATP) swabs, relative humidity, and temperature were also recorded for each sample site.

Results: No air samples tested positive for *Listeria* spp. (n = 0/170). *Listeria* spp. were detected in 12 of 18 (66.7%) DCs, with a total prevalence of ca. 5% (49/982 surface swabs). *Listeria* spp. prevalence varied between the 18 DCs, with a range of 0% (0/982) to ca. 33% (16/982). Floor-related samples (e.g., floor

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cracks, seams, epoxy patches) yielded the most *Listeria* spp. positive samples (17/182), with other positive samples being detected from cleaning areas and equipment, such as dust mops/pans and squeegees (9/152). ATP swabs yielded a range of 107.43 to 1,451.53 average relative light units between the DCs.

Significance: Findings suggest *Listeria* was more prevalent in sites that were hard to clean and sanitize, and also had infrequent sanitation schedules. Thus, *Listeria* may pose a food safety risk in DCs, and control strategies should be targeted to address harborage sites and sanitation regimes.

P2-155 Isolation of *Salmonella* spp. from Fresh Produce Sold at Farmers' Market and Urban Gardens

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* is one of the major foodborne bacteria responsible for produce-related outbreaks and has shown resistance to antibiotics. Although National Antimicrobial Resistance Monitoring System (NARMS) monitors antibiotic resistance in foodborne *Salmonella*, it does not monitor *Salmonella* in fresh produce. Therefore, determining resistant *Salmonella* in fresh produce is of upmost importance.

Purpose: To determine the antibiotic resistance of *Salmonella* isolated from fresh produce associated with urban food production and distribution system.

Methods: Fresh produce samples were collected from four farmers' markets and three urban farms in metro Detroit and West Virginia from June to August 2019. The FDA-BAM method was used for *Salmonella* isolation and then conformed by PCR. Antibiotic susceptibility was determined by using the Sensititre™ NARMS Gram Negative Plate and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. *E. coli* ATCC 25922 was used as reference strain. After that QIAGEN Genomic-tip 20/G was used to extract high molecular weight genomic DNA for Oxford Nanopore whole genome sequencing.

Results: Out of 109 composite samples, . Out of 11 isolates subject to Sensititre, 3 were resistant to ampicillin, 2 to cefoxitin while resistance to azithromycin, chloramphenicol, tetracycline, amoxicillin/clavulanic acid, ceftiofur, and trimethoprim sulfamethoxazole was detected in only 1 isolate each. and one isolate was resistant to two drugs. The DNA for 7 antibiotic resistance isolates was successfully sequenced.

Significance: The data suggest that *Salmonella* isolated from locally-grown fresh produce may carry antibiotic resistance and pose a public health concern. The study will act as a footstep for further research to determine the virulence potential in *Salmonella*.

P2-156 On-Farm Environmental Assessment of Very Small to Small-Sized Strawberry Farms in the Southeastern United States

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Introduction: Fresh strawberries are subject to on-farm contamination as evident by the number of reported foodborne disease outbreaks attributed to strawberries. Very small strawberry farms are common in the United States, hence, ensuring the safety of fresh strawberries grown on those farms is important. Environmental attributes of individual farms can influence the successful implementation of risk management practices (RMP).

Purpose: We aimed to determine the frequency of environmental attributes associated with RMP of very small to small-sized strawberry farms (two acres or less) in the southeastern United States (SEUS).

Methods: The environmental attributes associated with eight RMP (worker health and hygiene, agricultural water, animal control, biological soil amendments of animal origin, harvesting and packing, storage and transportation, and post-harvest handling) of strawberry farms (2 acres or less) in SEUS states were assessed using a checklist and by creating a map of the farm. Descriptive statistics were performed to determine frequency of these attributes and environmental score for each RMP.

Results: Our analysis included 20 farms in 10 SEUS states. All farms (20) used plasticulture to grow strawberries and mixed cropping was used on 19 farms. All (20) used a drip irrigation system and 19 had an adequate number of sanitary facilities. Nearly all (19) did not have a body fluid spill kit. Also, over half (11) did not have a safe irrigation water source. The environmental scores showed that more farms addressed animal control attributes (90%, 18) with far less addressing food safety signage (55%, 11).

Significance: These findings can inform strawberry-specific safety interventions aimed to increase RMP implementation.

P2-157 Implementation of the Produce Safety Rule – Identifying Supplemental Training Methods to Expand the Reach Across the Global Supply Chain

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Introduction: There is a need for developing and testing supplemental training methods that take advantage of the use of technology for supporting successful implementation of the Produce Safety Rule (PSR) in Latin America countries.

Purpose: Evaluate food safety knowledge and attitudes, food safety norms and rules followed at the farms; and the access to and perception of the use of technology by Latin American growers. Explore if there is a significant difference in attitudes towards the use of E-learning between growers from different Latin American and Caribbean (LAC) countries

Methods: A needs assessment based on the Health Action Model was carried out. The model included 5 constructs: knowledge, normative, motivational, belief and environmental that were addressed in a sample of 4 countries: Chile, Peru, Mexico and Dominican Republic. Data collection was completed/achieved through individual interviews (N = 166) and focus groups (N = 12). Quantitative data from individual interviews were analyzed using SPSS (descriptive, one-way ANOVA, Chi-Square) and qualitative data from the open-ended section of individual interviews and focus groups was coded and analyzed using NVivo.

Results: Participants from Chile, Mexico and Peru achieved a significantly higher mean food safety knowledge score than participants from the Dominican Republic (6.778 ± 1.70 vs 5.583 ± 1.74). Most people expressed positive attitudes towards food safety, although participants from Mexico had significantly higher attitude scores than participants from Peru (4.159 ± 0.34 vs 3.955 ± 0.29). Results showed similarities among participants regarding technology use and access, no statistically significant differences were found. Growers from sample countries have similar access to smartphones and/or computers that can be used for educational purposes. In general, participants support the use of e-learning for training.

Significance: Results show Latin American growers are equally supportive of using device-based e-learning programs to support food safety training initiatives.

P2-158 Microbiological Quality of Fresh Produce from PSR-exempt Farms and Their Connection to Food Safety Environment and Handling Practices

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❖ ***Developing Scientist Entrant***

Introduction: Fresh produce is a food category often related to foodborne outbreaks as these products easily become contaminated with foodborne pathogens from the environment and poor handling practices. To reduce food safety issues the FMSA Produce Safety Rule (PSR) was created. However, some farms are PSR exempt due to their small size and may be at risk of produce contamination.

Purpose: Purposes for this study were: 1) identify potential relationship between food safety environment and handling practices on microbial quality of fresh produce and water used for irrigation from PSR exempt Alabama farms; and 2) identify food safety/handling practices implemented in each location.

Methods: A total of 5 locations were evaluated throughout Alabama, with 60 samples of produce (onions, tomatoes, squash, and others) and 15 of irrigation water. Produce samples were analyzed for aerobic plate counts and coliforms. Water samples were enumerated for generic *E. coli* and coliforms using the EPA 1604 method. Farmers were asked to complete a paper-based survey consisting of yes/no questions covering PSR topics. Bacterial loads from produce were compared using ANOVA and Tukey HSD. Simple linear regression was used to determine potential relationship between coliforms from produce and water samples.

Results: Coliform loads from produce were between 1.45-5.55 log CFU/g, aerobic plate counts between 1.66–5.72 log CFU/g. Coliforms in water were between 1.38-3.51 log CFU/100 mL and 0.30-1.60 log CFU/100 mL for generic *E. coli.* There was no relationship between water and produce microbial quality (P = 0.46). The survey responses showed that most of the locations implement food safety practices.

Significance: Identifying microbial indicator loads from fresh produce and gaps in handling practices can aid in determining if further actions are needed to reduce produce contamination on PSR exempt farms in the state of Alabama.

P2-159 Detection of *Salmonella* from Whole Fresh Peaches Using the Hygiena™ BAX® System

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Introduction: Tree fruits are generally perceived as 'low risk' from becoming contaminated with pathogens since the fruits are not in direct contact with soil, irrigation water or fertilizers. However, mangoes, papayas and most recently peaches, have all been vehicles in foodborne disease outbreaks due to the presence of *Salmonella*. Since these products are usually consumed raw, microbial food safety hazards must be identified.

Purpose: In response to the 2020 peach outbreak, this study was designed to assess the ability of a rapid commercial PCR method to detect *Salmonella* in whole fresh peaches compared to the U. S. FDA BAM reference method.

Methods: Whole peaches (1 per sample) were surface inoculated with a low-level (1.2 CFU/sample) and a high-level (12 CFU/sample) of *Salmonella* Enteritidis with additional samples prepared for MPN analysis. Following inoculation, samples were held at room temperature for 72 hours. Samples were then combined with a sufficient volume of UPB in order for the peach to float and incubated at 35°C for 22-26 hours. Paired samples were screened with 2 PCR assays and confirmed according to the culture procedures in the FDA BAM Chapter 5.

Results: Real-time and standard PCR assays returned positives for 17/20 low-spiked samples and 5/5 high-spiked samples. All PCR results were identical to culture. The probability of detection (POD) was used to compare the results between PCR and culture which determined no significant difference.

Significance: This study provides reliable and accurate results using the BAX® System PCR assays to rapidly screen whole fresh peaches for the presence of *Salmonella* following the FDA BAM reference enrichment method with 100% sensitivity and 100% specificity.

P2-160 Comparison of Ultraviolet Light (254 nm and 279 nm) Systems for the Inactivation of Feline Calicivirus in Buffer and Inactivation on Formica Coupons by 279 nm UV

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❖ ***Developing Scientist Entrant***

Introduction: Ultraviolet light (UV-C) technologies are being increasingly researched for disinfection of surfaces and liquid foods. Human norovirus outbreaks continue to pose public health concerns. Feline calicivirus (FCV-F9), a respiratory virus, is a cultivable human norovirus surrogate used to determine inactivation by various technologies.

Purpose: The objective of this study was to compare the UV-C dose-response curve of FCV-F9 in phosphate buffered saline (PBS) using the traditional 254 nm UV-system and a novel 279 nm UV-LED system followed by optimal UV-LED inactivation on Formica coupons.

Methods: Five-mL FCV-F9-PBS suspensions (~5 log PFU/mL) in 10 mL beakers were continuously stirred during 254 nm UV treatments with 0 to 17 mJ/cm^2 or a 279 nm UV-LED device with 0 to 4 mJ/cm^2. Next, FCV-F9 (100 µL) was spread on Formica coupons (3×3 cm^2), air-dried, and treated with optimal UV-LED at 279 nm from 0 to 39 mJ/cm^2 (N = 3). Optical properties of suspensions were measured using a UV-Vis spectrophotometer. Survivors were enumerated using plaque assays. Data of three replicates were statistically analyzed using mixed model analysis of variance (adjust=tukey, SAS).

Results: FCV-F9 titers in PBS decreased by 0.61 ± 0.15 log PFU/mL with 279 nm UV-LED at maximum dose (4 mJ/cm^2) with D_{10}-value of 6.7 mJ/cm^2, whereas with 254 nm UV, reduction of 1.74 ± 0.12 log PFU/mL was observed at the maximum dose of 17 mJ/cm^2 with a D_{10} of 8.7 mJ/cm^2. Thus, FCV-F9 shows 1.3 times higher UV-C sensitivity at 279 nm compared to 254 nm. FCV-F9 on Formica coupons decreased by 2.45 ± 0.05 log PFU/mL (D_{10}-value of 14.4 mJ/cm^2) with 279 nm UV-LED. Higher D_{10}-values were observed on Formica coupons due to the gradient in UV intensity resulting in non-uniform dose distribution.

Significance: This kinetic data is useful for calculating the desired UV-C doses for target FCV log reductions in suspensions or on surfaces.

P2-161 The Prevalence of Quaternary Ammonium Compound (QAC) Resistance in *Listeria monocytogenes* Isolated from South African Food Factories

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* is a ubiquitous, Gram positive bacterium that can grow at refrigerated temperatures and cause the human disease listeriosis when consumed. This organism can proliferate, form biofilms in the food factory and contaminate final product. Misuse of sanitizers, particularly Quaternary Ammonium Compound-based ones has led to the rise of resistant strains which can survive cleaning protocols and the application of the terminal disinfectant.

Purpose: The prevalence of QAC or other sanitizer resistance in South African food factories among *L. monocytogenes* strains was determined and to stress the importance of allowing ample time for cleaning in factories.

Methods: Two resistance genes that encode for QAC resistance in a total sample set of 51 environmental isolates and two controls *L. monocytogenes*

(ATCC 7644) and *L. innocua* (ATCC 33090) were screened using a conventional polymerase chain reaction (PCR) method. The resistant genes were *bcr*ABC and *emr*C both of which have been identified in clinical cases of *L. monocytogenes* internationally. Phenotypic resistance testing was then carried out using blank diffusion disks impregnated with 1% of QAC and non-QAC sanitizers (peracetic acid and a novel QAC-free sanitizer).

Results: Of the 51 factory strains, 34 tested positive for the *bcr*ABC resistance gene, 31 of the isolates tested positive for the *emr*C resistance gene and 24 of the isolates had both the *bcr*ABC and *emr*C genes. Nine of the strains contained neither the *bcr*ABC nor *emr*C. *L. monocytogenes* isolates from different factory environments differed in their phenotypic responses to different generations of QAC sanitizers. There was a correlation between presence of these resistance genes to phenotypic resistance expression.

Significance: These data suggest that resistance to sanitizers by *L. monocytogenes* is prevalent in South African food factories. It is of concern and stresses the importance of cleaning protocols to reduce end product contamination.

P2-162 Anti-Noroviral Efficacy of Hypochlorite-based Surface Sanitizers Designed for Food Industry Applications

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Introduction: Surface contamination plays a significant role in human norovirus (hNoV) transmission and sanitizing is an important control. The anti-hNoV efficacy of sodium hypochlorite (NaOCl) is established for use at high concentrations, producing regulatory and corrosivity concerns that limits its utility in food industry applications.

Purpose: To characterize the anti-hNoV efficacy of novel NaOCl-based surface sanitizers, with the goal of identifying candidates more relevant for use on food-contact surfaces.

Methods: Three candidate products (A – pH 6, 150 ppm NaOCl; B – pH 11, 250 ppm NaOCl; and C – pH 11, 750 ppm NaOCl) were screened. A positive hNoV GII.4 Sydney stool specimen was diluted to obtain inocula having ~2.5% and/or ~0.25% organic matter. Assays were done per ASTM E1053-11 on stainless steel coupons. Contact times ranged from 30 s – 5 min. After neutralization, remaining viruses on coupons were eluted in PBS. Eluates were subjected to RNase treatment, followed by RNA extraction and RT-qPCR, with extrapolation to standard curves to estimate virus inactivation. Product A was formulated to represent CloroxPro Clorox Anywhere Daily Disinfectant & Sanitizer, achieving high stability at non-alkaline pH for safe use on food-contact surfaces.

Results: At negligible soil load (0.25%), Products A and B produced 3.96 ± 0.05 and 4.16 ± 0.05 log reduction (LR) in genome equivalent copies (GEC) at contact times as little as 3 min. Product C (750 ppm NaOCl) produced a 4.27 ± 0.18 LR after as little as 30 s. These all constituted the assay limit of detection (LOD). At higher soil load (2.5%), Product C produced 3.95 ± 0.25, 4.07 ± 0.24 and 4.34 ± 0.22 LR after 3, 4 and 5 min, respectively (LOD 5.7 log GEC).

Significance: Product A, with hypochlorite concentration of 150 ppm, demonstrated significant anti-hNoV efficacy at contact times as low as 3 min on relatively clean surfaces. Food-contact safe hypochlorite-based products with anti-hNoV activity can be produced at lower active concentrations with careful attention to formulation.

P2-163 Withdrawn

P2-164 Inactivation of *Listeria* Biofilm on Food-Contact Surfaces by Saturated Steam Treatment

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria* forms biofilms on different food-contact surfaces and showed enhanced resistance to antimicrobial interventions. The contaminated food-contact surfaces were implicated in multiple listeriosis outbreaks.

Purpose: This study is to examine the efficacy of steam treatment against the biofilm of *L. innocua* on common food-contact surfaces using a pilot-scale steam treatment blancher.

Methods: The 7-day-old *L. innocua* biofilms on surface coupons (conditioned with/without organic matter) were treated with steam for 0-180 seconds. The survival of *Listeria* cells in the biofilm on respective surfaces post steam treatment was enumerated after detaching from each surface. The hydrophobicity and roughness of surfaces were further analyzed using the sessile drop method and a profilometer, respectively.

Results: Saturated steam at 100°C was effective in quickly inactivating *L. innocua* in biofilms on all tested food-contact surfaces with a 6-s steam treatment attaining a 2.4 - 3.2 log CFU/coupon reduction depending on the type of surface. However, the effectiveness of steam decreased dramatically during prolonged steam treatment with tailing effects more pronounced on rubber, low-density polyethylene (LDPE), polyvinyl chloride, followed by polyester and stainless steel. A 30-180 s steam exposure at 100°C caused a 4.0 - 6.4 log CFU/coupon reduction of *L. innocua* biofilm on stainless steel, and 3.1 - 4.8, 2.9 - 4.2, 2.7 - 4.5 and 2.6 - 3.3 log reductions on polyester, LDPE, polyvinyl chloride and rubber surfaces, respectively. Organic soil did not compromise the bactericidal effects of steam against *Listeria* biofilm on surfaces. Repeated steam exposure did not impact hydrophobicity and roughness parameters of stainless steel, polyester and rubber coupons, but decreased hydrophobicity of polyvinyl chloride and LDPE.

Significance: Data suggested that a short time of steam exposure alone or in combination with other interventions likely provides effective treatments to control *Listeria* biofilm on stainless steel, polyester and rubber surfaces. (WTFRC-AP-17-102A)

P2-165 Industrial Processes Inoculation with Surrogate Microorganisms Via Food Matrix for Cleaning and Sanitation Validation Trials

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Introduction: Cleaning and sanitation protocols are key items of food safety and quality plans, as it allows to maintain a safe and mastered environment all along the production process. Prior to ensuring good application of the designed protocols, reliable tools and methodology should be developed to assess the microbial efficiency of cleaning and sanitation procedures. Like for standard decontamination process, efficiency of cleaning and sanitation protocols can be addressed using surrogate microorganisms directly in plant environment, in real industrial conditions.

Purpose: The objective of the study was to evaluate the use of a food matrix to contaminate an abiotic surface with surrogate microorganism, in view of performing cleaning and sanitation protocols validation.

Methods: A dark chocolate preparation was heat-treated to achieve partial melting and stabilized at 40°C (104°F), then homogeneously inoculated with a dry, ready-to-use surrogate preparation. Inoculated chocolate was evenly distributed with a sterile brush on a stainless-steel plate and left for drying. A hot water flush at 60°C (140°F) for 5 min was applied on the stainless-steel surface. To finish, the surface was sanitized using IPA wipes. Between each step, 10 x 10 cm samples were recovered on the plate using swabbing methodology. For each sample, the surrogate concentration was then calculated to estimate the impact of each step.

Results: The surrogate concentration was 5.81 log CFU/cm² in the spread chocolate layer, 4.45 log CFU/cm² below the chocolate layer. After the hot water flush, surrogate concentration remained quite the same while no surrogate was detectable after IPA wipes sanitation.

Significance: These data show potential application of using a food matrix to homogenously inoculate an industrial equipment with surrogate bacteria, which can be of great interest in the view of validating cleaning and sanitation protocols. Additional experiments on a wider scope of matrices and surfaces are under evaluation to strengthen these results.

Poster

P2-166 Antimicrobial Activity of ClO2 Gas Against *Salmonella* Enteritidis on Almonds

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Introduction: Nuts, including almonds, are occasionally contaminated with *Salmonella* spp.
Purpose: In this study, we used chlorine dioxide (ClO_2) gas to inactivate *S. enterica* subsp. *enterica* serovar Enteritidis on almonds.
Methods: Almonds inoculated with *S.* Enteritidis were exposed to ClO_2 gas generated from 1.0 or 1.5 mL ClO_2 solution in a sealed container at 50 or 60°C and 43% relative humidity for up to 10 h.
Results: The concentration of ClO_2 gas peaked at 354–510 and 750–786 ppm within 0.5 h upon deposition of 1.0 and 1.5 mL of aqueous ClO_2, respectively, and gradually decreased thereafter. The number of *S*. Enteritidis on almonds treated at 50°C decreased by 1.7–2.3 log CFU/sample within 1 h of exposure to ClO_2 gas and decreased to below the detection limit (<1.7 log CFU/sample) at all ClO_2 concentrations after 8 h. At 60°C, the microbial population fell below the detection limit within 1 h, regardless of the volume of the ClO_2 solution supplied. Microbial survival on almonds treated with ClO_2 gas and stored at 12 or 25 °C was observed for up to 8 weeks and the organism was not recovered from the almonds treated for 10 h and stored at 12°C for 2 -8 weeks.
Significance: In future studies, ClO_2 gas treatment time, temperature, and concentration should be optimized to maximize microbiological safety and minimize changes in the overall sensorial quality of almonds.

P2-167 Evaluation of Human Specific crAssphage as a Novel Hygiene Indicator in South Korea

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Introduction: CrAssphage was evaluated as a source tracking marker for human excrement contamination, and has since been studied for water quality monitoring in wastewater and sewage its association with SARS-CoV-2 has also been confirmed.
Purpose: The study aimed to investigate crAssphage as a novel hygiene indicator for human fecal contamination in food and related environments.
Methods: A total of 95 human fecal samples, 32 fecal samples of wild animals, and 24 fecal samples of companion animals were collected. To determine the abundance of crAssphage, SYBR green and probe-based quantitative real-time PCR were confirmed and compared with *Bacteroides* marker HF183. In school cafeteria, we collected 25 surface samples and each 3 fecal samples from the workers. The environment surface of 8 pre-treatment areas, 7 cooking areas, 4 dining areas, and 6 other areas were collected through sampling swab.
Results: The human specificity and sensitivity was 40.6% and 100% for crAssphage, 29.3% and 94.6% for HF183, respectively. As a result of qPCR analysis, crAssphage was in the range of 4.28-8.19 log gene copies (GC)/reaction with probe-based and 5.09-8.24 log GC/reaction with SYBR green-based qPCR, and HF183 was 5.08-8.18 log GC/reaction with probe-based and 3.56-7.60 log GC/reaction with SYBR green-based qPCR. At school cafeteria, 42.9 % of workers, and 31.8% in environmental surfaces. Data may be added ongoing the experiment.
Significance: Our results show that crAssphage is specific in human in South Korea, and can be used as a hygiene indicator in school cafeterias, where many food poisoning accidents occur.

P2-168 Quantitation of *Listeria* spp. from Surfaces Using New Rapid Bioluminogenic Detection Swab and Ensure Touch Luminometer from Hygiena

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Introduction: Rapid surface detection swabs is an advancing field in microbiology, with new technologies for determining qualitative and quantitative level of pathogens being developed to assist the rapid hygiene monitoring of surfaces.
Purpose: This swab is a rapid bioluminogenic device that will detect *Listeria* spp from surfaces in a time-of-flight manner from <12 hours to 24 hours for confirmed negative. The system uses a novel bioluminogenic substrate in selective media to enlighten presence of *Listeria* spp. The poster will show the quantitative nature of the test and inclusivity exclusivity performed across a panel of *Listeria* and non-*Listeria* exclusive organisms.
Methods: A panel of 44 *Listeria monocytogenes*, 23 non-mono *Listeria* spp and 30 non-*Listeria* spp were spiked onto surfaces from a decreasing decimal dilution series from 10^{-1} to 10^{-10} dilution then swabbed. The swabs were incubated at 37°C and assayed every hour from 12 to 24 hours in the EnSURE Touch luminometer to estimate RLU signal.
Results: The data were analyzed to show which CFU level of *Listeria monocytogenes*, *Listeria* spp and non-*Listeria* were detected at each timepoint. For all *Listeria* species (n = 67) the mean CFU detected at 12 hours was 28,950 at 100% PoD, at 14 hours was 11,872 at 100% PoD, at 16 hours 2,895 CFU at 99% PoD, at 18 hours 289 CFU at 97% PoD, 20 hours 29 CFU and <22 to 24 hours 3 CFU at 70% PoD. The PoD at 24 hours does not reach 100% due to fractionality and dilution artefacts. Exclusivity organisms were detected at a mean CFU level of 19,388,846 CFU at 22 to 24 hours but no other levels were detected in under 24 hours.
Significance: The use of strategic incubation times, RLU thresholds allow users to flag when high *Listeria* CFU are found on surfaces allowing rapid remediation.

P2-169 Does Prior Exposure to Sanitizers Affect *Listeria monocytogenes* Ability to Form Biofilms and Intestinal Cell Interaction?

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Introduction: *Listeria's* ability to persist in the processing environment is well known but its ability to form biofilms and pathogenicity when survived to sublethal sanitizer treatments is not very clear.
Purpose: To study the ability of sanitizer-resistant *Listeria monocytogenes* cells in comparison with parent cells to form biofilms and intestinal cell interaction.
Methods: *L. monocytogenes* (2 strains) and *L. innocua* which were previously either not exposed (parent) or exposed (resistant) to varying degrees of chlorine and QAC based sanitizers were used in this study. Biofilm assay was performed by subjecting these strains to low levels of sanitizers (4, 8, 16, 32 ppm) in TSBY at 21°C. The biofilm formation after 24, 48 and 72 h were quantified by measuring the absorbance at 590 nm. Cell adhesion assays were carried out by infecting semiconfluent CaCo-2 cells with *L. innocua*. The percentage of adhesion was evaluated for both parent and sanitizer pre-exposed *L. innocua*. The data was analyzed by ANOVA using SPSS.
Results: No significant difference (P > 0.05) in the levels of biofilm was observed among the tested parent strains of *Listeria*. Strains which were preexposed to sanitizers showed a decreased biofilm formation compared to parent strains. Increasing the time from 24 to 72 h increased biofilm production. However, increasing the sanitizer concentration from 4 to 32 ppm did not show a significant effect except in case of benzalkonium chloride. Cell adhesion was found to be significantly higher (2.8 ± 0.3 fold, P = 0.03) in sodium hypochlorite treated *L. innocua* compared to the parent strain. However, no such changes were observed in benzalkonium chloride (0.85 ± 0.3, P = 0.42) and peroxyacetic acid (1.6 ± 0.6, P = 0.4) treated *L. innocua.*
Significance: The findings of this study help to understand environmental and human health implications of sub lethally injured *Listeria* cells to devise efficient preventive controls.

Poster

P2-170 AMP, ADP, and ATP Concentrations Differentially Affected by Fermentation Process

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Introduction: Adenosine triphosphate (ATP) is used as a target for surface hygiene assessment in food processing. There are evidences ATP is generated and depleted through various reactions involving adenosine di- (ADP) and monophosphate (AMP) homologues. These reactions occur as a function of cellular metabolism, yet there is little evidence describing fermentation influencing concentrations of these important metabolic compounds.

Purpose: This work determined concentrations of ATP, ADP, AMP, and AXP (sum homologue concentration) in foods subjected to fermentation as part of their manufacturing processes.

Methods: Using a luminometer/luciferin-luciferase technique, concentrations of adenylate homologues were calculated using standard curves of AMP, ADP and ATP across timeframes typical of fermentation processes. Multiple replicates generated data that were analyzed with ANOVA and pairwise comparisons of adenylate concentrations. Food examined included fermented meat products, yogurt and beer.

Results: There were several trends dependent on the unique circumstances and processing steps examined. One trend is that some products undergoing fermentation displayed multi-log differences in cumulative adenylate concentrations. A second trend was that, depending on the stage of fermentation and the application of additional processing steps, ADP or AMP concentrations became most prevalent. There were instances of multi-log-scale depletions of total AXP in some products that were fermented, then subjected to heating such as certain meat products. A final trend was that ATP concentrations were rarely more than a small percentage of the total AXP with the exception of yogurt and beer in the final stages of fermentation.

Significance: Adenylate concentrations change up to several orders of magnitude based resulting from fermentation steps common to food manufacturing. Depending on fermentation factors, both increases and decreases of adenylate concentrations were indicated. Such changes would foreseeably increase or decrease adenylate-based test sensitivities and should be considered when such technologies are utilized for hygiene assessment.

P2-171 Ultraviolet Light (UV-C) for the Inactivation of *Cronobacter sakazakii* Suspensions in Buffer and 2% Fat Milk

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Undergraduate Student Award Entrant

Introduction: *Cronobacter sakazakii* outbreaks are associated with high infant mortality, producing extracellular polymeric substances-forming surface biofilms. Light-based technologies for food contact surface decontamination at regular intervals show promise for the control of these resistant-biofilms.

Purpose: The objective of this research was to determine the effects of ultraviolet light (254 nm) against two *C. sakazakii* strains suspended in phosphate buffer saline (PBS) and 2% fat milk.

Methods: Overnight cultures of two *C. sakazakii* strains 29004 and 29544 grown with Tryptic Soy Broth were washed and resuspended in ~10 mL PBS (pH 7.2) or 2% milk (pH 6.6), then 0.75 ml fluid (1 mm in thickness) was treated with UV-C light (254 nm) at surface doses up to 139 mJ.cm^{-2} on a sterile coupon (2.25 cm diameter). Control and treated survivor microbial populations were enumerated after surface spread plating on Tryptic Soy Agar plates and incubation at 37°C for 24 h. Each experiment was replicated thrice and plated in duplicate. Data from the replicates were plotted in Microsoft Excel and analyzed.

Results: *C. sakazakii* 29004 showed a *D*-value (dose for 1 log reduction) of 6.70 mJ.cm^{-2} (1.45 min) in PBS, though the *D*-value in milk increased to 46.34 mJ.cm^{-2} (10.06 min). *C. sakazakii* 29544 was more sensitive to UV-C than the 29004 strain, with a D-value of 2.49 mJ.cm^{-2} (0.54 min) in PBS and a *D*-value of 29.26 mJ.cm^{-2} (6.34 min) in milk, using the linear model (R^2>0.91).

Significance: Thus, UV-C shows promise to inactivate *C. sakazakii* in PBS and 2% milk on contact surfaces. However, high exposure doses (treatment times) are needed for *C. sakazakii* inactivation in milk or fluids containing high levels of UV-C absorbing compounds, lipids and proteins which attributed to absorption and light scattering. Further studies are warranted to study *C. sakazakii* inactivation in dynamic fluid systems accounting for fluid optics.

P2-172 In Vitro Assessment of Co- and Cross-resistance Development in *Listeria monocytogenes* to Different Sanitizer Treatments

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Introduction: *Listeria* is a persistent problem in the food processing environment however its resistance development to commonly used sanitizers is not well understood.

Purpose: To study the effect of different sanitizers when used alone or by rotation on the survival and persistence of *Listeria monocytogenes*

Methods: Three sanitizers (sodium hypochlorite (SH), benzalkonium chloride (BAC) and peroxyacetic acid (PAA)) at different concentrations (4, 8, 16, 32, 64, 128, 256 and 512 ppm) and treatment times (30 s, 1, 2.5 and 5 min) were tested against *L. monocytogenes* (101M and F8385) and *L. innocua* at 10^{6-7} log CFU/mL by following microbroth dilution method. The survived cells at highest sanitizer concentration and treatment time were subsequently isolated and exposed to the same and/or different sanitizers to determine co- and cross-resistance. Multiple exposure tests were performed on both parent and sanitizer resistant strains in Mueller Hinton broth at different concentration of sanitizers (1-32 ppm) and progressive daily transfer to higher sanitizer concentrations. All the experiments were replicated twice, and the data was analyzed by ANOVA using Statistical Package for Social Sciences (SPSS).

Results: *L. innocua* found to be more susceptible to sanitizers treatment compared to *L. monocytogenes*. No significant difference *(P > 0.05)* in the reduction was observed between SH and BAC or SH and PAA for *L. monocytogenes*. A reduction of >5 log units was observed at 512 to 64 ppm compared with ≤ 3 log at 32 and 16 ppm. Increasing the treatment time increased the log reduction. No significant difference *(P > 0.05)* in the reduction was observed between 2.5 and 5 min or 30 s and 1 min treatment. Previously exposed strains were found to be more susceptible than the parent strains. *Listeria* found to survive a maximum of 64 ppm benzalkonium chloride and 128 ppm peroxyacetic acid in multiple exposure tests.

Significance: These findings help better understand the effect of rotation of sanitizers on *L. monocytogenes* and to develop better disinfection protocols.

P2-173 Confirmation of the Presence of *C. cayetanensis* and *C. parvum* from Environmental Water Samples

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Developing Scientist Entrant

Introduction: Throughout the scientific literature there is a lack of validated molecular detection approaches for *Cyclospora* and *Cryptosporidium* in environmental samples. Global trends indicate an increase in foodborne protozoan infections which affect public health within the United States as well as other countries. Gaps in scientific understanding of detection, environmental persistence and transfer, in addition to some lack of *in vitro* and *in vivo* methods to study protozoa viability has limited development of effective control strategies for this pathogen.

Purpose: Identification of *C. cayetanensis* and *C. parvum* and evaluation of sequencing data from environmental samples will aid in clarification of advanced molecular detection tools.

Methods: Seventy-two water samples were collected from various sites in Delaware and Maryland between June 2017 and October 2018. Filtration and concentration of water samples were performed in accordance with EPA 1623 method. DNA was extracted and presumptive results were determined by

quantitative polymerase chain reaction (qPCR). From the 72 environmental samples, 33 presumptive positive *Cyclospora* and 26 presumptive positive *Cryptosporidium* samples. A nested PCR method and Sanger DNA sequencing with the ABI Prism 3130 Genetic Analyzer were used to confirm sample identity and create neighbor-joining phylogenetic trees with bootstrap analysis used with 50 replicates.

Results: One sample, originated from a tidal brackish river, out of 13 presumptive positive environmental samples were identified as *Cyclospora cayetanensis* (99.01%) by sequence analysis. Phylogenetic tree analysis concluded close genetic similarities between *Cyclospora cayetanensis* and *Eimeria bakidnonensis* with 66% support at the node. Other organisms identified through DNA sequencing include genera *Isospora* (47%), *Eimeria* (42%), *Schellackia* (8%) and *Caryospora* (3%). The *Eimeria* animal origin included avian, rodent, bovine, porcine, fish and reptile. All 26 presumptive positive *Cryptosporidium* samples were confirmed positive after nested PCR.

Significance: Development of efficient methods for identification of protozoa is critical for produce safety and likely involves multiple molecular detection methodologies.

P2-174 *Cyclospora cayetanensis* Detection from Soil Samples

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Introduction: *Cyclospora cayetanensis* is a protozoan parasite that causes foodborne outbreaks associated with consumption of fresh produce. Contact with soil may represent an important vehicle in parasite transmission and in the contamination of crops.

Purpose: There is no available method for the detection of *C. cayetanensis* in soil. The present study compared two techniques for the detection of *C. cayetanensis* oocysts in soil: flotation in sucrose concentrated solutions and direct DNA extraction using commercial kits. The limit of detection was then evaluated in soil samples using the technique which showed the best detection results.

Methods: Ten samples of soil (10 g each) were seeded with 100 *C. cayetanensis* oocysts. DNA was directly extracted from five samples using a commercial DNA extraction kit for soil (Power Max Soil DNA isolation kit; MO BIO laboratories), which included the use of a bead-beater homogenizer in 50 mL tubes. The other set of five samples of 10 g of soil was processed by flotation in sucrose concentrated solution (specific gravity: 1.12) followed by DNA isolation and qPCR performed as indicated in the BAM Chapter 19b.

Results: *S*ignificant differences ($P = 0.001$) were observed among average CT values between the two techniques, with statistically lower average CT values (higher detection) in samples processed by flotation (35.1±1.1) *versus* direct soil DNA extraction by a commercial kit (36.7±1.2). The flotation technique was evaluated in soil samples seeded with known numbers of *C. cayetanensis* oocysts and as few as 10 *C. cayetanensis* oocysts in 10 g of soil samples could be detected.

Significance: A method was developed to detect *C. cayetanensis* in soil. The ability to detect *C. cayetanensis* oocysts in soil would help in outbreak investigations and would increase the knowledge on the epidemiology of this important parasite.

P2-175 Concentration and Detection of Human Noroviruses from Food and Environmental Samples Using Engineered Norovirus Binding Bacteria

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❖ ***Developing Scientist Entrant***

Introduction: Human Norovirus remains the ubiquitous cause of non-bacterial gastrointestinal infections across the world and a major cause of foodborne illness in the United States. Research in the field of Norovirus infection is potentially limited by the fact that the virus is present in very low levels in food and environmental samples. Due to this, sample concentration becomes extremely important prior to detection.

Purpose: The purpose of this study was to evaluate the use of representative bacterial strains generally found as part of the human gut microbiome and engineered strains expressing norovirus binding peptides as a tool for concentrating and detecting Human Norovirus (GII.4) from patient stool and environmental samples.

Methods: *Staphylococcus aureus, Enterobacter cloacae, Bacillus* spp*, E. faecium, Klebsiella* spp*, Citrobacter* spp and *H. alvei* were suspended with 100 µL of diluted stool sample containing norovirus GII.4 strain. Following incubation, RT-qPCR was performed for calculating removal of input virus from the supernatant.

Results: Following the suspension assay RT-qPCR, all but two strains (*Bacillus* and *Klebsiella*) were able to capture more than 50% of the input virus sample. All assays were performed in duplicate. Percentage of binding efficiency is determined by loss-to-supernatant (total input virus-virus in supernatant)/ total input virus. Further optimization of the protocol is required to test the capture efficiency of *Bacillus* and *Klebsiella*. *E. coli* carrying plasmids capable of expressing the Ice nucleation protein, Serine-Glycine spacer and norovirus binding peptide will be used for capture experiments.

Significance: While conventional concentration methods like PEG precipitation and membrane filtration while successfully allow for concentration of the virus, they are limited by the carryover of certain inhibitory substances which can be detrimental to downstream detection methods. We hope to circumvent these issues by using an engineered *E. coli* strain expressing a norovirus binding peptide since it can be easily and inexpensively adopted.

P2-176 Evaluation of Vacuum Evaporation as Method for the Concentration of Biological Analytes from Large Volumes of Water and Artificial Saliva

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Introduction: Molecular methods struggle to achieve sensitive detection of biological analytes when faced with large volume (>10 mL) liquid samples. Concentration of the analytes into smaller liquid volumes may overcome this problem. Vacuum evaporation was evaluated as a method to improve the sensitivity of detection of a model biological analyte (synthetic SARS-CoV-2) from water and saliva using a commercially available qPCR for SARS-CoV-2 as the detection method.

Purpose: To determine the feasibility of vacuum evaporation to improve the sensitivity of detection of a commercially available real time qPCR method for the detection of a model biological analyte (synthetic SARS-CoV-2) from large liquid volumes.

Methods: A vacuum evaporation apparatus compatible with 50 mL conical tubes was designed and an alpha prototype constructed**.** Synthetic SARS-CoV-2 was 100 µL used to inoculate 30 mL of deionized H_20 or artificial saliva. Following vacuum evaporation for ~6 h at 60°C to total dryness, samples were rehydrated in 500 µL buffer, taken through a brief RNA extraction-free lysis protocol and then tested by qPCR. Changes in Cq values from inoculation to post rehydration were used to calculate actual concentration factors achieved.

Results: Complete dryness from 30 mL of either liquid was achieved in approximately 6 h. Based on starting and final volumes, the volumetric calculated concentration factor was 60X (~5 Cq). Actual concentration factor achieved for water averaged 6.7 ± 0.43 Cq (high inoculum) or 7.6 ±0.43 Cq (low inoculum). For experiments with inoculated artificial saliva actual concentration factors achieved averaged 1.4 Cq ±0.58 (high inoculum). No detectable RNA was observed at the low inoculum for artificial saliva samples.

Significance: These results demonstrate the feasibility of using vacuum evaporation to improve the sensitivity of detection of biological molecules from large volumes of liquids. The technique may be broadly applicable across a variety of detection platforms.

P2-177 Capture Efficiency and Detection of Hepatitis A Virus on Strawberry Using Apolipoprotein H Coated Magnetic Beads

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Introduction: Foodborne viruses, like human norovirus (hNoV) and hepatitis A virus (HAV), cause lots of health-economic issues. Viruses found in incriminated food are difficult to detect with current standardized methods. It has been recently reported that apolipoprotein H (ApoH) can bind hNoV and has broad spectrum affinity.
Purpose: The purpose of this study was to develop a capture method based on the affinity between ApoH magnetic beads and HAV prior to qRT-PCR detection in different matrices.
Methods: HAV HM-175 and hNoV (strawberry only) were inoculated using five concentrations (10^5 to 10^1 genome copies) in 1 mL and 40 mL of CV-1 buffer (*ApoH Technologies,* La Grande Motte) and on fresh and frozen strawberries. Viral elution from strawberry was performed at room temperature during 10 min in 40 mL of CV-1 buffer. Capture of the virus using ApoH-coated beads (*ApoH Technologies*) was conducted at 4ºC during 15 min after a magnetization of 15 min. Viral extraction was carried out using Nuclisens© buffers and the miniMAG© process (*BioMérieux*, Marcy-l'Étoile). Subsequently, the genome copy concentration was detected by RT-qPCR and the absence/presence value was determined.
Results: Our results show that HAV was detected in 75%, 100% and 100% of 1 mL and 40 mL samples at 10^3, 10^4 and 10^5, respectively. HAV was also detected in 100% of fresh and frozen strawberry samples at 10^4 and 10^5. Finally, hNoV was detected in 100% of fresh and frozen strawberry samples at 10^5 only.
Significance: These data suggest that ApoH has the capacity to bind HAV. This detection method could be an alternative tool used to detect and prevent potential foodborne viral outbreaks.

P2-178 Copper Inactivation of Viruses Affected by Food Solids, pH, and Other Virus Surrounding Environments

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Introduction: Foodborne viruses can be transmitted indirectly by contact with surfaces. MS2 coliphage (a virus surrogate) is stable on glass, polymer and stainless-steel surfaces but is likely inactivated by interaction with copper (Cu) surfaces.
Purpose: This study was to evaluate the impact of foods (with and without particulates), various pH and other components on the interaction between Cu and MS2 in solution.
Methods: MS2 and Cu coupons were deposited into 2.5 mL of 0.5% beef extract (lab grade with pH 4.0, 6.8, 8.9), food grade beef stock (pH 6.7), and strawberry juice (pH 3.6, 70 µm pore-filtered and unfiltered). After the interaction for 20-mins x 150 RPM at 20°C, MS2 levels were quantified by a virus plaque assay. Cu concentrations were quantified by ICP-MS. Results are based on 2-9 trials completed in duplicate.
Results: When deposited directly onto Cu Coupons, MS2 (~5 log) in PBS was inactivated at a rate of 0.95-1.07 log/min ($R^2 \geq 0.85$). Complex components (e.g., food) altered the interaction between Cu and MS2 in suspension by slowing down inactivation. Acidic beef extract resulted in the greatest release of Cu into media, and thus, the greatest reduction of MS2 (0.9ac1 log) when compared to neutral and alkaline beef extracts. The MS2 reductions in the latter two were insignificant, $P = 0.27$ and 0.12, respectively. Among market foods, beef stock provided a stable environment for MS2 while both strawberry juices resulted in losses of MS2 population following Cu exposure. Greater inactivation of MS2 was seen in the filtered strawberry juice (≥ 2.81 log) than in the unfiltered strawberry juice (0.36 log).
Significance: These data suggest that low pH results in greater release of Cu into the virus surrounding environment that inactivates more MS2. This inactivation may be decreased or stopped by the presence of solids that interfere in the MS2-Cu interaction.

P2-179 Use of *In Vivo* Fingerpad Methods to Evaluate the Removal and Inactivation Efficacy of Human Norovirus in Hand Washing/Sanitizing Regimens

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Introduction: Systematic evaluation of hand hygiene efficacy using highly infectious, non-cultivable viruses is difficult. Lab-based whole hand methods (e.g., glove juice) are not feasible and their relevance to fingerpad methods is complicated by the absence of important actions like rubbing, rinsing, and drying.
Purpose: To compare the performance of an alcohol-based hand sanitizer to several liquid soap wash regimens, using lab-based fingerpad assays, for removal and/or inactivation of human norovirus (hNoV) on fingers.
Methods: One commercial alcohol-based hand sanitizer (85% ethanol) and one antimicrobial handwash soap (0.5 % BAK) were evaluated using a hNoV GII.4 Sydney stool inoculum. Fingerpad assays were done in accordance with ASTM E1838-17, with modifications. For the soap product, additional steps were designed to mimic a water rinse (10 s); water rinse followed by disposable paper towel drying; or a wash-dry-hand sanitizer regimen. Ten human volunteers were used in each study with a 30 s sanitizer/soap exposure time. Virus concentration on fingerpads was determined by RNase-RT-qPCR with extrapolation to standard curves to estimate virus inactivation/removal efficiency.
Results: The hand sanitizer produced 3.2 ± 0.3 log reduction in genome equivalent copies (GEC). A mock soap wash, and washing followed by paper towel drying, yielded 0.3 ± 0.18 and 1.2 ± 0.4 log GEC reductions, respectively. The wash-dry-hand sanitizer regimen produced a 3.3 $\pm$ 0.3 log GEC reduction, equivalent to complete removal/inactivation of virus.
Significance: The efficacy of soap and water in inactivation of hNoV from fingerpads, even when followed by a drying step, was low. This is likely an artifact of using the ASTM E1838-17 protocol, which is designed to evaluate efficiency of virus inactivation but not necessarily removal. Better methods are needed to evaluate the efficacy of hand hygiene regimens under circumstances more illustrative of real-world manipulations.

P2-180 Microbial Risk Assessment for Hepatitis A Virus Foodborne Illness by Oyster Consumption in Korea

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Introduction: Hepatitis A virus shows high contamination levels in an oyster. Thus, people may be infected with the hepatitis A virus by consuming raw oysters or insufficiently cooked oysters.
Purpose: The objective of this study was to evaluate the probability of hepatitis A virus foodborne illness by oyster consumption in Korea.
Methods: One hundred fifty-six raw oyster samples from markets were analyzed to evaluate the hepatitis A virus prevalence. Hepatitis A virus titers were enumerated in the oyster samples at 4°C−25°C. The hepatitis A virus titers were fitted to the Baranyi model to calculate shoulder period (h) and death rate (log PFU/g/h). These kinetic parameters were fitted to a polynomial model as a function of temperature. Distribution temperature and time were surveyed, and consumption data for oyster were surveyed. A dose-response model was also searched. With these data, the probability of hepatitis A virus foodborne illness was estimated through the Monte Carlo simulation with @Risk program.
Results: Hepatitis A virus was not detected in oyster samples, and thus, the Beta distribution (1, 157) estimated the initial contamination level to be -5.7 log PFU/g. The developed predictive models showed that the hepatitis A virus titers decreased under the investigated distribution conditions for

oysters; Uniform distribution (0.325, 1.643) for time and Pert distribution (10, 18, 25) for temperature. Average consumption amount of oysters per person was 1.82 g, calculated by the Pert distribution [RiskPert (1.8200, 1.8200, 335.00, RiskTruncate (0,236.8))] at 0.98% of frequency. Beta-Poisson model [1-(1+Dose/186.4)$^{-0.373}$] was the appropriate dose-response model for hepatitis A virus. The simulation showed that the probability of hepatitis A virus foodborne illness by oyster consumption was 1.66×10^{-9} per person per day.

Significance: The results indicate that there is possibility of hepatitis A virus foodborne illness caused by the oyster consumption in Korea.

P2-181 Quantitative Microbial Risk Assessment for Foodborne Viruses by Water Consumption

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Introduction: Water (ground water) is used for drinking, irrigation, and food production and processing. Contaminated water with foodborne viruses such as norovirus and hepatitis A virus (HAV) can lead to large-scale foodborne illnesses, and thus, their risks need to be evaluated.

Purpose: This study evaluated probabilities of norovirus and HAV foodborne illnesses by water consumption.

Methods: To investigate the prevalence of norovirus and HAV, 151 and 1,273 water samples from respective literatures were analyzed. Growth patterns for murine norovirus (norovirus surrogate) and HAV inoculated in water samples were investigated at 4 - 35°C. The viral titers recovered by plaque assay were used to develop predictive models, which describe the kinetic behaviors of the virus. Distribution temperature and time, and water consumption data were surveyed. Each dose-response model for both viruses was searched through literatures. With these data, probabilities of norovirus and HAV foodborne illness were estimated through the Monte Carlo simulation in @RISK.

Results: Of 151 samples, norovirus was all negative, and 1 in 1,273 samples was HAV positive. The developed predictive models showed that the norovirus and HAV titers were decreased at 4 - 35°C. Most people (95.7%) in Korea consume purified water and drink 979.7 mL of water on average per day. $_1F_1$ hypergeometric dose response model [Risk=1-(1+ηCV)$^{-r}$] (η, 2.55×10^{-3}; CV, dose; r, 0.086) was selected as an appropriate model for norovirus, and Beta Poisson model [Risk=1-(1+dose/β)-α] (α, 0.373; β, 186.4) was used for HAV. Simulations using all data showed that the probability of the foodborne illness by water were 4.3×10^{-14}/person/day for norovirus and 9.15×10^{-14}/person/day for HAV.

Significance: The results indicate that the risks for both norovirus and HAV foodborne illnesses by water consumption are low in Korea.

P2-182 Quantitative Microbial Risk Assessment for Norovirus Foodborne Illness by Cucumber Consumption

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❖ ***Developing Scientist Entrant***

Introduction: In many countries, the cases of norovirus foodborne illness is the highest in last 10 years, and the virus is occasionally detected in foods such as fresh vegetables and fruits, which are usually consumed raw.

Purpose: The objective of this study was to assess the risk of norovirus foodborne illness by cucumber consumption.

Methods: One hundred nine cucumber samples collected from markets were analyzed to detect norovirus. Predictive models were developed with the Baranyi model (primary model) fitted to murine norovirus titers collected at 4°C-25°C to calculate kinetic parameters such as μ_{max} (maximum specific growth rate; log PFU/g/h) and *LPD* (lag phase duration; h). The kinetic parameters were fitted with a secondary model. Consumption amounts and frequency for cucumber, and dose-response models for norovirus infection were surveyed. With the collected data, a simulation model was prepared for the Monte Carlo simulation. The probability of norovirus foodborne illness by cucumber consumption was calculated in @Risk.

Results: Of 109 samples, 4 samples were norovirus positive. By analyzing this result with the Beta distribution, initial contamination level was estimated to be -5.8 log PFU/g. The developed predictive models showed that the norovirus titers tended to be maintained, regardless of storage temperature. Thus, the secondary model was not developed. The Lognornal distribution showed that the average consumption amount was 72.08 g at 12.5% of frequency. The combination of these results with the $_1F_1$ hypergeometric dose–response model [P=1-(1+ηCV)$^{-r}$] (η, 2.55×10^{-3}; CV, concentration × volume=dose; r, 0.086)] showed that the probability of norovirus foodborne illness per person per day through the cucumber consumption was 3.79×10^{-9}.

Significance: This result should be useful in evaluating the risk of norovirus foodborne illness by cucumber consumption.

P2-183 Persistence of Phi 6 Bacteriophage on Human Fingerpad

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Poster

Introduction: Phi 6 (φ6) bacteriophage has emerged as a leading surrogate to study enveloped viral pathogens including SARS-CoV-2 due to structural similarities and BSL-1 status. φ6 survival time on human hands has implications for the food industry related to cleaning and sanitation of food processing facilities and food service settings.

Purpose: To determine the survival time of φ6 bacteriophage on human fingerpads.

Methods: Fingerpads were prepared by washing hands with antimicrobial soap for 30 s, rinsing thoroughly, and air-drying completely. Fingers were soaked in 70% ethanol for 30 s and air-dried completely. Designated areas on each fingerpad were inoculated with φ6 (7 log PFU). After 5 min of drying, φ6 was recovered at 0, 5, 10, and 30 min via 1) massaging fingerpads in a 2x3" plastic bag containing 2 mL phosphate buffered saline (PBS) for 1min, 2) vigorously swabbing fingerpads with a polyurethane swab containing 1.5 mL PBS for 10 s, and 3) rubbing fingerpads into a 6-well plate containing 2 mL PBS for 1 min. Samples were diluted, and φ6 was detected by the double agar overlay assay. Experiment was performed in technical duplicates with two experimental trials. One-way ANOVA with Tukey's significant difference test was performed based on recovery method at each time point (P = 0.05).

Results: No significant difference in log PFU reduction was observed between methods at any time point (P > 0.05). Among all methods, the log PFU reduction (mean ± std. dev) was 1.33 ± 0.59, 3.31 ± 1.20, 4.18 ± 0.64, and 5.40 ± 0.89 at 0, 5, 10, and 30 min, respectively.

Significance: The survival time of φ6 on human hands has implications for the food industry and future research focused on appropriate surrogates for enveloped viruses. These data will support future studies involving the transfer of enveloped viruses between hands and surfaces—critical knowledge to determine effective disinfection protocols to control SARS-CoV-2 on environmental surfaces.

P2-184 Evaluation of the Thermofisher Scientific Real-Time SARS-CoV-2 PCR to Detect Surface Contamination of SARS-CoV-2

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Introduction: Ensuring COVID-safe environments prevents the spread of SARS-CoV-2 in the workplace including the food industry. Limiting an individual's exposure to the virus through surface contact is achieved through effective surface cleaning as well as good hand hygiene. Environmental sampling for SARS-CoV-2 allows the monitoring of cleaning efficacy, which in turn provides assurance of appropriate control over a pandemic virus of global concern.

Purpose: The Thermofisher Scientific Real-Time SARS-CoV-2 PCR workflow is a complete solution for environmental monitoring. This study evaluated the use of the Thermo Scientific™ KingFisher mL Food Protection System and Applied Biosystems™ PrepSEQ™ Nucleic Acid Extraction Kit protocol in combination with the Applied Biosystems™ TaqMan™ 2019nCoV PCR Assay to detect SARS-CoV-2 surface contamination.

Methods: The ability of the Thermo Scientific Kingfisher mL protocol and Applied Biosystems Real-Time SARS-CoV-2 PCR to detect different levels of SARS-CoV-2 on stainless steel was assessed. Heat inactivated SARS-CoV-2 virus was diluted to obtain levels of 150 GU to 9,600 GU per coupon. Replicate

stainless steel coupons were inoculated with 100 µL of each dilution and allowed to dry. Pre-moistened swabs were used to swab the stainless steel surface and the assay was completed within 24 h. A comparison was also made with samples processed using an automated RNA extraction platform analyzed using the same Real time PCR assay.

Results: Findings of the study indicate that the Thermofisher Scientific Real-Time SARS-CoV-2 PCR workflow is a sensitive solution for SARS-CoV-2 detection on stainless steel detecting levels as low as 150 GU per 2"x 2" surface analyzed.

Significance: The Thermofisher Scientific Real-Time SARS-CoV-2 PCR workflow offers a timely complete solution for the detection of SARS-CoV2. This assay could be used by food business operators to monitor viral contamination of environmental surfaces as well as on food packaging.

P2-185 Scale of Analysis Drives the Observed Ratio of Spatial to Non-Spatial Variance in Fecal Indicator Bacteria Levels in Upstate New York Surface Water: Insights from Two Decades of Citizen Science Data

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Introduction: Programs for managing risks associated with agricultural or recreational surface water use rely on *E. coli* monitoring. However, recent studies reported substantial spatial and temporal variations in *E. coli* levels and different patterns of variation at different scales of analysis (SOA).

Purpose: To assess how SOA affects the percent variance in *E. coli* levels attributable to spatial and temporal factors, and to quantify associations between environmental factors and *E. coli* levels.

Methods: This study used publicly-available citizen science data from NY waterways collected between 2002 and 2020 (Samples = 11,326). Bayesian models of coregionalization were used to quantify the percent spatial variance (PSV) and the associations between environmental factors and *E. coli* levels. All analyses were performed separately for each waterway (*N* = 55), each watershed (*N* = 4), and for all data combined (state-level).

Results: The PSV depended on the SOA. Spatial factors accounted for less variance in *E. coli* levels than temporal factors in waterway models (Interquartile Range [IQR] for PSV = 7-13%) but not in watershed (IQR = 21-27%) or state (IQR = 33-37%) models. *E. coli* levels were strongly seasonal, between 0.3 and 2.3 log higher in August than in April depending on waterway, water type, and SOA (median log difference between August and April = 0.55; IQR = 0.46-0.65). There were also positive effects of storm events compared to baseflow (IQR = 0.38-0.53 log CFU/100 mL), wastewater outfall presence (IQR = 0.26-0.59 log CFU/100 mL), and turbidity (IQR = 0.39-0.50 log CFU/100 mL). While the magnitude of effect for most factors was constant across all SOA; the effect of point source presence (e.g., wastewater outfall) was higher in waterway models than in watershed and state models, but the opposite was observed for variables related to non-point sources (e.g., percent watershed under pasture).

Significance: This study demonstrates the importance of scale when designing monitoring studies, analyzing water quality data, or using the results to guide management decisions.

P2-186 Fecal Contamination of Northeastern Streams That Span an Urban-Rural Gradient is Associated with Land Use and Physicochemical Water Quality

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Introduction: Fecal contamination of agricultural water has been associated with multiple enteric disease outbreaks and food recalls. Thus, it is important to understand factors associated with fecal contamination of agricultural water sources. Fecal indicator bacteria (FIB) are used to monitor surface water for potential fecal contamination.

Purpose: The purpose was to characterize associations between environmental factors, and (i) fecal indicator bacteria (FIBs) levels, and (ii) host-specific fecal marker detection.

Methods: This study used citizen science data collected from 23 sites along 3 waterways, each of which spanned the rural-urban gradient between Syracuse, NY and its agricultural hinterlands. Between 2008 and 2017, 2,816 water samples were collected, and *E. coli, Enterococcus,* and/or fecal and total coliform concentrations enumerated. A subset of samples (*N* = 30) were tested for human and ruminant fecal markers. Water quality (e.g., pH, turbidity) and weather (e.g., temperature, rainfall) data were also collected for each sample. Bayesian mixed models, which are robust to small sample sizes, were used to characterize the relationship between each microbial target, and land use, water quality and weather factors. For each model, probability of direction and region of practical equivalence overlap (ROPE) were calculated to characterize the direction and strength, respectively, of the association.

Results: All FIB showed evidence of strong seasonal trends with FIB levels being substantially higher in summer compared to all other seasons. For example, *E. coli* levels were 0.6 log [95% Credibility Interval (CI) = 0.3-1.0] higher in July compared to November. Fecal contamination was also strongly associated with land use. Log *E. coli* concentration (log) increased by 0.3 (CI = 0.2-0.4) for each percent increase in pasture cover in a 1,098 m buffer around the sample site. Similarly, for each percent increase in pasture cover within 122 m, the probability of detecting ruminant contamination increased by 30% (CI = 10-50%).

Significance: These findings highlight the importance of considering non-point sources of contamination when developing strategies for managing produce safety hazards associated with fecal contamination of agricultural water.

P2-187 Risk-based Evaluation of Treatments for Water Used at a Pre-Harvest Stage to Mitigate *E. coli* on Fresh Raspberry in Chile

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❖ ***Developing Scientist Entrant***

Introduction: Water has long been identified as one of the most significant sources of microbial contamination in produce, which can negatively impact human health. Previous results of a quantitative microbial risk assessment model indicated that water used for pesticide application is the main entry point of generic *E. coli* in raspberries produced in small Chilean orchards.

Purpose: The purpose of this study was to identify water treatments that can effectively mitigate *E. coli* in water and are feasible to be implemented at small-scale raspberry farms.

Methods: To compare the efficacy of various treatments in controlling *E. coli* in water, a rapid systematic review of studies in English and Spanish was conducted by searching electronic databases including Web of Science Core Collection (1900-2019), Scopus (1959-2019), Medline (1950-2019) and CABI (1910-2019). The search focused on established water treatment technologies applied in freshwater sources, excluding those at proof-of-concept stage. The feasibility analysis covered technological, managerial, and sustainability criteria, considering Chile-specific situations.

Results: From 11,762 identified citations, 42 were considered relevant. Articles corresponded to filtration-based interventions (22), chemical treatments (10), ultraviolet light (UV) (8), and combined treatments (2). Results showed variable efficacy against *E. coli,* but UV light and a multi-barrier approach (ozone-chlorine) exhibited higher log inactivation levels (> 7 log). Based on the risk-based evaluation, a ≥3-log reduction was recommended for groundwater sources, while more effective technologies should be considered for surface water to reach an efficacy of up to 6-log reduction.

Significance: Albeit significant data gap in the current literature on disinfection methods applied in agricultural water at a pre-harvest stage was identified, our study critically reviewed and analyzed data currently available in literature, results of which can assist the Chilean food safety authorities with science-based decision on water treatment method adoption and implementation.

P2-188 Withdrawn

P2-189 Comparison of Culture and PCR-based Methods for the Detection of *Salmonella* spp. and *Listeria monocytogenes* in Nontraditional Irrigation Water on Maryland's Eastern Shore: A Conserve Study

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❖ ***Developing Scientist Entrant***

Introduction: Non-traditional waters (NTW), while possible alternatives to traditional irrigation sources, can harbor *Salmonella* spp. *(Sal)* and *Listeria monocytogenes (Lm)*. Culture-based methods (CM) to detect these pathogens are time-consuming; the development of rapid detection (PCR-based, PM) methods would be beneficial to produce growers.

Purpose: To evaluate the detection of *Sal* and *Lm* by CM and PM in NTW.

Methods: Samples were collected by filtration through modified Moore swabs (MMS) from October 2016-October 2018 from two reclaimed water plants (RW- MA01, MA02), one tidal/brackish river (TR- MA08), and three non-tidal freshwater creeks (NC- MA03, MA07, MA09) on Maryland's Eastern Shore. A modified MPN procedure with three volumes (0.1 L, 1 L, and10 L) was employed. MMS samples were enriched in Universal Pre-enrichment Broth. For PM, DNA from UPB enriched samples (n = 163) was quantified by multiplex real-time PCR (RT-PCR) specific for *Sal* and *Lm*. For CM, enriched samples (n = 155) were subjected to secondary enrichment followed by plating on selective media, and RT-PCR confirmation of presumptive isolates. Differences in *Sal* and *Lm* between CM and PM were tested using Wilcoxon signed rank tests.

Results: *Sal* and *Lm* MPN levels were greater for PM than CM in 2 sites; and *Lm* MPN levels were greater for CM than PM in 1 site. *Sal* and *Lm* MPN values generated from PM were significantly (P < 0.05) greater than MPN values from CM for MA01 and MA02. Seasonality showed *Lm* MPN values significantly (P < 0.05) greater from CM than from PM during spring. *Sal* MPN values were significantly (P < 0.0001) greater from PM than CM during winter. *Lm* MPN values in NC were significantly (P < 0.05) greater from CM than PM.

Significance: This study shows that PM can overestimate *Sal* and *Lm* levels in specific circumstances. Analysis by PM can be used to prioritize water samples for further pathogen analysis or quantification.

P2-190 Recovery of *Salmonella enterica* and *Listeria monocytogenes* in Surface Waters By Rapid and Culture-Based Methods: A Conserve Study

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Introduction: *Salmonella enterica* (*Se*) and *Listeria monocytogenes* (*Lm)* can be present in irrigation water, potentially contaminating produce and causing foodborne illness. Accelerating the testing or screening process for *Se* or *Lm* would benefit many produce safety stakeholders in their irrigation water management.

Purpose: To determine if a rapid method (RM) to detect the presence of *Se*/*Lm* in surface waters is equivalent to using a culture-based method (CM).

Methods: Water from six surface or reclaimed sites in the Mid-Atlantic U.S. were surveyed by filtering three separate volumes (10 L, 1 L, and 0.1 L) through Modified Moore Swabs (MMS) at 170 sampling events from September 2016–October 2018. For CM methods, MMS were incubated in universal pre-enrichment broth (UPB), followed by enrichment in selective broths and isolation on selective agar for *Se* and *Lm* detection. For RM methods, DNA extracted from UPB pre-enrichments was amplified using multiplex real-time PCR specific for *Se* and *Lm* to determine their presence/absence. For CM methods, presumptive isolates were confirmed by RT-PCR. Odds ratio (ORs) with 95% confidence interval (CI) were calculated to evaluate associations between RM and CM findings.

Results: CM detected *Se* and *Lm* in 50% (n = 168) and 31% (n = 170) of sampling events, respectively. The absence/presence of *Se* as determined by RM was significantly (P < 0.001) associated with results of CM (OR:16.61, CI:7.20,38.34). Similar associations between RM and CM results for *Lm* were also found (OR:7.13, CI:3.40,13.96). When analyzing MMS from 10 L volumes, OR for *Se* (23.85, CI: 10.06,56.57) and *Lm* (11.27, CI: 4.35, 29.17) detection by RM were significantly (P < 0.001) associated with results by CM.

Significance: Rapid methods used for the detection of *Salmonella* and *L. monocytogenes* provide similar levels of accuracy compared to culture-based methods, and may expedite screening and testing times for these pathogens in irrigation water.

P2-191 Withdrawn

P3-01 Analysis of FSMA Produce Safety and Preventive Controls for Human Food Trainings in the Western Region of the United States

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❖ ***Developing Scientist Entrant***

Introduction: The Western Regional Center to Enhance Food Safety (WRCEFS) is one of four U.S. regional centers that coordinates Food Safety Modernization Act food safety training programs in 13 states and two territories. Two types of trainings are currently offered, grower trainings (GTs) available through the Produce Safety Alliance (PSA) and Preventive Controls for Human Food trainings for preventive controls qualified individuals (PCQI) available through the Food Safety Preventive Controls Alliance (FSPCA).

Purpose: Evaluate the knowledge gained by participants in PSA and FSPCA trainings in the western region.

Methods: In-person (n = 57 PSA; n = 5 FSPCA) and remote delivery (n = 22 PSA; n = 3 FSPCA) trainings were conducted in six states. Trainers used standardized questionnaires to assess pre/post-training knowledge of participants on preventive controls (15 questions) or produce safety (25 questions) information. Data were averaged across the states and the region, and overall participant numbers and training sessions were reported.

Results: Overall, 2,211 participants (GTs-PSA: 1,981; PCQI-FSPCA: 230) attended 111 training sessions (PSA: 97; FSPCA: 14). Of those, 1,820 participants (GTs: 1,643, PCQI: 177) took pre/post-knowledge surveys. The range of PSA trainings per state was 4-36, while the range of FSPCA trainings was 2-4. Overall, the average pre/post-test scores for PSA trainings were 16.39 (66%) and 20.79 (83%), respectively, with 18% increase in knowledge gain. Both in-person (4.34) and remote (4.76) PSA trainings had similar knowledge gain. For FSPCA trainings, the average pre/post-test scores were 6.53 (44%) and 8.55 (57%), respectively, with 13% increase in knowledge gain. A slight decrease in knowledge gain was seen for remote FSPCA trainings (1.26) compared to in-person

trainings (2.33).

Significance: Gains in participant knowledge were seen across all states in both trainings. PSA grower trainings knowledge gain and post-test scores demonstrate a strong understanding of the material, whereas the lower scores of the FSPCA trainings warrant further analysis.

P3-02 Improving Access and Motivation for Small and Medium Processors in the Northeast to Comply with FSMA's Preventive Controls Rule

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Introduction: Small and medium-sized food processors face significant regulatory hurdles to implementing Preventive Controls (PC) for Human Food found within the Food Safety Modernization Act (FSMA). Therefore, new strategies are needed to help increase their knowledge and motivation in building and implementing food safety plans for their businesses.

Purpose: This project developed targeted educational and technical support interventions specific to the needs of small food processors by providing a 3-part virtual training series, including, a 1-hour webinar, a 3-hour workshop, and 3-day PCQI certification course designed specifically for small and medium-sized processors.

Methods: Pre- and post-program evaluations were administered to measure knowledge and food safety behavior throughout each educational intervention. Results were statistically analyzed (*t*-test) using SAS software and reported at $P < 0.05$.

Results: The educational interventions resulted in 81 students participating in the 1-hour webinar. While the overall results reported that this first intervention did not have a statistical change in overall knowledge, there was a 75% retention rate of participants that chose to participate in the second intervention (3-hour workshop) and 25 participants that attended the third (the 3-day PCQI training). Program evaluations from the later interventions are currently pending analysis, however, participants stated that they were "better prepared", the training made things "more attainable and less intimidating" and "gave them (me) the tools" for developing and implementing scale appropriate food safety plans. Results suggest that the use of a 3-part intervention series increased overall knowledge, confidence, and the likelihood of building and implementing a food safety plan for their respective businesses.

Significance: Providing targeted educational programming that caters to the scale appropriate needs of participants helps to increase the number of processors nationwide in developing and implementing food safety plans thereby reducing the overall risk of foodborne illness.

P3-03 Evaluation of the Southern Center for FSMA Training

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Introduction: Compliance with the Food Safety Modernization Act's Produce Safety Rule (PSR) and Preventive Controls for Human Foods Rule (PCHF) requires training, outreach, and technical assistance for owners and operators of small and medium-sized farms, beginning farmers, socially disadvantaged farmers, small food processors, and small fruit and vegetable wholesale merchants.

Purpose: The Southern Center for FSMA Training (SC) is a consortium of 22 institutions aimed at enhancing produce safety in 13 southern states and 2 territories through a variety of educational means, including conducting and evaluating standardized courses (Produce Safety Alliance (PSA) and Food Safety Preventive Controls Alliance (FSPCA) Preventive Controls Qualified Individual (PCQI)) targeting the PSR and PCHF.

Methods: Validated pre/post-tests were given at PSA and FSPCA PCQI courses to assess short-term knowledge gains from October 2019-September 2020 across the SC. A quantitative evaluation was conducted with participants four months after training to evaluate medium-term outcomes of behavior change related to food safety practices.

Results: FSPCA PCQI post-test scores ($n = 20$) were not significantly higher than pre-test scores ($T = -1.56$, $P < 0.05$), indicating there was not a significant increase in knowledge after participation in the training. Of seven practices included in the FSPCA PCQI behavior change surveys ($n = 20$) the most frequent behavior changes were recommending the training to others (30%), fine-tuning existing food safety plans (15%), and implementing new food safety plans (15%). PSA post-test scores ($n = 987$) were significantly higher than pre-test scores ($T = -39.69$, $P < 0.05$), indicating a significant increase in knowledge. Of 13 practices included in the PSA surveys ($n = 48$) the most frequent behavior changes were creation or modification of food safety record-keeping systems (44.2%) and beginning to write or modify farm food safety plans (44.2%).

Significance: Trainings have increased stakeholders' produce safety knowledge, food safety practices, and preparation for the PSR and PCHF.

P3-04 A New Communication Tool across Agencies

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Introduction: The credibility of a governmental agency depends in part on the accuracy of information it provides, timeliness of sharing information, and transparency. During foodborne illness outbreaks, consumers and retailers look to the U.S. Food and Drug Administration (FDA) as a credible and trustworthy source of information to educate, inform, and provide advice to protect themselves or their customers.

Purpose: To demonstrate the FDA Coordinated Outbreak and Response Evaluation Network (CORE) Communications Team novel approaches and

strategies to educate and communicate to the public during foodborne illness outbreaks and protect public health.

Methods: In November 2020, FDA, the Centers for Disease Control and Prevention, and the U.S. Department of Agriculture Food Safety Inspection Service developed new communication tools to notify the public of outbreaks earlier in the investigation. FDA's CORE has developed a new tool to publicly share the status of outbreak investigations being coordinated by the CORE Response Teams, the CORE Investigation Table.

Results: The CORE Investigation Table is a tool that allows FDA to share information quickly and accurately, even in the early stages of an outbreak investigation when significant details may be yet unknown, and thus reinforces the need for consumers to adhere to general food safety advice. Additionally, it provides an easy to access and centralized list of ongoing outbreaks, which streamlines review for consumers and retailers. After six months of the table's deployment, FDA will evaluate the table and reach out to stakeholders to see if improvements can be made. A survey will be deployed via a Survey Monkey link in summer of 2021 to obtain feedback that stakeholder groups may have.

Significance: The CORE investigation table is one of many of FDA's recent innovations in communications strategies. CORE responds to approximately 20-30 FDA-food related outbreaks per year, and issues around 15 outbreak advisories a year. This new tool enables the Agency to be more proactive and forthcoming in sharing as much detailed information as possible, while promoting transparency and maintaining credibility and the public trust.

P3-05 Evaluation of the Online Produce Safety Alliance Grower Training Course

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Introduction: The Produce Safety Alliance (PSA) Grower Training (GT) was designed to meet the educational requirement in § 112.22(c) of the FSMA Produce Safety Rule (PSR) in approximately eight hours of in-person learning. Online delivery of the PSA GT was developed to meet the needs of growers for whom the in-person course is not a good option due to schedule, location, or other challenges. It is a self-paced course that is more robust and demanding due to innovative add-ons and completion is expected to take 15-30 hours over a three-week period.

Purpose: Evaluation data from 295 participants who completed the Online PSA GT from February 2020 to February 2021 were analyzed to assess perceived knowledge gain, confidence in implementing food safety practices, demographic information, and the impact of engagement elements (i.e., videos, quizzes, case studies, discussions with instructor feedback) on participants' understanding.

Methods: Descriptive statistics were analyzed with SPSS, with the Pearson chi-square test used to identify relationships between demographics, previous produce safety training, and course sufficiency.

Results: More than 97% of participants (n = 295) indicated that the Online PSA GT was sufficient to guide them in implementing regulatory requirements, with 87.8% (n = 206) responding that they agreed or strongly agreed that the required discussions helped with understanding how the PSR applied to their farm. Prior to attending the Online PSA GT, 28.1% (n = 206) of participants had no previous online course experience and 76.2% (n = 295) had no previous produce safety or Good Agricultural Practices training. Previous produce safety training and demographics had no significant relationship with course sufficiency.

Significance: Participants are enrolling in the Online PSA GT despite a higher registration cost and increased participation requirements. These results indicate this delivery method is an innovative way to meet the educational requirement in § 112.22(c) that increases understanding and is valued by participants.

P3-06 Use of In-Depth Interviews to Identify Barriers to Consumers Adopting a Recommended Food-Handling Practice

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Introduction: Despite the U.S. Department of Agriculture recommendation that consumers not wash raw poultry, some consumers continue to practice this behavior even when they are aware of the recommended behavior.

Purpose: The purpose of this study was to utilize the qualitative research method of in-depth interviews to better understand why individuals may be resistant to the message to not wash raw poultry so that future education messages can address identified barriers.

Methods: A questioning route was developed based on the theory of planned behavior. It allowed for both structured and flexible questions to be asked throughout the interview depending upon individual participant responses. Recruitment was conducted via social media and snowballing. Eligible participants were those who washed or rinsed raw poultry despite being aware of the recommendation not to wash it. Interviews lasted approximately 60 minutes and were conducted via Zoom. All interviews were recorded and transcribed. Thematic analysis of the data was performed to identify barriers to consumers adopting the recommended behavior.

Results: Twenty-four interviews were completed. Preliminary thematic analysis revealed that cultural identity contributes to consumers' food handling practices. Where and from whom individuals learned how to cook contributed significantly to their food handling practices. Individuals felt that their washing procedures were not increasing their risk for contracting foodborne illness. Rather, they felt that their cleaning procedures after washing the chicken were sufficient to prevent foodborne illness. Consumers also indicated that they preferred food safety recommendations be provided by more relatable sources and include messages about why the behavior was unsafe. Trust in food processing procedures was also a common theme.

Significance: The results of this study highlight the need to develop targeted messages that explain the importance of preventing cross-contamination that may occur from washing chicken. Further development and improved dissemination of the message are needed to better reach individuals who are resistant to changing these behaviors.

P3-07 Assessment of the Effectiveness of a Piloted Online Delivery of Personal Health and Hygiene Program for Small Food Processor in Iowa

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Introduction: Implementing best practices for compliance with Good Manufacturing Practices (GMPs) is rooted in good personal health and hygiene programs. Personnel is considered critical sources of contamination of food contact surfaces, thus causing foodborne illness.

Purpose: This study's objective was to assess the changes in participants' attitudes, perceptions, intentions, and behavior towards food safety practices using the four constructs from the Theory of Planned Behavior.

Methods: Seventeen participants enrolled and completed an online pilot module on cGMPs on personnel. The effectiveness of the online module was measured by examining participants a pre-and post-assessment. Descriptive statistics (frequency, percentage, mean, and standard deviation) and Wilcoxon signed ranks tests were used for the analysis.

Results: Eleven people (females, n = 9; race: white, n = 9, African American, n = 1, Asian, n = 1; Age: M = 34.2, SD = 13.9) completed pre and post-test assessments of perception of personal preparation, intention to use safe food practices, perceived social pressure to use safe food practices, attitude toward safe food practices in a public environment for food sales, and stage of change for food operation. Pre- and post-assessments were matched for the analysis. On all constructs of perception of personal preparation, intention to use safe food practices, perceived social pressure to use safe food practices, attitude toward safe food practices in a public environment for food sales, and stage of change for food operation, there were slight increases in the scores from pre-test to post-test. The changes from pre to post were not statistically significant.

Significance: These findings were used to improve the transition of the remaining modules. The transition will include additional resources to reinforce participants' practices to positive food safety actions on the food production floor.

P3-08 South Eastern United States Hydroponic Grower Food Safety Needs Assessment

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Undergraduate Student Award Entrant

Introduction: The Produce Safety Rule, based on the Food Safety Modernization Act (FSMA), aims to reduce the risk of microbial contamination on farms proactively; however, there are no hydroponic-specific guidelines for growers practicing Controlled Environmental Agriculture (CEA).

Purpose: The purpose of this project was to conduct one-on-one interviews with hydroponic growers and identify current practices, resources, and pain points Based on this, a needs assessment was carried out and food safety modules were designed.

Methods: Qualitative structured interviews were conducted with hydroponic crop growers and subject matter experts (SMEs) to identify general and food safety challenges that hydroponic growers experience. The goal was to determine baseline food safety knowledge, current sanitation practices, and problems that growers encounter. To this end, data was collected from 7 hydroponic growers and SMEs in the state of Texas.

Results: Growers who were interviewed conveyed the need for food safety training specific to their farm type. Qualitative interview data was categorized manually, and the following 4 themes were identified to include in hydroponic specific training: a. food safety record keeping; b. cost of hydroponic materials; c. training based on crop distinctions; and d. equipment maintenance.

Significance: This study was the first step to design a food safety toolkit specifically for hydroponic growers. The toolkit will be designed by gaining valuable insight directly from stakeholders and grower input will be obtained during each design phase.

P3-09 Food Safety Smart Tools: Big Data, from Hazard Analysis to Weather Forecasting for a Revolutionary Future Smart Food Safety Management

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Introduction: Big Data are actually in common use in different field, also available for Food Industries. We proposed the use of Big Data to develop Hazard Analysis, assess weather forecasting, assess geopolits situations and finally predict and assess future global pandemy.

Purpose: We developed new predictive tools for a smarter food safety management.

Methods: We asked to available existing Big Data platforms to be used by a group of 3 food industries from July to December 2020. First platform used was one for International Hazard assessment to help industries verifed hazard linked to geographical origin; second platform used was specialized in Geopolitics prediction able to assess semestral political trends in a specific country; third platform was able to predict weather condition with an accuracy of 6 mounth to better schedule and prevent agronomic strategy, climate disaster, international transport; fourth platform was able to predict future public health pandemy.

Results: The 6-month predictive assessment was used by an Olive Oils plant, a Cheese Plant and a Canner of Vegetable products. All the predictions were successfully used by Olive Oils plant and Canner reducing by 65% lost of agricultural raw products during their seasonal harvest time, optimizing their manufacturing process and identifying new safe market for trading with new trade opportunity estimated in a +10% in average sales in comparison to last 2019. The Cheese plant avoid to sent in wrong period its sea container choosing faster period thanks weather prediction (15% more faster than in the same period of 2019).

Significance: An unbelievable plus feedback to increase strategic procedures and develop a real stronger food safety.

P3-10 Improving Preventive Controls Prerequisite Education Programs to Address Food Safety Knowledge Gaps for Small- and Medium-sized Food Processors

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Developing Scientist Entrant

Introduction: Foodborne illnesses affect over 48 million people every year. Challenges regarding foodborne outbreaks stem from lack of education and technical support for food processors that are directly working with food products. Previous work indicates that the lack in understanding limits small processors' capacity to properly establish appropriate food safety controls.

Purpose: This study conducted a comparative evaluation series to determine knowledge of small and medium-sized processors within the Northeast prior to and following a 1-hour educational webinar on Preventive Controls for Human Foods.

Methods: Food safety knowledge assessment evaluations were distributed to 30 participants as a pre- and post-survey for a 1-hour webinar. The survey consisted of 15 questions where participants had to rank their knowledge of food safety topics on a scale from 1-5, 1 being no knowledge and 5 being extremely knowledgeable. Knowledge assessments were statistically analyzed (*t*-test) to determine a change in knowledge after the educational intervention. All processors participating had varying levels of food safety knowledge prior to attending.

Results: Overall, the results showed that the average score for each question was lower after the participants took the 1-hour webinar. When a two-tailed *t*-test was conducted, responses from 30 participants reported that there were no statistical differences between pre- and post- overall knowledge. Interestingly, there was a 75% retention rate of participants that chose to participate in an additional 3-hour workshop following the webinar that provides further details pertaining to food safety risk and food safety regulations. Post-program interviews confirmed that the lack of increase in knowledge attributed to self-discovery about certain food safety practices/knowledge.

Significance: The survey results indicate that participants may have learned that there is more education needed for food processors to help manage food safety risks. This is important as additional awareness and/or interventions may help to improve food safety practices.

P3-11 Evaluation of a Virtual Food Safety Hands-on Program for the Future Food Industry Workforce: A Pilot Test of the Sanitation Module

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Developing Scientist Entrant

Introduction: University students majoring in Food Science or related areas are the future workforce of the food industry. Hands-on experience can guide them to understand better food industries' procedures to comply with food regulations, such as the food safety regulation. Virtual serious games can be a solution to provide feasible hands-on food safety training for university students willing to work in the food industry.

Purpose: Develop virtual food safety hands-on training modules for university students, and evaluate the effectiveness of one of the modules, using the Theory of Planned Behavior.

Methods: A virtual hands-on serious game was developed by Purdue Envision Team and reviewed by food safety experts. This abstract will present the evaluation of the module "sanitation." The evaluation measurement (pre- and post-surveys) was developed based on the Theory of Planned Behavior. The surveys consist of 20 and 19 questions from the pre- and post-survey, respectively, including questions of sociodemographic characteristics and questions measuring sanitation knowledge, attitude, subjective norm, perceived behavioral control, behavior changed intention, and the program's format. Students

were recruited through an email invitation.

Results: Thirty-nine university students completed both pre- and post-surveys to evaluate the "sanitation" module. Among participants, 67% were females, 38% were majoring in Food Science, and more than two-thirds (69%) did not have prior food safety training. The module significantly increased participants' awareness of the cleaning and sanitizing procedures and their confidence and willingness to perform these procedures. Participants agreed that the program's delivery format met their expectations and was helpful in understanding the topic. Also, participants had some suggestions to improve the module. The most mentioned was to incorporate audio in the demonstration.

Significance: The findings will support developing virtual food safety educational tools to provide hands-on training for those willing to enter the food industry workforce.

P3-12 A Year in Review: Western Regional Center to Enhance Food Safety Engagement with a Regional and Global Community through Web-based Platforms

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Introduction: The Western Regional Center to Enhance Food Safety (WRCEFS) coordinates Food Safety Modernization Act food safety training programs and supports food safety trainers in 13 western states and 2 territories. Although WRCEFS has had an online presence through a website (https://agsci.oregonstate.edu/wrcefs) for several years, no data were available prior to 2020 to understand the Center's regional and global reach and online engagement.

Purpose: Identify the main audience and their interaction with the WRCEFS web-based platforms.

Methods: Google analytics was used to track activity on the WRCEFS website from January 22nd to December 31st, 2020. The data analyzed included number of users, page views, devices used to access the page, viewer demographics, and types of documents/themes that are most viewed. Twitter activity was tracked with Twitter analytics.

Results: The WRCEFS website was viewed 9,614 times by 3,091 users. Combined, these viewers logged 4,541 sessions, accessing the website in 70 countries and territories, and 828 cities. Over 75% of viewers (2,334) used desktop computers to access the website, whereas mobile and tablet viewers accounted for 25% of viewership (629 and 129 viewers, respectively). The most common ways viewers reached the website was through directly typing in the website URL (38%; 1,184), referrals from other websites (29%; 919), and searches by search engines (25%; 812). The three most commonly viewed pages were the COVID-19 and food safety resource page (4,510 views), homepage (2,016 views), and the resource page (566 views). On Twitter, 64 tweets were created. These tweets received 20,681 impressions, 762 engagements, and 74 retweets.

Significance: Using analytics data helped improve the WRCEFS website and attract new users, increasing the Center's overall reach and impact. This work highlights the value of using analytics data to better understand online communities and tailor design and content to better serve the target audience(s).

P3-13 Food Safety Knowledge across Generations Among Cambodian and Laotian Refugee Communities: Community Bases Cross-Sectional Study

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Introduction: Foodborne illness is a public health hazard that knows no boundaries. Each year, one in six Americans get sick from eating contaminated food. Although there are many factors that influence risks for foodborne illnesses, most are related to poor food handing behaviors. Certain minority groups within the U.S. have a higher risk than the general population for developing foodborne illnesses. Although there have been studies conducted in the U.S. to investigate food safety practices of minority populations, little research has been conducted in Cambodian and Laotian refugee communities.

Purpose: The purpose of the study was to assess community members' knowledge of safe food handling practices for preparing foods.

Methods: A cross-sectional study was conducted by surveying food safety knowledge of 48 male and female Cambodian-Laotian adults over age 18. A food safety questionnaire was loaded into iPads in English, Khmer, and Lao (community members' native languages). Chi square test of association was performed to test for significant associations between socio-demographics and food safety knowledge/attitudes.

Results: The majority of the participants appeared to have adequate knowledge regarding the personal hygiene practice of hand washing, time-temperature control, and cross-contamination practices. Most of the participants agreed that anyone can get sick from food poisoning and believed that food poisoning could occur this year. However, 60.4% did not know the correct reheating temperature to prevent food poisoning. Overall lack of food safety knowledge was significantly associated with ethnicity, age, and place of birth ($P < .05$).

Significance: There is a need for the development of educational messages and for raising consumer awareness of food safety practices among Cambodian and Laotian communities in the U.S. Efforts should be made to educate all ages but with emphasis on young and elderly community members because of their tendency to have lower abilities to ward off diseases.

P3-14 Food Safety Education Among Food Handlers in China: A Review

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❖ ***Developing Scientist Entrant***

Introduction: Food safety is a significant public health challenge in many countries. Approximately 200 million foodborne illnesses occur annually in China. Many foodborne illnesses have been linked to food handlers' improper food handling practices. Thus, an increasing number of studies investigated food handlers' food safety education needs and developed food safety education programs in the country, but there is no comprehensive review of those efforts.

Purpose: Understand food safety knowledge, attitudes, and practices of food handlers in China and evaluate the effectiveness of previous food safety education interventions.

Methods: A search of online databases and journal archives was conducted in English and Chinese to locate published studies on food safety education among food handlers in China. The inclusion criteria are (1) reporting primary research data from mainland China; (2) in English or Chinese between 2000-2020; and (3) assessing food safety knowledge, attitudes, and practices, or evaluating the effectiveness of food safety education programs for Chinese food handlers.

Results: Thirty-five studies were selected. Many food handlers lacked understanding of foodborne pathogens and were not aware of certain safe food handling practices. Cleaning and sanitation practices, leftover foods handling, and temperature control were the least recognized food handling practices. Moreover, it was also reported that the Chinese food handlers did not always follow food safety practices such as using cooking thermometers (94%), using cooler bags for raw meat transportation (90%), and using soap when washing hands (84%). Thirteen studies evaluated the effectiveness of food safety education interventions in China with knowledge change as the major indicator of the intervention effectiveness. All interventions increased food handlers' knowledge, eight improved attitudes, and only six improved behavior.

Significance: The findings support the need for food safety education programs for food handlers and consumers in China and identified the areas where the knowledge gap was greatest.

P3-15 Evaluation of Asian Restaurant Managers' and Chefs' Food Safety Knowledge and Practice of Handling Dried Wood Ear Mushrooms in the United States

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❖ ***Developing Scientist Entrant***

Introduction: Dried wood ear mushroom is a common food ingredient in Asian cuisine. Like other low-moisture foods, historically dried wood ear mushroom has not been considered as a source for foodborne pathogens. A recent *Salmonella* Stanley outbreak was associated with wood ear mushrooms sold to restaurants, which sickened over 50 individuals with six hospitalized. The mechanism by which contaminated dried wood ear mushroom leads to human illness has not been elucidated.

Purpose: This study evaluated Asian restaurant managers' and chefs' food safety knowledge and practices of handling wood ear mushrooms using telephone interviews.

Methods: The interview assesses restaurant managers' and chefs' (1) food safety knowledge and handling practices of dried wood ear mushrooms; (2) perceptions of potential risk of low-moisture food ingredient; (3) knowledge of and practices after hearing about a food recall. The interview questions were reviewed by two food safety research experts and pilot tested among 3 restaurant managers prior to administering. All participants were restaurant managers or chefs who had experience handling dried wood ear mushrooms in the United States.

Results: Fifteen restaurant managers or chefs participated in the interview. Most participants were working in an Asian cuisine restaurant. Almost all participants had heard of food recalls before, but none of them had heard of recalls related to dried wood ear mushrooms. One-third of the participants perceived that low-moisture foods had no or very low microbiological food safety risks. Fourteen participants mentioned that they would clean the utensils and equipment after handling wood ear mushrooms, but only half of them would sanitize after cleaning.

Significance: Findings could help food safety professionals increase the effectiveness of educational programs directed toward restaurants, especially those deliver ethnic cuisine with dried wood ear mushrooms. The study will also shed light on food recall communications among this hard-to-reach audience.

P3-16 Developing Food Safety Education Program for Low Socioeconomic Families with Young Children: An Application of the Theory of Planned Behavior

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❖ ***Developing Scientist Entrant***

Introduction: Consumers of low-socioeconomic families are reported to have poorer health conditions and face more challenges such as limited knowledge and resources to follow food safety practices. Young children are at high risk of contracting foodborne illnesses due to their immature immune systems. Thus, it's critical to develop an effective food safety education program for the food handlers in low-socioeconomic families with young children.

Purpose: To develop a virtual food safety education program for the primary food handlers in low-socioeconomic families with young children and evaluate the effectiveness of the program using the Theory of Planned Behavior (TPB).

Methods: The virtual food safety education program evaluation included pre-survey, two weekly one-hour online course sessions, and post-survey. The course materials were developed based on the core four food safety practices of FightBAC! The course materials were delivered in both English and Spanish, with one trained native speaker delivered to each language group; all Spanish materials were translated and reviewed by three bilingual researchers. Participants (n = 60) were recruited by collaborating extension agencies and organizations by word-of-mouth, with 30 participants for each language group.

Results: Overall results of two language groups were reported. Most participants were female and with one to three children. After attending the program, participants' food safety knowledge score and self-reported behavior score increased statistically significantly from 5.32 to 7.43 (out of 8.00) and from 24.78 to 29.30 (out of 35.00), respectively. All TPB constructs' scores, including attitude, perceived behavioral control, subjective norm, and behavior change intention, were statistically significantly improved. Participants with a higher education level tended to have a greater increase in their perceived behavioral control.

Significance: This virtual education program improved low-income participants' food safety knowledge and behavior change. The findings provide insights for researchers, educators, and government agencies in developing and evaluating virtual food safety education programs.

P3-17 Consumers' Reactions to Allergen Advisory Labeling: Results of a Qualitative Study

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Introduction: This study was conducted as part of the U.S. Food and Drug Administration's (FDA's) strategy for exploring advisory labeling that clearly communicates information about the potential presence of major food allergens.

Purpose: To learn how consumers understand and react to allergen advisory statements on food labels.

Methods: We conducted eight focus groups in three cities (Alexandria, VA; Austin, TX; Mokena, IL) with food-allergic individuals and caregivers of food-allergic individuals, segmented by education level (lower and higher) (N = *76*). Participants viewed nine advisory statements on mock food products (dark chocolate bar, loaf of bread).

Results: Participants had mixed reactions to allergen advisory statements; some identified positive attributes (e.g., clear, not confusing; information provided helps inform purchase decisions based on severity of the consumer's allergy) and some identified negative attributes (e.g., confusing, contains insufficient information so the burden is on the consumer to decide, statements are complex or lengthy). Participants believed different allergen advisory statements conveyed varying levels of risk. For example, some participants believed "May contain traces of milk" meant the product is more likely to contain the allergen than a product with "May contain milk," but to others, the word "traces" meant less risk. The exception was the statement "Not suitable for people with milk allergies," which communicated to most participants that the allergen was definitely in the product. Many participants believed that allergen advisory statements should be mandatory and be limited to a small number of approved statements.

Significance: Although there was no clear consensus among participants on a preferred allergen advisory statement, results showed statements are interpreted differently depending on wording. Quantitative research is needed to determine the set of allergen statements that would be most useful to consumers.

P3-18 Extension Food Technology is Opening the Doors to Community Food Safety Education through Online Virtual Platforms

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Introduction: Delivering food safety education is a challenge because of the nature of topic and time it takes to deliver a certification programs. Online virtual platforms such as Zoom™ and Clickmeeting™ allow for shorter meetings over several days or weeks. This provides small food business and home food processors an opportunity to meet their training needs.

Purpose: Evaluate online programs presented to various groups from 15 to 75 years in age, high school and college students, consumers to food industry professionals. Three different audiences were evaluated: youth (12), community (16) and industry professionals (60).

Methods: Participants of online food safety courses are asked for their evaluation of the course they had just taken. They are asked several questions dealing with issues ranging from course content to online class environment and their general perceptions of the courses.

Results: The data demonstrate that most participants feel that they have increased their knowledge and skills to use the course materials to implement food safety practices in food processing applications. The "satisfactory" score was the lowest value given for these three questions. Additionally, participants expectations for the food safety course were found acceptable. All groups evaluated see the value of training online as well as time savings of not travel to a training location. Although the community group still prefer face to face training.

Significance: The online food safety programs have been well received by most participants. The main drawback of this course format is internet infrastructure and poor conductivity during sessions. Although the training delivered online meets participant expectations, food safety practices must be emphasized in the workplace or kitchen, to be fully implemented and effective in preventing food born illness. A follow up survey must be done to fully assess if knowledge and skills are fully implemented.

P3-19 Food Safety Management Training for Small and Emerging Food Businesses: Integrating a Food Safety Culture from Concept to Commercialization

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Introduction: The development of shared-use processing facilities and incubator kitchens has created opportunities for small food businesses, yet this audience is most challenged with navigating through regulation and food safety compliance while still delivering a safe, high quality food product.

Purpose: The purpose of this project is to provide a focused educational delivery of customized training to food entrepreneurs to understand critical food safety considerations from concept to commercialization. This project will increase market opportunities for locally produced food products by providing food safety training relevant to food product development.

Methods: (1) Conduct a needs assessment specific to the food safety educational and training opportunities necessary to support small and emerging food businesses. (2) Develop a curriculum and online training tools, field a pilot test and evaluate the food safety educational program that addresses the needs specific to small and emerging food businesses that will enable FSMA compliance. (3) Implement a food safety program specific to small and emerging food businesses to enhance food safety culture.

Results: The needs assessment overall knowledge scores were for content categories were 90 ± 16 for general food safety, 84 ± 12 for prerequisite programs, and 77 ± 15 for hazard analysis and preventive controls (n = 47). While knowledge scored were high, implementation of employee food safety training (62%), employee health and hygiene training (55%), and record keeping of employee training activities (43%) were low. More than half (55%) of respondents indicated they would like educational and training opportunities.

Significance: While knowledge scores were higher than expected, there is still a gap in implementation practices. Ninety-five percent of participants operating out of incubator kitchens (n = 22) and 56% of participants operating out of shared use facilities (n = 18) indicated training was required, highlighting the need for targeted training to meet the needs of the small food businesses.

P3-20 Science in a Box – A Solution to Continued Food Science Outreach during the COVID-19 Pandemic

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Introduction: Early exposure to STEM principles is crucial to enhance interest in food safety careers. Establishing accessible and interactive STEM learning experiences that introduce early concepts of food safety, particularly for underrepresented minorities, became more evident during the recent pandemic since most outreach activities have been interrupted.

Purpose: This project aims to develop self-contained food science kits and test their efficacy during activities with underrepresented minorities.

Methods: Food science kits and activities were designed and constructed to adhere to key principles, including: 1) self-contained, i.e., carrying all the materials and information needed to perform the activities, 2) low cost to allow broad production, 3) safe for use by minors without supervision and easy to clean to reduce the risk of viral transmission, and 4) portability for easy shipping and distribution. Boxes were prepared in compliance with these requirements, distributed via community partners, and sessions were hosted virtually. Pre and post program surveys were administered and statistically analyzed (t-test) to evaluate science motivation on self-efficacy, self-determination, intrinsic motivation, science learning value, and career motivation.

Results: Since June 2020, 4-modules were created resulting in ~60 participants engaged in activity sessions lasting 1.5 hours on average. Participants reported increased self-efficacy and science learning value, with increased concept comprehension. In virtual sessions, when discussions on the daily relevance of science, careers in STEM, and independent inquiry were emphasized, comprehension and interest in STEM improved.

Significance: Hands on, and accessible science learning opportunities are critical for students to develop a lifelong love of science and introduce career entry for food safety positions. The development of science boxes that can be deployed in underserved communities can overcome barriers such as location and socioeconomic status. These boxes can modify student attitudes towards science, an essential component of promoting lifelong science learning and literacy.

P3-21 Nationwide Survey of Women's Healthcare Providers' Role in Food Safety Guidance during Pregnancy and Postpartum Care

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Introduction: Women's healthcare providers (OB/GYNs, nurse practitioners, and physician assistants) may serve as primary resources for providing food information to pregnant and postpartum patients; however, food safety information (e.g., specific foods to limit or avoid, recommended food-handling practices) may not be emphasized.

Purpose: The purpose of this study was to assess whether women's healthcare providers disseminate food safety information to prenatal and postpartum patients, and to determine common themes and practices surrounding information dissemination.

Methods: A 52-item online survey was developed using data collection tool, QualtricsXM. Survey questions included the following topics: nutrition education, lactation, food safety, and the coordination of care with dietitians and lactation support. Medical programs were identified and compiled from the American College of Obstetricians and Gynecologists (ACOG) and the Association of American Medical Colleges (AAMC) listings. Inclusion criteria for survey participants were as follows: 1) identified as a practicing OB/GYNs (attendings and residents), nurse practitioner, or physician assistants; and 2) provided care to pregnant and/or postpartum patients. A total of 172 OB/GYN medical program directors, administrative staff, and department chairs of teaching

hospital programs, accounting for all states, were contacted in January 2021.

Results: Risks associated with raw/undercooked seafood(21), undercooked meat and poultry(21), toxicity attributed to fish consumption(21), contact with cat feces(18), and ready-to-eat foods(18) were commonly discussed with patients (n=25; check all that apply). Risks associated with raw/undercooked flour(2), soft-serve ice cream (1), leafy greens/sprouts(2), and pre-cut fruits and melons(2) were rarely mentioned to patients.

Significance: Women may be at a higher risk of contracting a foodborne illness when pregnant. Survey results can be used to fill gaps through a variety of methods such as developing trainings and continued education for women's healthcare providers; connecting women's healthcare providers with Registered Dietitians and food safety specialists; and providing women's healthcare providers with resources designed for pregnant and postpartum patients.

P3-22 Food Safety Knowledge and Behavior of Foodservice Staff Who Serve Immunocompromised Patients

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Introduction: Patients diagnosed with cancer undergoing treatment with chemotherapy may suffer from neutropenia as a result of immunosuppression. Neutropenic patients often have impairment in one or more protective barriers such as mucosal lining, which can lead to increased susceptibility to infections. A neutropenic diet has been used to offer a solution to aid in reducing the risk of infections associated with bacterial translocation in this patient population.

Purpose: To assess the food safety knowledge and behavior of foodservice staff who serve immunocompromised patients.

Methods: A questionnaire was designed and preliminary pilot testing completed. It was then administrated to foodservice staff at local cancer centers to test for initial validation. Data was analyzed using descriptive statistics.

Results: Staff were not consistent on knowledge of diet restrictions, particularly raw fruits and vegetables. When comparing groups, the front of the house was more knowledgeable about diet restrictions and importance of food safety behavior than the back of the house. After reliability and validity were confirmed and final questionnaire produced, it was deemed efficient and effective for use in the target population.

Significance: Due to inconsistent implementation and lack of efficiency of neutropenic diet, hospitals are in transition from neutropenic diet to FDA's guidance of *Food Safety for Older Adults and People with Cancer, Diabetes, HIV/AIDS, Organ Transplants, and Autoimmune Diseases*. Results from this study will serve as baseline for an effective transition.

P3-23 Use of the Health Belief Model to Identify Food Safety Risks for Older Adults

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❖ ***Developing Scientist Entrant***

Introduction: Older adults suffer greater consequences from foodborne illness. In the United States *Salmonella*, *Campylobacter* and *Listeria* are responsible for over two-hundred thousand illnesses among seniors each year.

Purpose: The purpose of this study was to determine food handling and consumption practices which may represent food safety risks for older adults. Additionally, the research sought to identify facilitators and barriers to safe food handling behaviors by seniors.

Methods: Six virtual focus groups were conducted via Zoom. The questioning route included questions to determine current food handling and consumption practices of foods that could be contaminated with *Salmonella*, *Campylobacter* or *Listeria*. Questions were also designed to determine why older adults may follow or ignore safe food handling recommendations. The Health Belief Model behavioral theory supported question development and thematic analysis of the focus group responses.

Results: Thirty-nine adults between the ages of 56 and 80 participated in focus groups with an average of six participants per group. Participants were from across the U.S. and represented a range of racial/ethnic backgrounds. Analysis indicated that a large number of participants reported not eating potentially hazardous ready-to-eat foods, such as lunch meats, pre-cut fruit and deli salads. Those who did eat deli meat were very resistant to the recommendation to reheat it prior to consumption. Participants widely reported consuming undercooked eggs and many were unaware of the recommendation to not wash raw poultry. Most older adults did not perceive themselves to be at increased risk for foodborne illness or complications from it.

Significance: Results of this qualitative research will inform development and administration of a national survey to older adults to determine whether these findings are applicable to a larger sample of seniors. Ultimately qualitative and quantitative results will inform development of a targeted food safety education message for older adults.

P3-24 Food-Handling Practices of Active Food Delivery Service Users

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❖ ***Developing Scientist Entrant***

Introduction: The global pandemic has resulted in consumer lifestyle changes, one of which is how consumers order their food items. Handwashing has been a major campaign during the pandemic along with sanitizing of high-use surfaces. Online food delivery services such as grocery delivery, restaurant and meal kits deliveries have increased food purchasing options. Consumers also engage in more home cooking; this inadvertently will lead to more food safety concerns. Studies have showed that poor food handling practices and improper handwashing can contribute to a rise in foodborne illness.

Purpose: The purpose of this study was to understand food-handling practices of active food delivery service users (meal kit, prepared food, and grocery delivery).

Methods: A self-report survey was provided to 657 consumers through Qualtrics XM between July and August 2020. Demographic information along with multi-choice and open-ended questions were asked to determine temperature abuse, food handling and hand washing practices.

Results: Of the 657 consumers surveyed in this study, results revealed that 38% of consumers wash their hands 20 seconds or longer, 56% of respondents reported that they sanitize their food packaging, and 5% wash their food items with soap.

Significance: There is need to develop consumer-based food safety education material in these three main areas to ensure safety when using food delivery services. Food safety educators can use findings from this study to develop food safety educational materials specific to food delivery service users.

P3-25 A Study to Determine the Barriers and Solutions to SALSA Certificated Businesses Transitioning to BRCGS START Intermediate! Food Safety Scheme

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Introduction: The Welsh Government has recognized there is a need to support small food-manufacturing businesses to obtain third party food-safety certification. A number of businesses are SALSA (Safe and Local Supplier Audit) certificated and the recent launch of BRCGS START! Scheme has highlighted the need from industry and scheme owners to understand the differences in implementation of both schemes to inform the business owners in the selection of an appropriate scheme. ZERO2FIVE (endorsed by the scheme owners) engaged with businesses to understand the barriers to transitioning from SASLSA to BRCGS Intermediate.

Purpose: To determine the different requirements between the SALSA and BRCGS START! To develop tools to support businesses to select and implement the appropriate third party Food Safety standard (FSS).

Methods: A study was designed to 1. Identify the differences in the SALSA and BRCGS START! Standards. 2. Evaluate the significance of the differences to food manufacturers in implementing the standard's requirements via interview (*n* = 396 questions) and factory inspections (*n* = 6) 3. Develop tools to support the businesses in the selection and implementation of the most appropriate Food Safety Scheme.

Results: Participation in the study took 6 months to complete. SALSA certificated small businesses contributed to the program (*n* = 6). Sixty-six clauses were identified as requiring further investigation for compliance to BRCGSSTART! Intermediate with each of the businesses. Sixteen clauses were identified as most difficult to comply with e.g., foreign body detection. A decision tree and a self-assessment tool were defined for business use.

Significance: This study determined the barriers and solutions a SALSA Certificated Company would need to overcome in order to transition to BRCGS START! Intermediate. The development of a decision tree and self- assessment checklist enabled the companies to select an appropriate FSS to implement which will support new market entry and business growth.

P3-26 The Impact of COVID-19 on Farmers' Markets Nationwide

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❖ ***Developing Scientist Entrant***

Introduction: The COVID-19 pandemic has required that farmers markets (FM) adapt to changing federal and local regulations to remain open.

Purpose: The purpose of this study is to understand how farmers markets have altered their health, hygiene and food safety practices in response to the COVID-19 pandemic in order to inform strategies on how best to assist the markets moving forward.

Methods: A Qualtrics survey was distributed to FM managers nationwide through email listservs using COVID-19 project stakeholders, Extension Agents, and other various collaborative networks. Questions were asked about FM's cleaning and sanitation practices, and health and hygiene practices to prevent COVID-19 spread; as well as where they are obtaining COVID guidance. Survey results were collected from November to December 2020.

Results: Completed surveys were collected from 121 FM managers across 21 states. The majority of markets (63%) were never closed during COVID, but some (36%) markets closed for a short time. For markets to remain open or reopen, FM managers implemented practices such as spacing booths apart, postponing market events, and placing physical markers for customers. As a result of COVID, about three-quarters of markets began providing hand sanitizer and started displaying hand washing signage. The majority of markets (94%) reported both cleaning and disinfecting surfaces with non-porous tabletops and cash box/card readers being the most common. Two major challenges faced by FMs were mask compliance by vendors and customers and obtaining proper guidance and information for COVID.

Significance: This study provides data on the implementation of health and hygiene practices that affect food safety practices and the challenges faced by farmers markets COVID. These results will aid in developing specialized, tailored training opportunities that are unique to the challenges FMs have faced.

P3-27 Effectiveness of a Multi-State COVID-19 and Food Safety Outreach Campaign Utilizing Science-based Communication Strategies

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Introduction: Amid the COVID-19 pandemic NC State Extension was presented with a unique opportunity to address public health and food safety concerns across the food sector. A coordinated outreach campaign provided timely, peer-reviewed, science-based information through social, online and traditional media channels. Multiple organizations were offered rebranded materials to expand reach.

Purpose: This multi-state COVID-19 and food safety outreach aimed to utilize the Safe Plates Food Safety Information Center and its established content development process to produce and disseminate public health communication related to COVID-19 and food safety utilizing science-based information and delivery strategies.

Methods: As SARS-CoV-2 information evolved, resources were continually updated with best available science and guidance from CDC, FDA and USDA. Food safety experts across the country peer reviewed all campaign materials. Strategies included disseminating new materials and hosting short campaigns on topical issues. Facebook Live, Instagram Live and "Ask Me Anything" Twitter events were hosted as a forum to pose questions to experts in real time. Social media analytics were reported utilizing Hootsuite and Google Analytics to track social media reach, engagement and webpage traffic. Cision was used to document overall news media hits.

Results: Across the United States, forty-four states utilized resources including NC State Extension branded and rebranded materials. One hundred fifty resources were produced in English, Spanish, Mandarin and Haitian Creole covering a range of COVID-19 topics. Over 706 rebranded materials were developed for twenty organizations. In 2020, the campaign disseminated 767 consumer messages, reaching 465,207 individuals across three social media channels. There were 39,668 visits on the COVID-19 and food safety resource webpage throughout 2020. Over 960 news outlets shared NC State Extension's expert interviews focused on COVID-19 and food safety.

Significance: Findings provide insight and guidance on communication strategies and dissemination methods of a coordinated public health communication campaign focusing on COVID-19 and food safety.

P3-28 Exploring Food Safety Messages in an Era of COVID-19: Analysis of Youtube Video Content

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❖ ***Developing Scientist Entrant***

Introduction: Although COVID-19 has not been declared a foodborne illness, food safety has been a major topic of concern, causing consumers to seek food-handling information through different media during the pandemic. YouTube is a popular video-sharing platform for disseminating information but has been found to spread contradictory and misleading health information.

Purpose: This study characterized the food safety information by reviewing the topics covered, assessing the accuracy of the information, use of government citations, and professions of the spokesperson in YouTube videos during the COVID-19 pandemic.

Methods: A YouTube search was conducted in June 2020 using the following keywords: "Food and COVID-19," "Food safety and COVID-19," and "Groceries and COVID-19." Videos were sorted by view count and only included if it was (1) English-speaking, (2) more than 500 views, (3) location specified as from the United States or Canada, and (4) less than 20 minutes. A coding system was created and implemented to classify the food safety information in the videos.

Results: Eighty-five videos met all criteria. Over half, 59%, presented handwashing procedures, 22% showed kitchen disinfection, and 69% showed concern for "take-out" or "grocery store" practices. Over a quarter of the videos (33%) explicitly mentioned that food was not hazardous while some (20%) mentioned food packaging as potentially hazardous. Most videos cited government agencies and had a host or guest who was a healthcare professional or professor/expert. Of the overall citations, three videos were not aligned with a government agency's guideline. Two of these three were presented by a healthcare professional.

Significance: The findings of this study highlight the need for educational interventions to increase YouTube authors' awareness of food safety, and provide insights on the social-media-use for food safety dissemination. Educators can campaign the need for web presenters to provide accurate information and then offer training/materials to presenters.

P3-29 Food-Handling Practices in the Era of COVID-19: A Mixed-Method Longitudinal Needs Assessment of Consumers in the United States

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❖ ***Developing Scientist Entrant***

Introduction: The COVID-19 pandemic has changed how consumers view food and food safety. Although SARS-CoV-2 is not a foodborne pathogen, consumers still expressed concerns about getting sick from foods, which may change their food handling practices.
Purpose: Using surveys and online focus group discussions, researchers utilized a longitudinal qualitative-quantitative mixed-methods approach to assess food handling practices during the COVID-19 pandemic.
Methods: Five waves of surveys (April to August 2020; *N* = 3,584, with over 700 each month) were distributed to an online U.S. consumer panel, managed by Qualtrics, Inc., and screened to include only primary food preparers and grocery shoppers. The online focus groups (May to July 2020) were conducted via WebEx and volunteers (*N* = 43) were recruited from the first wave of survey respondents. Topics for both studies included both COVID-19 and food safety practices, and anticipated practices after the COVID-19 pandemic.
Results: Handwashing increased in response to the pandemic for both survey respondents and focus group participants. However, participants anticipated their levels of handwashing after the pandemic would decrease; some focus group participants noted that lower level of hand hygiene practice could be due to human nature "kicking in." In all five months, the survey reported increased produce-washing, both with water only and with water plus soap. Some focus group participants justified their use of soap to wash produce as a preventative measure to "kill" the virus. This study also reported an increase in food-thermometer use during the pandemic. Social determinants like gender, income, education, and age impacted levels of certain food safety practices throughout the pandemic.
Significance: The findings indicated that the COVID-19 pandemic affected consumers' food handling behavior change. Not all the changes comply with the food safety recommendations. This timely information can guide future food safety education and communication during health crises and pandemics.

P3-30 Effect of Flour Outbreaks and Recalls on Consumer Knowledge and Behavior

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Introduction: The knowledge and behavioral effects from information conveyed to consumers through outbreaks and recalls has not been sufficiently examined for flour; a food that has only recently been associated with pathogenic bacteria.
Purpose: This study examines the effects of recent flour outbreaks and recalls on consumer knowledge and behavior related to flour safety.
Methods: Theoretically, publicity associated with bacterial outbreaks and recalls provides consumers with knowledge about the potential risks from consuming raw flour; resulting in behavioral change. We empirically examine this relationship by using multinomial and binary Logit regression models to estimate the effect of being in a state that has had recent flour recalls and/or outbreaks on safety knowledge and behavior. We combine state level data from a recent large (*n* = 1045) study on flour knowledge and behavior with FDA recall data and CDC outbreak data. The effect of outbreak and recall size are also examined.
Results: Between 2015 and 2019 pathogen-contaminated flour resulted in 30 recall events, 206 product recalls and 6 outbreaks. Nevertheless, 85% of consumers are unaware that these flour recalls and outbreaks have occurred. Preliminary results suggest that being in a state with recent large recalls and/or outbreaks does not significantly affect knowledge or behavior related to the risks from contaminated flour. Survey respondents who were unaware of a recall event involving flour were less attentive to lot number OR 0.16 (0.09; 0.26)*****, storage instructions OR 0.58 (0.40; 0.84)*****, and organic certification of flour packages 0.58 (0.39; 0.86)***** than those who were aware of flour recalls.
Significance: A better understanding of how consumers are impacted by information conveyed through recall and outbreak events can help us leverage these events to improve knowledge, change behavior, and reduce foodborne illness.

P3-31 Impact of the COVID-19 Pandemic on Foodborne Disease Healthcare-Seeking Behavior and Diagnoses

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Introduction: The COVID-19 pandemic has significantly changed the way people around the world go about their daily lives and many have speculated that the pandemic has affected the diagnosis and reporting of other infectious diseases, such as foodborne illness. However, few studies have empirically examined changes in disease incidence and healthcare-seeking behavior during the pandemic.
Purpose: The objective of this study was to examine changes in healthcare seeking behaviors and diagnostic practices around foodborne illness during the COVID-19 pandemic.
Methods: A retrospective cohort study of 157,585 individuals with a history of foodborne disease was undertaken using electronic medical record data from The Ohio State University health system. Data, including age, gender, race, ethnicity, zip code, medical history, discharge diagnosis codes, stool samples collected and resulting lab tests, and number of visits were obtained for all patients diagnosed with a possible, probable, or confirmed case of foodborne disease from 2012 through 2020. Regression models were used to compare the incidence rates of various foodborne diseases as well as associated healthcare-seeking behaviors, patient visits, and diagnostic orders during the pandemic (March 24 – October 24) to the same time frame in previous years.
Results: Early results showed a significant change in incidence rates for three foodborne pathogens during the pandemic. *Campylobacter*, *Toxoplasma gondii*, and *Salmonella* all saw significant decreases in incidence from 2019 to 2020 with *Campylobacter* showing the largest decrease from 1.9 per 100,000 person-years in 2019 to 0.6 per 100,000 person-years in 2020 (*P* = 0.012). Patient visits decreased in March and April of 2020 but returned to normal in June and surpassed the previous year's total visits.
Significance: The results suggest that the pandemic has reduced the incidence of some foodborne diseases, and only initially decreased healthcare seeking behavior.

P3-32 Comparison of Statistical Methods for Identifying *Salmonella* Contamination Risk Factors of Whole Chicken Carcasses

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella*, a leading cause of foodborne illness in the U.S., is often attributed to consumption of chicken and other poultry products. Studies characterizing *Salmonella* contamination and/or evaluating processing interventions are highly variable in statistical methods. Complexity of the food system makes analyzing data from these studies challenging since many of the assumptions made by common analytical approaches are violated. Further, sampling often introduces longitudinal correlation to food microbiological data that must be accounted for during analyses.

Purpose: The goal of this study was to evaluate the appropriateness of three approaches for analyzing food microbiological data and the impact of longitudinal correlation on conclusions.

Methods: As a case study, risk factor analyses for *Salmonella* contamination of whole chicken carcasses were conducted using regulatory data collected by the U.S. Department of Agriculture's Food Safety and Inspection Service between May 2015 and December 2019 from 203 regulated establishments. Three statistical models – logistic, generalized estimating equation, random effects – were fit for *Salmonella* presence/absence with establishment demographics and inspection history included as potential covariates. Odds ratios (OR) and 95% confidence intervals (CI) for risk factors were compared across models.

Results: While slaughtering turkey (in addition to chicken) was associated with *Salmonella* presence across all models, conclusions drawn from the three models differ. Specifically, this association was statistically significant in the logistic model (OR = 1.32; 95% CI: [1.049-1.670]), which does not account for longitudinal correlation, but was nonsignificant in the generalized estimating equation (OR = 1.34; 95% CI: [0.848-2.114]) and random effects (OR = 1.28; 95% CI: [0.789-2.091]) models, which do account for longitudinal correlation.

Significance: This work demonstrates that choice of statistical approach can significantly alter results – especially when longitudinal correlation is present – due to methods having different targets of inference. These results support a renewed focus on statistical methodology in food safety.

P3-33 Monitoring Environmental Contaminants in Meat, Poultry, and Egg Products

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Introduction: The United States Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS) protects the public's health by ensuring the safety of meat, poultry, and processed egg products. As part of this mission, FSIS considers chemical contaminants as part of its food safety programs.

Purpose: To manage potential public health risks, FSIS scientists identify and prioritize hazards associated with chemical residues and contaminants, test thousands of food samples each year, and analyze and interpret collected data.

Methods: Under the U.S. National Residue Program for Meat, Poultry, and Egg Products (NRP), FSIS develops a comprehensive sample collection and testing program on an annual basis. Tissue samples are collected by FSIS inspectors shortly after slaughter and sent to a specialized FSIS laboratory for analysis. In addition to testing for veterinary drugs and pesticide residues, FSIS has incorporated a focus on chemical contaminants. Currently, the Agency is collecting data on the levels of certain metals and certain per- and polyfluoroalkyl substances (PFAS). Dioxins and dioxin-like compounds in USDA-regulated products are analyzed through periodic surveys.

Results: FSIS will present current results of its chemical contaminant program. This will include new metals testing data (18 different metals) that was collected in 2020 and 2021. PFAS testing (16 different compounds) is newly expanded to chicken, pork, and *Siluroformes* fish muscle tissue, in addition to cattle. FSIS will provide public health context to these results, as well as further insight into the future of its contaminant testing program and how it integrates with other parts of the NRP.

Significance: The data collected will help determine Agency priorities for sampling and testing programs and could also inform future policy changes and Agency decision-making.

P3-34 Shiga Toxin-producing *Escherichia coli* (STEC) in the Raw Pork Production Chain: A Cause for Concern?

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❖ ***Developing Scientist Entrant***

Introduction: Retail pork implicated to Shiga toxin-Producing *Escherichia coli* (STEC) contamination highlight the need for increased awareness of pork as a potential source of STEC infection. However, little is known on the prevalence, ecophysiology, growth, and potential survival of STEC in raw pork products.

Purpose: The objective of this review study is to contribute to our understanding of raw pork products as potential carriers for STEC into the food supply.

Methods: Primary literature reporting the prevalence of STEC in the raw pork production chain were collected from several databases including Scopus, Sceilo, and PubMed published between 2001 and 2020, and summarized and critically analyzed.

Results: The reported prevalence rate of *stx*-positive *E. coli* isolates in live swine, slaughtered swine, and retail pork samples around the world ranged from 4.4 (22/500) to 68.3% (82/120), 22 (309/1395) to 86.3 (69/80) %, and 0.09 (1/1167) to 80% (32/40), respectively, depending upon the sample categories, detection methods, and the hygiene condition of the slaughterhouses and retail markets. In retail pork, among the regulated non-O157 serogroups (O26, O45, O103, O111, O12, O145), O145 were prevalent in the U.S., Europe, and Asian studies. Serogroup O26 was prevalent in the U.S., Europe, and Africa. Serogroup O121 was only reported in the U.S. Serogroup O91 was reported in the U.S., Asia, and South American retail pork samples. The most common virulence gene combination in the retail pork around the globe were as follows: the U.S.; serogroup O157+*stx*, regulated non-O157+*stx*, unknown serogroups+*stx+eae*; Europe; unknown serogroups+(*stx+eae, stx2+eae*, or *stx1+stx2+eae)*, Asia; O157+*stx1+stx2+hlyA*, Unknown+*stx1+eaeA+hlyA,* or only *eae*, Africa; O157+*stx2+eae+hlyA.*

Significance: STEC strains derived from retail pork fall under low to moderate risk categories capable of causing human disease. This review will contribute to understand the ecology of STEC in retail pork and aid in the development of prevention and control strategies.

P3-35 Overview of *Salmonella* Outbreaks Linked to Tuna Imported from Southeast Asian Countries, Existing Challenges, and Potential Prevention Efforts

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Introduction: *Salmonella* is a foodborne pathogen that can cause human illness, including bloody diarrhea, fever, and abdominal cramps. From 2007 to 2021, FDA responded to seven outbreaks of *Salmonella* infections associated with tuna imported from Southeast Asia; involving 205 cases and 31 hospitalizations from 23 states. The recurrence of *Salmonella* outbreaks associated with imported tuna suggests deficiencies with food safety practices in this region.

Purpose: The purpose of this study was to review outbreak investigation information regarding contaminated imported tuna from Southeast Asia and identify investigational challenges, regional tuna contamination factors, and prevention strategies.

Methods: FDA's Coordinated Outbreak Response and Evaluation Network collected, reviewed, and analyzed epidemiologic, laboratory, traceback, surveillance, firm inspection, and prevention data from the seven *Salmonella* outbreaks associated with tuna. Significant similarities and differences within the data were further evaluated, such as case exposure, product/environmental samples, and product origin; lessons learned and existing regulatory challenges were identified. Conclusions were made from the analysis to determine prevention efforts and proposed actions.

Results: The outbreak analysis identified trends in the tuna source, lack of traceability, insanitary conditions, and cross-contamination, which revealed the following existing challenges: weather-related contamination and FDA's regulatory authority to inspect foreign fishing vessels where cross-contamination occurred. During six of the seven outbreaks, tuna was supplied by firms from Southeast Asian countries; the majority from Indonesia. However, a single common Indonesian supplier of the contaminated tuna could not be identified. FDA Import Alerts and Bulletins support the assertion that the contamination was not a single firm issue, as they list 13 firms for tuna products from Indonesia. Differences among the outbreaks included the *Salmonella* serotype, tuna product type, and exposure routes.

Significance: Considering the challenges highlighted in the data, proposed prevention strategies include: focused review of FDA inspection reports to determine the potential root-cause; education and outreach to Indonesian firms via sanitation lectures; consumer messaging; and a region-wide import alert for tuna.

P3-36 Developing an Expert Model for the Diagnosis of Foodborne Illness

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Introduction: Current guidance and training for clinicians on how to diagnose foodborne disease (FBD) have not been analyzed from a decision science perspective, which could contribute to under-diagnosis.

Purpose: This study develops intuitive descriptions of the expert (normative) model for how clinicians should make decisions about diagnosing FBD, which can be used to assess the degree of alignment with this approach among clinicians.

Methods: The Infectious Disease Society of America Clinical Practice Guidelines for the Diagnosis and Management of Infectious Diarrhea Guidance is the primary source of information on diagnosing FBD for clinicians at Ohio State University, and the CDC's Primer for Diagnosis and Management of Foodborne Illness is a secondary source for this study. Based on this guidance, the decision process was represented in the form of both a decision flowchart and an influence diagram. An influence diagram is a display of a decision problem that includes uncertainties in the decision and captures their influence on the outcomes of interest. Interviews with food safety and clinical experts were used to update and validate the model.

Results: In the flowchart, specific conditions are described for ordering stool/blood cultures, or toxin tests. In the influence diagram, seven factors were identified as impacting the likelihood of contracting FBD, and four factors were identified as increasing the likelihood that the infectious diarrhea was caused by other factors. In addition, two factors were identified as being relevant for use in clinical judgement.

Significance: The validated expert model can be used to assess how well healthcare providers follow the normative model, resulting in improved diagnosis of FBD by the healthcare providers. This will greatly improve the data on FBD that is generated from surveillance efforts, which will, in turn, lead to improved quality in the burden of disease estimates and, subsequently, drive improved risk management.

P3-37 Comparative Review of the Evolution of Antibiotic-Resistant *Salmonella* Surveillance in Different Countries

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Introduction: Antimicrobial resistance (AMR) in foodborne pathogens such as *Salmonella enterica* is a major public health concern. AMR surveillance systems between food producing and consuming countries need harmonization to ensure policies are supported by proper information. Understanding surveillance differences between countries is important to perform comparative foodborne AMR risk assessments.

Purpose: Compare surveillance of AMR *Salmonella* in food between the EU, UK, and USA, and identify differences and data gaps that may impact quantitative measurements of AMR *Salmonella*.

Methods: Regulations and directives from the EU, UK, and USA pertaining to AMR *Salmonella* surveillance in food from the last 20 years will be examined. Compulsory and voluntary sampling programs for *Salmonella* will be identified to compare between: sampling rationale, sampling scheme, and data availability. For AMR surveillance, the antimicrobials tested and corresponding cutoff values between sampling and testing protocols will be compared over time. In addition to classic sampling methods, WGS is increasingly being employed in AMR surveillance systems. Important sequencing and analysis parameters will be compared to identify commonalities and possible improvements between regions.

Results: An objective, thorough review of sampling programs and testing protocols over time will provide information into AMR *Salmonella* surveillance in food in the EU, UK, and USA. Identification of data gaps, and significant differences in surveillance programs will provide researchers needed information to make necessary adjustments before comparing prevalence values between nations, or within a nation over time.

Significance: Providing a historical description of the evolution of AMR/*Salmonella* surveillance programs, including which AMUs and cutoffs have changed is essential information towards performing objective comparisons between countries. This is also useful to perform quantitative risk assessments.

P3-38 One Health Enteric Package v1.0: Expanded and Standardized Metadata for Enteric Genomic Epidemiology in the U.S.

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Introduction: Genomic data is intrinsically standard across all life on earth, making these data an incredibly useful tool for food safety surveillance and outbreak investigations. However, contextual data associated with these genomes (where, from what, by whom, and when the sample was collected), are generally *not* inherently standard, requiring consistent terminology to perform meaningful comparisons. An early global standard was key for establishing basic interoperability for inferring relationships between genomes, but, more sophisticated analyses to identify risk, root cause, source attribution, and other relationships require richer, more structured contextual data, or metadata, to accompany each genome.

Purpose: We propose an expanded, standardized, metadata package to be used for all US contributors across our open genomic epidemiology network for enteric surveillance.

Poster

Methods: The Genomics for Food Safety (GenFS) metadata working group reviewed the existing metadata standard, *National Center for Biotechnology Information* (NCBI) pathogen metadata package, and identied core attributes to retain while flagging ones that were not utilized or relevant to our mission. A new set of attributes were also developed to standardize and expand upon existing free-text fields, like "isolation source", providing machine-readable, ontologically structured metadata describing samples taken from across the One Health arena.

Results: The One Health Enteric Package v1.0 marks the first major update to the NCBI pathogen metadata template for use in genomic foodborne pathogen surveillance. This release includes the same core set of fields utilized in the NCBI pathogen package plus a set of custom fields specific to our community.

Significance: Expanding and standardizing metadata can significantly impact One Heath goals by allowing for sophisticated data science analyses investigating links between genomic data and source data, e.g. machine learning or population-adapted genome-wide association studies (GWAS) to test hypotheses around evolution of virulence, stress tolerance, antimicrobial resistance, risk assessment, source attribution, among others.

P3-39 Revisiting Our Knowledge on *Listeria sensu stricto* Species by Predicting Traits from Whole Genome Sequencing Data

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Introduction: Whole genome sequencing (WGS) is becoming the method of choice for rapid and accurate identification and characterization of bacteria.

Purpose: Here, we describe and validate the use of a genotyping functionality implemented in the BioNumerics software platform to predict phenotypical characteristics of all *Listeria sensu stricto* species based on genomic data.

Methods: Virulence and resistance factors of *Listeria* spp. were collected. A serovar prediction based on the absence/presence of markers was also implemented. This was used to build a tool in BioNumerics and to subsequently screen a database containing de novo assemblies of all *Listeria* isolates in the SRA database of NCBI until June 2018 (appr. 20,000). Strains for which no serovar could be detected were mapped against a core genome of *Listeria sensu stricto* species.

Results: The predicted serovar matched the serovar supplied by the submitter in 92% of isolates. For 0.4% of isolates, no marker for the genus *Listeria* could be found. No markers for *Listeria monocytogenes* were found for 0.3% of isolates and the same percentage of runs had a previously undescribed combination of markers. The presence of both virulence and resistance markers correlated strongly with both species and serovar though many isolates with deviating patterns could also be seen. One strain could not be classified within the existing species. A phylogenetic tree based on the core genome analysis, suggests this strain shares an ancestor with *L. monocytogenes* and *L. innocua*, the predicted phenotypic profile also confirmed this.

Significance: The tool allowed us to perform a large-scale prediction of phenotypic characteristics. The joint analysis of this information with genetic analyses is very useful to increase our knowledge on *L. monocytogenes* and related species and contribute to a better control of listeriosis.

P3-40 Rapid Identification and Molecular Characterization of *Salmonella enterica* Isolates from Pecan Orchards through Whole Genome Sequencing

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❖ ***Developing Scientist Entrant***

Introduction: Whole genome sequencing has become an indispensable tool in food safety and outbreak surveillance.

Purpose: This study was conducted to evaluate the performance of Minion sequencing for rapid identification and molecular characterization of *S. enterica.*

Methods: Ten *S. enterica* isolates obtained from pecan orchards were sequenced using Minion and Illumina NextSeq 500. The reads of Minion were time-based subsampled to determine the earliest identification turnaround time for each isolate.

Results: Species-level identification was achieved at 15 min of sequencing run. All the antigens related to serotyping based on the Kauffmann White Scheme were predicted with 8-hour run. Assemblies obtained from the subsampled reads were compared with assemblies obtained from the full reads dataset, as well as hybrid assemblies from the reads from Minion and Illumina. The results showed that the best values of continuity (N50, size of the longer contig, and the entire number of contigs) ($P < 0.05$) were obtained at and after 8 hours of sequencing runs. However, under a stringent BLASTp search (percentage of identity of 95 % and query coverage of 85 %) against the Comprehensive Antibiotic Resistance Database (CARD) and Virulence Factor Database (VFDB) using the proteins annotated from each of the time-based assembled genomes as well as the hybrid assemblies, none of these time-based sequencing runs were sufficient to generate hits significantly similar ($P < 0.05$) to those obtained from the hybrid assemblies. Nevertheless, it was possible to obtain an average of 67.14% and 74.12% of the hits obtained from the hybrid assemblies using the CARD and VFDB, respectively, where no significant changes were observed after 8 hours compared to the complete datasets ($P < 0.05$).

Significance: These results demonstrated that Minion could offer an effective tool for the rapid identification of *S. enterica* isolates and certain capabilities for their characterization.

P3-41 Whole Genome Sequencing-based Characterization of *Cronobacter sakazakii* Strain Isolated from Tilapia

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Introduction: *Cronobacter sakazakii* is an emerging, opportunistic, Gram-negative, human-pathogenic foodborne bacterium that causes life-threatening meningitis and necrotizing enterocolitis, predominantly in neonates. It has also been reported to cause serious illness in immunocompromised individuals. It can survive in severe dry conditions and has been linked to powdered infant formula (PIF) contamination worldwide. However, it has also been recovered from a wide variety of foods and environmental samples. Currently, the application of Whole Genome Sequencing (WGS) has enabled bacterial typing and has been widely used for precise strain identification to understand the transmission of disease. In this study, we describe the draft genome sequence of a *Cronobacter sakazakii* strain SRL-95, isolated from food.

Purpose: The major objective of this study was to identify the human-pathogenic strain of *Cronobacter sakazakii* recovered from food by performing WGS analysis.

Methods: A *Cronobacter sakazakii*-like Gram-negative bacterial isolate was recovered from smoked Tilapia. Preliminary identification of the recovered isolate was completed on bioMerieux VITEK 2 system, real-time PCR and MALDI-TOF mass spectrometry analysis. Subsequently, WGS was performed on an Illumina MiSeq system, using a Nextera XT DNA library preparation kit and a 250-bp paired-end read MiSeq Reagent v2 kit (500-cycle), following manufacturer's instructions and protocols.

Results: The VITEK MS system could provide species identification with high confidence value (>99%) to *Cronobacter sakazakii* strain SRL-95. WGS analysis ascertained the sequence type (ST) of the genome of the recovered *Cronobacter sakazakii* strain SRL-95. The *Cronobacter* sp. multilocus sequence typing website (http://pubmlst.org/cronobacter) confirmed that *Cronobacter sakazakii* strain SRL-95 belonged to ST 145. Furthermore, the genome sequence of *Cronobacter sakazakii* strain SRL-95 was 4,643,111 bp in length with a G+C content of 56.8% and the draft genome was distributed in 67 contigs.

Significance: WGS technology can be applied for the strain identification of the foodborne *Cronobacter sakazakii* known to contaminate PIF and cause fatal disease in humans.

P3-42 Scope and Challenges of Louisiana Retail-Foodservice Businesses That Perform Specialized Processing

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Introduction: The Louisiana retail-foodservice businesses are regulated under the Louisiana Administrative Code. Some of these businesses may also perform specialized processing as described in the Food Code §3-502.12. However, the inspection of specialized processing normally varies from one inspector to another due to lack of standardized training. Businesses are, thus, left with confusion and challenges.

Purpose: To assess the regulation and food safety training needs of food businesses in Louisiana from sanitarians' perspective.

Methods: Questionnaire responses were collected through Qualtrics®. Descriptive statistical analyses were conducted in Microsoft Excel. To access the landscape of the crossover businesses in Louisiana, frequencies and percentages were used to describe the specialized processes conducted in retail businesses. Business owner's awareness of regulation, food safety education needs, as well as sanitarians' awareness and confidence data were collected on a 3-point Likert scale. Percentages were calculated against total responses in each specialized process, regulation item, or food safety training program.

Results: One hundred seventy-one responses were collected. Results showed that top three specialized processes were reduced oxygen processing (ROP) (40%), using of additives such as vinegar as a method of preservation (21%), and smoking as a method of preservation (20%). ROP of raw single ingredient (26%), hot smoking (88%), and sushi (25%) rated the most popular product within each category. However, only 15% businesses who perform ROP were considered having sufficient trainings, and 13% for smoking foods or sushi. Number of sanitarians who reported "not confident when inspecting" reached 27-35%. Food establishments not aware of regulation (35%) and no knowledge and resources to develop the systems to comply (35%) were top two challenges.

Significance: The findings from this study will serve as a needs assessment for food safety educational material development for the state sanitarians as well as local businesses who perform specialized processing.

P3-43 Transfer of Indicator *Escherichia coli* to Romaine Lettuce Grown in Organic and Conventional Fields Amended with Animal-based Soil Fertilizers in the Southwestern Desert, 2019–2020

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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 untreated BSAAOs are at increased risk for microbial contamination, but composted and treated BSAAOs also have the potential for cross-contamination.

Purpose: To investigate the persistence of indicator *E. coli* in soil amended with different animal manure types and the potential transfer to lettuce grown in organic and conventional fields in the southwestern desert.

Methods: Heat treated poultry pellet (HTPP), bovine manure compost (CMP), and raw poultry litter (PL) were tilled separately into eight replicate soil plots in the southwestern desert. Organic and conventional plots were inoculated with a manure slurry of indicator *Escherichia coli* (10^8 MPN/g). Controls included un-amended (UA) and un-inoculated (UI) plots. Lettuce was planted 12 days post-application (dpa). Soil samples were collected from dpa 0-369. Lettuce was harvested on 111 and 118 dpa. *E. coli* presence and concentrations were quantified using direct plating and MPN methods.

Results: *E. coli* contamination was discovered on lettuce leaves cultivated on all manure-amended and unamended plots on 111 and 118 dpa. *E. coli* levels enumerated from lettuce leaves grown in manure amended soil was found to be significantly higher (P-value < 0.005) than lettuce in unamended plots (average, 1.7×10^0 and 3×10^{-1} MPN/g, respectively). Also, lettuce cultivated on PL-amended plots had significantly higher (P-value < 0.005) concentration of *E. coli* than those grown on HTPP and CMP amended plots (average, 2.5×10^0, 1.2×10^0 and 1.4×10^0 MPN/g).

Significance: The long-term survival of *E. coli* in manure-amended soil and transfer to romaine lettuce was observed. Our findings suggest longer (>120 days) wait periods may be needed to protect leafy greens from microbial contamination via raw animal manure.

P3-44 FSIS Siluriformes Sampling at Five Years

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Introduction: The US Department of Agriculture's Food Safety and Inspection Service (FSIS) ensures meat, poultry, and processed egg products are safe, wholesome, and accurately labeled. In December 2015, FSIS acquired responsibility for federal *Siluriformes* (catfish) inspection and has been collecting microbiological and chemical residue samples since May 2016.

Purpose: With several years of data now collected and analyzed, and in light of FSIS' recent Strategic Assessment of Sampling Resources (SASR) Evaluation, the purpose of reviewing this data is to obtain a comprehensive and up-to-date understanding of microbiological and chemical hazards in catfish.

Methods: This review will build on previously published works and will be informed by an assessment of the existing literature on microbiological and chemical hazards associated with domestic catfish slaughter/production. Additionally, it will include findings from a comprehensive data analysis of the over 10,000 routine microbiological samples (specifically for *Salmonella*) and chemical residue samples FSIS has collected since implementation of the catfish inspection program.

Results: Conclusions will be shared. Areas of discussion will include observed trends in microbiological sampling, specifically for *Salmonella*, as well as chemical residue sampling for a variety of hazards. As appropriate, regional, seasonal, and other trends will be included in the discussion.

Significance: By routinely analyzing results from the Agency's sampling programs, FSIS can ensure it is using its resources effectively and efficiently. For catfish, results from this review could inform future catfish sampling efforts and the development of other policy options.

P3-45 Noteworthy Updates and Expansion of the USDA's Food Safety and Inspection Services Accredited Laboratory Program

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Introduction: The USDA's Food Safety and Inspection Service (FSIS) Accredited Laboratory Program (ALP) is proposing changes in statistical methods and expanding the ALP to include pathogens and additional chemical residues.

Purpose: This presentation describes FSIS' Proposed Rule to update and modernize the ALP regulations published in the Federal Register in December 2020.

Methods: The FSIS ALP offers voluntary certifications for non-Federal laboratories. Due to current federal regulations, the ALP is restricted in the types of accreditations offered and how proficiency testing (PT) results can be scored. The regulations prescribe specific analytes within the field of chemistry: the analysis of food chemistry (moisture, protein, fat, and salt) and select chemical residue classes. The proposed regulatory changes provide flexibility to expand accreditation offerings to foodborne indicator and pathogen analytes and additional chemical residues under FSIS' jurisdiction. Because the Pasteurized Egg Products Recognized Laboratory (PEPRLab) Program has been discontinued, the ALP expansion also proposes to include accreditation offerings for pathogens in egg products. Additionally, the ALP would like the flexibility to adopt more robust statistical tools for scoring PT results than the

Cumulative Sum (CUSUM) scoring prescribed in the regulations.

Results: The regulatory changes described in the Proposed Rule would provide a variety of chemical and biological accreditation and proficiency testing options to any interested non-Federal laboratories.

Significance: Additional offerings for pathogens and chemical residues and updated statistics through the modernized ALP will provide greater confidence to those in the industry who rely on ALP labs to conduct testing. It may also open a potential pathway for establishments to voluntarily submit industry data to FSIS.

P3-46 Shiga Toxin-producing *Escherichia coli* (STEC) Recovered from Verification Sampling of Raw Beef Products Collected By the United States Department of Agriculture Food Safety and Inspection Service

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Introduction: The Food Safety and Inspection Service (FSIS) collects samples from federally inspected establishments to verify that meat, poultry and egg products are not contaminated with bacterial pathogens. FSIS has multiple routine verification and follow-up sampling projects to detect *Escherichia coli* (*E. coli*) O157:H7 and non-O157 STEC in raw beef products. The Agency uses the results from these sampling projects to identify adulterated raw beef products and facilitate removal of these products from commerce.

Purpose: To determine the occurrence of the top seven serogroups of STEC adulterants (O157:H7, O26, O45, O103, O111, O121, and O145) in all beef product categories tested by FSIS.

Methods: Between June 2012 and December 2020, FSIS tested for *E. coli* O157:H7 in samples of raw ground beef, raw ground beef components other than trim, and bench trim from cattle not slaughtered onsite. In contrast, beef manufacturing trim samples produced from cattle slaughtered onsite were analyzed for both O157:H7 and non-O157 STEC. Follow-up samples collected in response to a positive finding from initial routine verification samples derived from specific beef products were also collected and tested for the presence of STEC.

Results: A total of 167,073 raw beef product samples were tested for *E. coli* O157:H7, and 220 (0.13%) were positive. Of 44,457 samples, 316 were positive for non-O157 STEC (0.71%). STEC recovered from beef manufacturing trim was O103 (0.42%), O157:H7 (0.23%), O26 (0.15%), and O111 (0.11%). Other non-O157 STEC were recovered from beef manufacturing trim at lower frequencies: O145 (0.022%), O45 (0.020%), and O121 (0.016%). Approximately 45 million pounds of contaminated raw beef products were converted to cooked product or otherwise held from commerce.

Significance: FSIS raw beef verification projects have been effective in helping to protect public health by detection of STEC adulterants and removal of these products from commerce.

P3-47 Analysis of Methodology Used to Classify Produce Commodities as Rarely Consumed Raw

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Introduction: As part of the FSMA Final Rule on Produce Safety, produce commodities that are rarely consumed raw (RCR) are exempt from minimum science-based standards for safe growing, harvesting, packing, and holding, of fruits and vegetables grown for human consumption. Data from the 2003 – 2010 National Health and Nutrition Examination Surveys (NHANES) and the EPA's Food Commodity Intake Database (FCID) have been used to determine which commodities should be included on the RCR list. Since foods that are deemed to be RCR are exempt from important food safety regulations, the development of the RCR list needs to be informed by the best available data and appropriate statistical analyses.

Purpose: This study examines the impact of the statistical methodology, including the validity of assumptions, and the recency of data on the designation of RCR commodities with low reported consumption.

Methods: NHANES dietary recall data will be used to estimate: 1) percent of the population that consumes commodities with low reported consumption in any form; 2) percent of the population that consumes these commodities uncooked; and 3) number of eating occasions in which commodity is consumed uncooked. Trend analyses will be used to evaluate the appropriateness of combing data across NHANES cycles. The impact of using one day versus two days of dietary recall data will also be examined.

Results: Several considered RCR produce commodities (e.g., arugula, boysenberry, yam bean, pomegranate) showed significant cycle-to-cycle variation in the proportion of the population consuming the products overall and in uncooked forms. For example, of the 48 considered produce commodities, 27% did not meet the criteria for RCR for at least one cycle between 2003 – 2017 and 16.7% did not meet the criteria for RCR using 2017-2018 data.

Significance: This work will advance public health by informing the designation of commodities exempted from federal regulations.

P3-48 Annual Sampling Plan and Sampling Summary Reporting at the United States Department of Agriculture's Food Safety and Inspection Service

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Introduction: The U.S. Department of Agriculture's Food Safety and Inspection Service (FSIS) verifies the safety of meat, poultry, and egg products through robust product sampling for pathogens or chemical residues. Each year, FSIS establishes sampling priorities and goals through its Annual Sampling Plan.

Purpose: There is a natural life cycle for the Annual Sampling Plan and Sampling Summary Report within FSIS where the plan anticipates the results to be included in the summary report, and the summary report informs the public of the results and refines the development of the next plan. During the summary report development, FSIS identifies areas to improve public health safety, reporting, and transparency. This includes developing key sampling priorities for the Fiscal Year (FY) 2021 Annual Sampling Plan.

Methods: FSIS evaluated sampling results from 10 different commodity groups to detail sampling allocations and activities in a FY summary report to inform future FSIS sampling plans and to provide public transparency. FSIS analyzed and summarized over 129,000 samples that generated over 2.7 million testing results. For example, *Salmonella* prevalence in raw ground beef from FSIS-inspected establishments was 2.25% (1,211/10,683) in FY 2019 and 2.89% (1,200/10,540) in FY 2020, and *Salmonella* prevalence in beef trimmings was 1.44% (489/4,076) in FY 2019 and 2.01% (488/4,108) in FY 2020.

Results: Beginning with results from FY 2019, FSIS compiled data from its sampling programs and provided context and key activities, which affected them throughout the year. The summary report addresses microbiological, chemical, and other sampling program results in a standardized format that can be compared across FYs.

Significance: Highlights of what the report provides readers, why it is significant, and a review of the content will be presented for discussion. This will also include examples of how the summary report informed the development of the FY 2021 Annual Sampling Plan.

P3-49 Evaluation of Decreased Recalls Recommended by the USDA Food Safety and Inspection Service (FSIS) in Calendar Year (CY) 2020

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Introduction: FSIS recommended fewer recalls involving meat, poultry, or egg products in calendar year (CY) 2020 than previous years CY 2006 to present.

Purpose: FSIS conducted this study to determine likely causes of the decrease in recommended recalls and to evaluate what this decrease signifies.

Methods: FSIS examined its historical recall and Public Health Alert (PHA) data from CY 2006 to present, including cases analyzed and closed without recall or PHA. FSIS examined the number of new illness outbreaks requiring Agency investigation from CY 2017 through CY 2020, data from the Consumer Complaint Monitoring System for CY 2019 and CY 2020, and laboratory sampling data from the Public Health Information System for CY 2019 and CY2020. Recall and PHA data were then evaluated against data regarding illness investigations, consumer complaints, FSIS laboratory sampling, and total cases submitted for recall analyses over time.

Results: FSIS analyzed more laboratory samples, received fewer consumer complaints, received fewer new illness outbreaks for investigation, and elevated fewer cases for recall analysis in CY 2020 than in previous years. FSIS implemented procedural changes resulting in an increased use of PHAs as a public notification tool. The total number of recalls and PHAs in CY 2020, while lower, is consistent with previous years with similar caseloads. For instance, in CY 2009, FSIS analyzed approximately 151 cases and recommended 69 recalls and PHAs, whereas in CY 2020 FSIS analyzed 137 cases and recommended 49 recalls and PHAs.

Significance: During the COVID-19 pandemic, FSIS maintained all regulatory functions including inspection of all FSIS-regulated products in federally regulated facilities to ensure a continual safe food supply. While total cases also decreased, likely due to various factors, a decrease in the proportion of recalls and PHAs to total cases signifies an overall reduction in public exposure to hazardous and mislabeled foods.

P3-50 *Salmonella* Isolates and Antimicrobial-resistance Trends in FSIS Sampling for the National Antimicrobial-resistance Monitoring System (NARMS), 2014–2019

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Introduction: The National Antimicrobial Resistance Monitoring System (NARMS) is a national surveillance system that monitors changes in the antimicrobial susceptibility of enteric bacteria found in clinical, retail, and food animal samples in the US. The United States Department of Agriculture, Food Safety and Inspection Service (FSIS) samples certain food animal species and products for *Salmonella* and other bacteria in regulated establishments as part of NARMS.

Purpose: We evaluated trends in *Salmonella* serotypes and antimicrobial resistance (AMR) in certain food animal species and products sampled from 2014 through 2019 as part of FSIS NARMS sampling. These data were further evaluated by geographic region and product volume.

Methods: Samples analyzed included cecal (intestinal) samples from food-producing animals at slaughter and product samples tested as part of verification testing. *Salmonella* isolates were analyzed by serotype and antimicrobial susceptibility and by species (chicken, turkey, beef, and swine) to evaluate differences of *Salmonella* serotypes among the products. Trend was assessed using Kendall tau correlation coefficient at $P < 0.05$.

Results: A total of 7,908 *Salmonella* isolates were recovered from cecal samples collected from chicken (19%), turkey (4%), beef (31%) and swine (47%) and 15,785 *Salmonella* isolates were recovered from PR/HACCP chicken (65%), turkey (8%), beef (11%) and pork (16%) product samples from 2014 through 2019. Overall, pan-susceptibility was 64% among cecal and 45% among product samples. Between 2018 to 2019, multi-drug resistance increased by 5% (21% to 26%, respectively) in product samples.

Significance: To address AMR and *Salmonella* in food, an in-depth understanding of short- and long-term trends is essential. The findings from this analysis can inform the development of robust and effective pre- and post-harvest strategies by facilitating and fostering exchange of ideas, collaborations, and innovation and lead to further strengthening of public health.

P3-51 ARS Studies Addressing FSIS Research Needs

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Introduction: USDA's Food Safety and Inspection Service (FSIS) is a food safety regulatory agency that uses a science-based approach in carrying out its mission: issuing regulations, writing guidelines, evaluating new technologies, and developing laboratory methods for FSIS verification sampling programs.

Purpose: Using the latest science is of paramount importance and aligns with FSIS' Strategic Plan. FSIS requires additional studies to fill data gaps relevant to its mission's essential functions.

Methods: FSIS identifies data gaps by evaluating:

- Data FSIS collects.
- Questions received through askFSIS by establishments and Agency personnel in the field.
- Changes in laboratory technology.
- Changes in processes establishments implement.
- Causes for recalls and outbreaks.

For data gaps that support policy and verification activities, FSIS collaborates with USDA's Agricultural Research Service (ARS). Discussions focus on the study design that fulfills research needs.

Results: ARS research findings and methodology have been successfully transferred into FSIS laboratories and FSIS guidance. Examples include:

- The "Multi-residue Method" used by FSIS laboratories
- The new Buffered Peptone Water (nBPW) transport media used by FSIS laboratories
- ARS Predictive Microbiology Information Portal models for *Clostridium perfringens* in uncured beef, chicken, and pork referenced as a validated modeling program in FSIS' Guideline for Stabilization.

Research is still needed on fermented, salt-cured, and dried products:

- Approaches to control pathogens in dried and fermented products and dry-cured ham.
- Drying times are needed for different diameter dry and semi-dry fermented sausages because most research is only conducted with one size sausage diameter and drying times differ by diameter.

Significance: The outputs of ARS research have been used to develop FSIS policies and procedures that improve food safety. To ensure science is available to guide new policies and program initiatives, FSIS encourages researchers to apply their expertise to address FSIS priorities and encourages funding agencies to consider FSIS priorities when developing research opportunities.

P3-52 UV-C Treatment of Papaya Epicarp to Inactivate *Salmonella* spp., and the Effect of Fruit Ripeness and Storage Conditions

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Introduction: Papayas have been associated with frequent outbreaks of *Salmonella*. The efficacy of novel treatment interventions like UV light to mitigate the risk has not been well reported.

Purpose: To study the effect of ripeness level and storage conditions on the survival of *Salmonella* spp., on papaya epicarp after UVC treatment

Methods: Papayas of 3 commercial ripeness levels (0, 50, and 100%) were cut into 3 cm^2 size samples and spot inoculated with 25 µL of nalidixic acid (50 µg/mL) adopted *Salmonella* spp (4-strain) to an initial load of 10^{3-4} log CFU/sample. The inoculated samples were subjected to UV-C treatment at 12.5 mW/cm^2 for 0 to 15 min. After the treatment, a batch of samples were processed in duplicates immediately (0 h) in 10 mL BPW and plated on XLDN. Other

batches were stored in environmental chamber at 4, 12 and 21°C and 90% RH for up to 3 days to determine log survivors after 24, 48 and 72 h, respectively. Two duplicate samples were analyzed in each experiment and the experiments were conducted in replicates, and the data was analyzed by ANOVA using SPSS.

Results: Storage temperature and ripeness level has showed a significant effect ($P \leq 0.05$) on the survival of *Salmonella.* Increasing the storage temperature from 4 to 21°C and ripeness level from 0 to 100% increased the log survival. After 3 days of storage 21°C, *Salmonella* levels increased by 1.45 and 0.5 log CFU/sample at 100 and 0% ripeness levels, respectively. While a decreasing trend was observed for all ripeness levels at 4 and 12°C. Whereas, samples subjected to UV-C treatment has showed a significant reduction at all ripeness levels and storage conditions. However, the interaction effect of epicarp extracts especially at low ripeness levels still need to be determined.

Significance: These findings show promise to utilize UV light as potential surface treatment interventions to mitigate the risk of *Salmonella* in papayas.

P3-53 Evaluation of Ultraviolet (UV-C) Technology for the Reduction of Aflatoxin B1 in Almond Milk

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❖ ***Developing Scientist Entrant***

Introduction: In almond milk (AM), aflatoxin B_1 (AFB_1) and endospores are of major concern. The formation of short-lived reactive species initiated by UV-C photons causes photolysis of AFB_1, hence UV-C light at 254 nm could be an effective tool for inactivating spores and degradation of AF via photo-oxidation.

Purpose: To evaluate the UV susceptibility of endospores and AFB_1 in AM.

Methods: AM samples were spiked with 1μg.mL^{-1} of AFB_1 and *B. cereus* endospores (~ 7 log CFU/mL) separately, optical properties of test samples were mathematically calculated. The volumetric fluence rate (mW.cm^{-2}) was calculated based on the optical properties using computational fluid dynamics. Samples were treated under a low-pressure mercury (40 W) lamp emitting irradiation at 254 nm. Quantifiable UV doses ranging from 0 to 120 mJ.cm^{-2} was delivered. AFB_1 in samples (control and UV treated) were quantified using reverse phase high performance liquid chromatography coupled with fluorescence detector. Survival population of endospores were enumerated using standard plate count method with detection limit of 2 log CFU/mL.

Results: The delivered average fluence was determined (0.015622 mJ.cm^{-2}) based on absorption coefficient (1407 m^{-1}) and scattering coefficient (3668 m^{-1}) of AM. AFB_1 degradation kinetics showed log-linear relationship with UV dose with rate constant 0.0083 cm^2.mJ^{-1}. The quantum yield of AFB_1 was 3.35×10^{-4} and the energy consumed was 7.82×10^{-19}J. At 120 mJ.cm^{-2}, 72.4 ± 0.03 % ($P < 0.05$) AFB_1 was degraded, which is sufficient to treat AM with AFB_1 conc. up to 28 μg.L^{-1} (recommended limit is 8 μg.L^{-1}) AFB_1. At 36.5 mJ.cm^{-2}, 4.5-log reduction of *B. cereus* was observed ($P > 0.05$) with D_{10}-value 9.2 mJ.cm^{-2}.

Significance: The efficacy of UV-C technology in reducing AFB_1 and spores in AM; a highly opaque fluid, is clearly demonstrated. Further studies are focused on assessing the quality of UV-treated AM.

P3-54 Microbial Safety and Quality Assessment of Whole Milk Processed Using a Pilot-Scale Dean Flow UV System

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❖ ***Developing Scientist Entrant***

Introduction: Alteration in the taste/flavor and nutritional composition of thermally processed milk has led to an interest in novel technologies such as UV-C light.

Purpose: To evaluate the efficacy of a pilot-scale Dean Flow UV System against *Listeria monocytogenes* ATCC 19115 and *Bacillus cereus* ATCC 14579 endospores in whole milk (WM) and assessment of the volatiles.

Methods: Dean flow UV system comprised of a food-grade Fluorinated Ethylene Propylene (FEP) tube (0.64 cm diameter) wrapped in a serpentine path around 500 W UV-C (254 nm) lamp, cooling fan, peristaltic pump, lamp sensor, inlet and outlet tanks. WM was inoculated with test *microorganisms* (~7 log CFU/mL) and passed through the system at flow-rates of 16, 32 and 48 gal/h. Survivors after treatment were enumerated using the serial dilution plate count method (Detection limit = 2 log CFU/mL). The volatile profile of the samples was evaluated using a gas chromatography electronic nose. All experiments were done in triplicate. The total number of samples was eighteen.

Results: *L. monocytogenes* and *B. cereus* endospores were reduced >5 log and >3 log, respectively. With the increase in flow rate homogeneous dose distribution efficiency was increased which was attributed to the increase in Dean number. The D_{10}-values were observed to be 3.26 ± 0.21 and 8.91 ± 0.07 mJ/cm^2 for *L. monocytogenes* and *B. cereus* endospores, respectively. No significant impact on the volatile profile of the WM was observed ($P > 0.05$).

Significance: UV technology can be implemented in milk processing plants to produce safer and better quality milk. This technology can also be integrated to reduce bacterial counts to improve milk quality in parts of the world where there is a lack of a reliable energy supply and high cost of refrigeration, specifically in milk chilling stations.

P3-55 Wavelength Specific Inactivation of Vegetative Bacteria and Endospores By Germicidal UV-C Light in Liquid Suspensions

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Introduction: The UV action spectrum of a microorganism is a measure of inactivation effectiveness as a function of wavelength. UV inactivation of microorganisms was studied extensively at 254 nm using a low-pressure mercury lamp (LPM). Recently, UV light-emitting diodes (UV-LEDs) have emerged as a new source for UV radiation generation. UV-LEDs at various wavelengths can be manufactured using different semiconductor materials.

Purpose: This study investigates the comparative effect of UV-C light at various wavelengths (254, 265, 279 nm) on vegetative bacteria and endospores.

Methods: Absorption spectra of microbial suspensions in phosphate buffer saline were scanned in the range of 240-290 nm using a Cary UV-Vis spectrophotometer (Agilent Technology, Santa Clara, CA). The spectral irradiance of lamps was measured in the range of 200-400 nm using a spectrometer (QE pro series, Ocean Optics, Dunedin, FL). Average dose delivery through the fluid was measured by considering absorbance and irradiance in the range of the UV light spectrum. *E. coli* O157:H7 and *B. cereus* ATCC 14579 endospores were used. After treatment, enumeration of microorganisms was performed by serial dilution plate count method (detection limit 2 log CFU.mL^{-1}). Total number of sample 9 and replicates 3.

Results: For *E. coli*, UV-C light at 265 nm showed higher inactivation rate (0.39 ± 0.003 cm^2/mJ) in comparison to other 254 nm (0.36 ± 0.005 cm^2/mJ), and 279 nm (0.35 ± 0.006 cm^2/mJ). Similarly, for *B. cereus* endospores, UV-C light at 265 nm showed higher inactivation rate (0.118 ± 0.001 cm^2/mJ) than 254 nm (0.112 ± 0.001 cm^2/mJ), and 279 nm (0.104 ± 0.002 cm^2/mJ). Overall, the data show UV-C light at 265 nm efficient against *E. coli* and *B. cereus* endospores.

Significance: This study is useful for the selection of UV-C wavelength to inactivate vegetative bacteria and bacterial endospores in liquid foods.

P3-56 Microbial Validation of Radio Frequency Assisted Pasteurization of Whole Milk Powder

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❖ ***Developing Scientist Entrant***

Introduction: Food pathogens, such as *Salmonella* species, might be more heat resistant and survive for a longer time in milk powder due to its low water activity. Radio frequency (RF) heating may be a promising technology for milk powder pasteurization due to its volumetric and rapid heating in foods.
Purpose: The purpose of this study was to validate RF heating as a means for inactivating *Enterococcus faecium* NRRL B-2354 (*E. faecium*), a potential surrogate for *Salmonella enterica*, in whole milk powder. The effect of soybean oil as a surrounding media was also investigated as compared with air.
Methods: *E. faecium* and *Salmonella* cocktail (*S.* Montevideo, *S.* Agona, *S.* Tennessee, *S.* Enteritidis) were inoculated in whole milk powder and equilibrated at 0.35 a_w and 30°C. The milk powder container with/out immersing in soybean oil was heated in the RF oven until the cold spot reached to 75°C. The sample was put in an ice bath after holding in the RF oven. Survivor curves were plotted and *D*-values obtained from isothermal treatment by TDT cells were calculated. The log reductions (mean ± std dev of 3 replications) were calculated for each system and compared with ANOVA.
Results: The *D*-values of *E. faecium* and *Salmonella* cocktail varied from 16.81 to 11.36 min at 75°C, 12.03 to 6.44 min at 80°C, and 5.61 to 3.26 min at 85°C, respectively. Heating the whole milk powder in the RF oven to 75°C resulted in 0.11 ± 0.05 and 0.16 ± 0.11 log reductions in *E. faecium* and 0.81 ± 0.10 and 0.79 ± 0.01 log reductions in *Salmonella* cocktail inoculated milk powder when heated in air and soybean oil, respectively. Holding the milk powder at 75°C after RF heating can further increase the log reductions to satisfy FDA requirements.
Significance: Surrounding milk powder with soybean oil might be effective to increase the RF heating rate. The data obtained may be useful for future scale-up of the system for commercial application in food powder pasteurization.

P3-57 Developing a Practical Phantom Dosimeter for Ensuring the Safety and Quality of the X-Ray Irradiated Blueberries

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❖ ***Developing Scientist Entrant***

Introduction: Ionizing irradiation has been studied/utilized for control of insects and prevention of foodborne illness of fresh fruits and vegetables, without significant impact on physicochemical quality. However, it is a challenge for low-energy ionizing irradiation to measure the dose distribution throughout the entire product, such as a case of blueberries, in an industrial process. Providing more accessible quantification methods for validating dose distribution throughout foods would ensure safety and quality.
Purpose: This study's objective was to develop a phantom colorimetric dosimeter for measuring the dose distribution of blueberries.
Methods: A cylindrical phantom dosimeter (60 mm diameter/15 mm thickness) was constructed using paraffin wax, methyl yellow dye, and chloroform, which was irradiated at a known dose of 0, 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 kGy using an X-ray irradiator at 70 kV. Subsequently, the color change (from yellow to red) was quantified by a colorimeter (Konica Minolta CR400) using the CIE color scale, and a calibration curve was developed. For validation, model blueberries (18 mm diameter) were made from the same wax solution, irradiated, then quantified for internal and surface doses. The dose distribution was validated with real blueberry dose measurements at the surface, middle, and bottom of the blueberry with a solid-state dosimeter (RaySafe Solo RAD).
Results: The calibration data was fit to the Hill-Langmuir equation with an R^2_{adj} of 0.66. The model blueberry validation applied to the calibration equation was estimated with an R^2_{adj} of 0.99, and 0.97 for surface and internal dose exposure, respectively. The average dose difference between surface and internal measurements was 0.0129 ± 0.0081 kGy across all samples.
Significance: The validation of a dosimetry method for the practical assessment of dose distribution through solid foods helps develop optimized irradiation treatment methods for food safety and quality control.

P3-58 Effects of Ohmic Heating on Microorganisms Elimination and Foaming Ability of Liquid Whole Egg

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Introduction: Liquid egg is a convenient ingredient greatly used in bakeries and the food industry. Thermal pasteurized (TP) liquid egg usually results in the poorer forming ability by the protein denatured. Ohmic heating (OH) could uniformly heat the product to eliminate pathogenic microorganisms and maintain the food quality in short time company with thermal and non-thermal effects.
Purpose: This study was to evaluate the impacts of OH and TP treatments on liquid egg under lower temperature conditions and to identify the thermal or non-thermal effects for the reduction of the total counts and *Salmonella* spp.
Methods: Eggs were provided by local farm, and then washed, broken, homogenized, and refrigerated under 4°C in the day before used. The pH value and conductivity of the samples were measured. The TP treatments were using water bath for 45, 50, 55°C. The OH treatments were set at the voltage of 30 V, 20-50 Hz of the frequency for 45, 50, 55°C and holding 1 minute. The total counts and the *Salmonella* spp. were enumerated to determine the reductions (log CFU/g), and then calculated the *D*-value of each temperature of samples. Foaming abilities were calculated as overrun (%).
Results: The initial counts of the samples were 8.58 to 8.84 log CFU/g, pH values and conductivity of liquid whole egg were 7.83 and 6.45 mS/cm, respectively. *D*-value of OH in 45, 50, 55°C were 0.33, 0.30, 0.25 minute, respectively. The foaming abilities of OH-treated samples were non-significant different ($P > 0.05$) with the unpasteurized one.
Significance: The samples treated with 55°C could achieve >4-log reduction and maintain the foaming ability. The application of OH treatment result in non-thermal effects, electrical breakdown or electroporation, to eliminate the microorganisms and preserve the quality. This finding parameters may apply to industries to improve the liquid egg quality and food safety.

P3-59 Removal of Bacterial Cells, Biofilms and Catfish Processing Residue on Food Contact Surface by Pneumatic Driven Swipe of Steam and Steam-Jetted Hot Water

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Introduction: Effect of continuous steaming on pathogen survival has not been reported in the literature. We used a new Clean-In-Place (CIP) technology, Optima Steamer™ SE-II to clean and sanitize subjects on the continuous belt by swiping steam or steam-jetted hot water (SJHW) combined with pneumatic-driven nozzle installed on the Optima pneumatic conveyor-belt cleaning tool.
Purpose: The objective is to remove bacterial cells, biofilms and catfish processing residue on food contact surfaces by this automatic steamer technology.
Methods: *Escherichia coli* ATCC 25922 and *Listeria innocua* ATCC 33090 were used as surrogates for *E. coli* O157:H7 and *L. monocytogenes* to form inoculum and biofilm on PVC and stainless-steel coupons. Hygiena SystemSURE Plus ATP monitoring system and UltraSnap Surface ATP test swabs were used to study the effectiveness of cleaning catfish meat fouling.
Results: Inoculum of *E. coli* and *L. innocua* on the surface of PVC and stainless-steel coupons were reduced to under detection limit (2.3 log CFU/coupon) after heating with SJHW at 100 psi with 16 cm/30 seconds conveyor speed. Both steam and SJHW reduced the *E. coli* biofilm on PVC coupons from 7.18 ± 0.72 log CFU/coupon to under detection limit after the swipe with pressure higher than 40 psi at 4 and 8 cm/30 seconds conveyor speed. Spray of 100

Poster

psi steam at conveyor speed of 4 cm/30 seconds caused 5.5 log CFU/coupon reduction of the *L.* innocula biofilm on stainless steel. Both steam and SJHW could clean the stainless-steel plate and PVC cutting board contaminated by catfish meat to under suggested values (<500 RLUs) while SJHW was more effective.

Significance: The CIP steam operation provided a faster, less labor-intensive, and utilized less water, less wastewater and no chemical exposure risk and can be useful to enhance safety in the catfish and other food processing industry.

P3-60 Inactivation of *Geobacillus stearothermophilus* Spore in Flour on Different Food Processing Surfaces during Superheated Steam Treatment: Influence of Heat and Moisture Transfer

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Introduction: Superheated steam is an emerging technology for sanitation of food processing surfaces. However, there is limited data on how microbial inactivation on different surface materials is affected by temperature and moisture changes during superheated steam treatment.

Purpose: The objectives were to investigate how the inactivation of *Geobacillus stearothermophilus* spore on different surface materials was affected by the temperature and moisture changes during superheated steam treatment.

Methods: Stainless steel, concrete, and rubber coupons (34.9 mm × 23.8 mm × 4.8mm) were coated with wheat flour film (0.30 mm) inoculated with *G. stearothermophilus* ATCC7953 spores (7.51 ± 0.23 log CFU/g). The samples were treated in the preheated chamber (125°C, 170°C, and 250°C) to investigate the spore inactivation. The surface temperature and the moisture content of flour film were monitored. The experiments were carried out in triplicate. The heating rate (*k*) and moisture diffusivity (*D*) parameters were estimated to describe the heat and moisture transfer characteristics.

Results: Regardless of superheated steam temperature, the surface temperatures on concrete increased faster (k > 1.89/min) than on stainless steel (k > 0.65/min). As a consequence, concrete surfaces showed faster dehydration ($D > 1.17\times10^{-4}$ mm/s) than stainless steel ($D > 0.76\times10^{-4}$ mm/s). Rubber coupons could not withstand temperature >170°C and were destroyed. The k and D on rubber at 125°C were 2.34/min and 0.92×10^{-4} mm/s, respectively. At 250°C, a 5-log reduction of *G. stearothermophilus* spore was achieved in 180 s on stainless steel (5.61 ± 0.17 log reduction) and 240 s on concrete (5.00 ± 0.28 log reduction). Faster inactivation on stainless steel can be explained by the higher initial condensation (13.3 ± 0.4%) and lower D (1.27×10^{-4} mm/s) on stainless steel compared to those on concrete (2.3 ± 0.1% and 4.66×10^{-4}mm/s, respectively). At 170°C, a 5-log reduction was achieved in 1,200 s for both stainless steel and concrete (5.44±0.17 log reduction and 5.03±0.17 log reduction, respectively) while only 0.97±0.16 log reduction was achieved at 125°C in 1,800 s.

Significance: The results provide useful insights on microbial efficacy of superheated steam sanitization on different surface materials.

P3-61 Inactivation of *Salmonella* on Mung Bean Sprouting Seeds Using Dry Heat Treatment

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❖ ***Developing Scientist Entrant***

Introduction: Sprouts are widely consumed in the world due to their many health benefits. Unfortunately, numerous outbreaks linked to seed sprouts have occurred in the USA and other countries for the past two decades. Currently, effective seed decontamination interventions have not been developed.

Purpose: The objective was to determine the effects of dry heat (temperature and time) and water activity of seeds on *Salmonella* inactivation and seed's viability.

Methods: Mung bean seeds were dip-inoculated with a four-strain *Salmonella* cocktail to a final level of ~6 log CFU/g. The inoculated seeds along with un-inoculated seeds were dried to water activities of 0.2 – 0.4 using saturated salt solutions. Un-inoculated seeds and seeds inoculated with *Salmonella* were placed in glass tubes, sealed and treated in hot air at 70 and 75℃ for selected time intervals. The inoculated seeds were used for determining *Salmonella* counts and un-inoculated seeds were used for growing sprouts.

Results: Water activity level of seeds played a critical role in *Salmonella* inactivation by dry heat and seed's viability. Increasing water activity of seeds improved *Salmonella* inactivation by dry heat, but decreased seed's viability. To achieve a > 5-log reduction of *Salmonella* on seeds, seeds with water activity levels of 0.2, 0.3, and 0.4 need to be treated at 75℃ for 30, 22, and 19 hours, respectively. Decreasing treatment temperature from 75 to 70℃ increased the treatment time needed to achieve a > 5-log reduction of *Salmonella,* but better preserved seed's viability. When un-inoculated seeds with 0.2 water activity were treated at 70℃ for 60 hours, they successfully germinated and grew to a sprout weight that was almost identical to that of the un-treated seeds.

Significance: Dry heat treatment with proper control of seed's water activity could be an effective method for seed's decontamination while preserving seed's viability.

P3-62 Effects of *Debaryomyces hansenii* Isolates on Microbial Flora in Dry-Aged Beef during Dry-Aging

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❖ ***Developing Scientist Entrant***

Introduction: Dry-aging is a method of ripening beef by exposing the carcasses to various microorganisms, but the safety of the dry-aged beef is uncertain. Some yeasts, such as *Debaryomyces hansenii* can be used to enhance flavor and taste in the manufacture of dry-aged beef. Therefore, it is necessary to evaluate whether the growth of the yeast inhibits the growth of microbial flora.

Purpose: This study evaluated the effect of isolated yeasts on inhibiting the growth of microbial flora in dry-aged beef, and also on the improvement of tenderness and flavor in low grade beef.

Methods: Five *D. hansenii* isolates (SMFM201812-1, SMFM201812-3, SMFM201905-4, SMFM201905-5, SMFM201905-15) from dry-aged beef, which have no hemolysis and cytotoxicity, were inoculated on 600 g of the topside beef for dry-aging at 6 log CFU/mL. After 4 weeks of dry-aged periods, prevalence of microbiological contaminants, and quality factors related to meat texture quality such as pH and shear force were measured in dry-aged beef. Flavor related factors such as lipid rancidity, fatty acid compositions, and free amino acid compositions in dry-aged beef were measured.

Results: The samples inoculated with *D. hansenii* SMFM201905-5 had the lowest total bacterial cell counts, and pathogenic bacteria were not detected in all samples. The pH and lipid rancidity were measured at an acceptable intake level. Considering the shear force, tenderness of *D. hansenii* SMFM201812-3 inoculated group was significantly low (P < 0.05). As a result of measuring the fatty acid, total content of *D. hansenii* SMFM201812-1 and SMFM201812-3 inoculated beef were higher than other groups (P < 0.05). Moreover, the total free amino acid content of *D. hansenii* SMFM201812-3 and SMFM201905-5 inoculated beef were higher (P < 0.05) than *D. hansenii* SMFM201812-1 inoculated beef.

Significance: The effects of *D. hansenii* strains on inhibiting microbial flora and improving qualities in dry-aged beef are variant. Thus, these effects should be considered, when selecting *D. hansenii* strains for both safety and quality in dry-aged beef.

P3-63 Microbial Validation of Biltong Processing to Achieve 5-Log Reduction of *L. monocytogenes* and *E. coli* O157:H7

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❖ ***Developing Scientist Entrant***

Introduction: 'Biltong' is a South African style dried beef product that is marinaded and dried at moderate heat but still must accommodate USDA-FSIS safety concerns for process validation by demonstrating a 5-log pathogen reduction.
Purpose: Our objective was to achieve a 5-log reduction of *L. monocytogenes* and *E. coli* O157:H7 without a heat lethality step and evaluate the individual contribution marinade components (spice, salt, vinegar) to the overall reduction on inoculated beef.
Methods: Beef was cut into small 'steaks' (0.75-in x 2-in x 3-in) and inoculated with a 4-serovar mixture of acid-adapted *L. monocytogenes* or *E. coli* O157:H7 spread on the surface of the beef. Beef pieces were vacuum-tumbled with either a mixture of spice, salt, and vinegar, or just individual marinade components and marinaded for 30 minutes. Beef was then dried in a temperature-controlled humidity oven for 10 days (75°F/23.9°C; 55% RH). All trials were performed in duplicate replication (with triplicate samples at each time point; $n = 6$) and RM one-way ANOVA to determine significant differences ($P < 0.05$).
Results: The combination of spice, salt, and vinegar resulted in a reduction of >5 log and >6 log with drying of 8 and 10 days, respectively. The greatest reduction of *L. monocytogenes* from individual marinade components was exhibited by vinegar (>5 log) followed by salt (4.7 log) and spice (4.1 log). Combined ingredients of marinade also resulted in a reduction of >5 log with drying period of 4 days for *E. coli O157:H7*. Salt and vinegar when treated individually lead to similar reduction of (5.48 log) and (5.49 log) followed by spice (4.11 log). Water activity for 10 days of drying was 0.79.
Significance: This is the first published work achieving >5-log reduction of *L. monocytogenes* and *E. coli* O157:H7 with biltong, thereby validating this process for USDA-FSIS approval that is sought by many upscale food stores to ensure product safety.

P3-64 Effectiveness of Elevated Hydrostatic Pressure and Mild Heat Against Pressure-Stressed, Habituated, and Wild-Type *Listeria monocytogenes*, *Listeria innocua,* and *Staphylococcus aureus*

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Introduction: As a commercially feasible and efficacious alternative to thermal pasteurization, high-pressure processing could assure safety of an array of products while providing co-benefits such as superior organoleptic characteristics and fresh-like qualities.
Purpose: This study investigated decontamination effects of high hydrostatic pressure against wild-type, pressure-stressed, and three-day habituated phenotypes of *Listeria monocytogenes*, *L. innocua,* and *Staphylococcus aureus* in orange juice and milk.
Methods: Pressure intensity of 350 and 500 MPa were used at 4.4 and 60.0°C for treatments lasting up to seven minutes. Temperature was controlled using a stainless-steel water jacket surrounding the pressure chamber mechanically connected to a refrigerated circulating water bath. Results were statistically analyzed using ANOVA followed by Tukey-adjusted means separation.
Results: While <2 log CFU/mL reduction ($P < 0.05$) of *L. monocytogenes*, *L. innocua,* and *S. aureus* were observed after treatments of up to seven minutes at 500 MPa/4.4°C, similar treatments at 60.0°C resulted in >5 log CFU/mL reductions ($P < 0.05$) of pathogens inoculated in milk. Similar microbial reductions were observed when pathogens were inoculated in acidic environment (*e.g.,* orange juice) and when treated at 350 MPa at 4.4°C as well as 60°C. Thus, under conditions of this experiment, we observed mild heat could appreciably augment effectiveness of high-pressure processing. Three tested phenotypes of these Gram-positive pathogens showed comparable sensitivity ($P \geq 0.05$) to treatments, indicating a validated treatment against wild-type cells could almost certainly be efficacious against other phenotypes as well.
Significance: Pressure treatments of 500 MPa at 60.0°C resulted in ≥ 5 log reductions of pathogens and more than 90 to 99% of pathogens could be eliminated at 500 MPa at 4.4°C. *L. monocytogenes* and *L. innocua* exhibited comparable sensitivity to hydrostatic pressure. Similarly, *S. aureus* exhibited comparable sensitivity relative to *L. monocytogenes.*

P3-65 Survival of Acid-adapted, Pressure-resistant *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* during Cold Storage in HPP-treated Juices

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❖ ***Developing Scientist Entrant***

Introduction: HPP juice manufacturers are required to demonstrate a 5-log reduction of the pertinent microorganism to comply with FDA Juice HACCP. There is currently no consensus on validation approaches for shelf-life studies in terms of storage conditions, sampling times, or temperature abuse.
Purpose: Compare HPP inactivation and post-HPP survival of acid-adapted *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* in apple and orange juice during a 75-day shelf life using quantitative and qualitative assessment.
Methods: Bacterial strains *E. coli* O157:H7 TW14359, *Salmonella* Cubana and *L. monocytogenes* MAD328 were acid-adapted in intermediate pH 5.0 TSBYE. Individual strains were inoculated into apple juice (pH 3.50 ± 0.20) and orange juice (pH 3.88 ± 0.10) at approximately 6.00 log CFU/mL, pressure treated at 586 MPa (180 s, 4°C initial) and stored at 4°C for remainder of shelf life. Inoculated, untreated controls were included. Triplicate samples with duplicate analyses. Quantitative plating and qualitative enrichments were conducted on days 1, 2, 3, 4, 5, 10, 15, 35, 55, and 75 with temperature abuse on days 3 (30°C, 2 h) and 12 (30°C, 2 h and room temperature, 1 h).
Results: Significantly ($P < 0.05$) greater log reduction occurred for *S.* Cubana (approximately 3 logs) and *L. monocytogenes* MAD328 (>5 logs) compared to *E. coli* O157:H7 TW14359 (<1 log) following initial post-HPP analyses. While complete inactivation was not seen, bacterial inactivation exceeded 5-log reduction in both HPP-treated juices immediately following pressure treatment for *L. monocytogenes* MAD328, after 24 hours in cold storage for *S.* Cubana, and after 4 days of cold storage for *E. coli* O157:H7 TW14359. Contradictory positive quantitative and negative qualitative analyses resulted for *E. coli* O157:H7 TW14359. Recovery of *L. monocytogenes* MAD328 in orange juice was observed with prolonged cold storage time on days 35 and 75.
Significance: These results suggest in certain juice types a cold holding time following pressure treatment may be required to achieve a 5-log reduction.

P3-66 Comparison of Inactivation of *Salmonella* spp. by High Pressure Processing in Ground Chicken Meat Sources Used in Raw Pet Foods

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❖ ***Developing Scientist Entrant***

Introduction: Raw pet foods present a unique microbial safety challenge in that the products are made of raw meats, but are considered ready-to-eat foods for companion animals. High-pressure processing (HPP) is used by some manufacturers to control pathogens in their products, while minimizing organoleptic changes. The composition of meats and meat byproducts used in pet food formulations can affect the efficacy of HPP.
Purpose: The objective of this study was to compare the initial inactivation of *Salmonella* spp. by HPP treatment and their rates of recovery post-HPP in four ground chicken matrices with different fat and bone contents.

Methods: Four ground chicken matrices – 100 % breasts (S1), 10% backs and 90% breasts (S2), 100% backs (S3) and 100% deboned backs (S4) – were inoculated with a five-strain cocktail of *Salmonella* spp. Levels of fat and calcium (used as an estimate of bone content) were measured for each matrix. The inoculated samples were treated with HPP at 450 and 590 MPa for 3 min at 4°C. *Salmonella* inactivation was determined by subtracting the HPP-treated samples from pre-HPP samples, immediately, post-HPP treatment. Pathogen recovery in all four matrices was monitored for 14 days of refrigerated storage.

Results: Fat increased from S1 < S2 < S3 < S4, while calcium increased from S1 < S4 < S2 < S3. More than 5-log reduction of *Salmonella* was observed in S1 and S2 at 450 MPa, while 590 MPa was required in S3 and S4. In general, the rate of pathogen recovery post-HPP increased with increasing fat and calcium contents. S4 had the highest pathogen recovery, owing to its high-fat content.

Significance: This study showed that the efficacy of HPP treatment is dependent on product formulation. Relatively high fat and bone contents may offer a baroprotective effect on pathogens that, despite achieving a 5-log reduction immediately post-HPP, allows sublethally injured cells to recover over the product shelf life.

P3-67 Recovery of Sub-Lethally Injured *Salmonella spp.* in Ground Chicken Breast by High Pressure Processing on Different Plating Media

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Introduction: High pressure processing (HPP) has been recognized by the U. S. National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 2005) as non-thermal technology for commercial pasteurization of ready-to-eat meat products. A verification of HPP effectiveness is generally conducted by quantifying post-treatment survival cells. The enumeration media often overlook the recovery of sub-lethally injured cells imparted by HPP, thus resulting in an overestimate of the lethality of the treatment.

Purpose: The objective of this study was to investigate the most appropriate recovery media for sub-lethally injured *Salmonella* spp.

Methods: Ground turkey breast meat were inoculated with five *Salmonella* strains and were treated with high pressure at 0.1 (Control), 300, 450, and 590 MPa for 3 min at 4°C. Samples were enumerated immediately and 24 h post HPP-treatment. Four recovery plating media were evaluated: (a) nonselective tryptic soy agar (TSA) overlayed with a selective media, xylose lysine deoxycholate (XLD); (b) thin layer of XLD onto TSA; (c) a modified TSA with 0.3% Ferric Ammonium sulfate to differentiate *Salmonella spp.* from background microflora; and selective media, XLD, which was used as a reference to recover healthy cells. The proportion of sublethally injured cells was expressed as the percent difference of cell counts from those recovered with XLD only.

Results: No significant differences in sublethally injured cell counts were observed among all three of recovery media at 450 and 590 MPa ($P > 0.05$). At 300 MPa, a higher proportion of sublethally injured cells were recovered in modified TSA compared to the TSA with XLD overlay and thin layer TSA over XLD ($P < 0.05$), while the sublethally injured cells count in overlay and thin layer agar were statistically the same ($P > 0.05$).

Significance: This study highlighted the importance of recovery of sublethally injured cells post-HPP treatment to alleviate the overestimation of treatment lethality, thus improving the accuracy of microbial analysis in HPP validation study.

P3-68 Long-Term Survival Phase Cells of *Escherichia coli* O121 and *Salmonella* Typhimurium Exhibit Increased Tolerance to Atmospheric Cold Plasma on Artificially Inoculated Wheat Grains

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❖ ***Developing Scientist Entrant***

Introduction: Raw wheat-based foods contaminated by enteric pathogens pose a serious food safety risk. Novel technologies such as atmospheric cold plasma (ACP) can reduce pathogens in the stationary phase (STA) on wheat grains, however, there are no published reports on the tolerance of the long-term survival phase (LTS) cells of enteric pathogens to ACP.

Purpose: The purpose of this study was to evaluate the tolerance of *Escherichia coli* O121 and *Salmonella enterica* Typhimurium in the LTS phase to ACP on wheat grains and the extent of sub-lethal injury in survivors.

Methods: Samples (10 g) of wheat grains were inoculated with *E. coli* O121 or *S. enterica* Typhimurium ATCC 14028 cells in the STA or LTS phase to obtain an initial count of ~7.0 log CFU/g. Inoculated grains in sealed plastic bags with atmospheric air were exposed to ACP (44 kV) for 0 (control), 4, 8, 12 and 16 minutes, then held at 22 ± 1°C for 24 h. Survivors were evaluated by surface plating samples on Thin Agar Layer (TAL) media and appropriate selective agar, and bacterial colony counts were performed after 24 h (TAL) and 48 h (selective agar) of incubation (35°C).

Results: ACP tolerance of *E. coli* and *S*. Typhimurium LTS cells was higher than that of STA cells ($P < 0.05$). For both pathogens, STA cells were undetected (<1.0 log CFU/g) on TAL medium or SEL agar after ACP treatment for 16 min. In contrast, LTS cells were consistently detected on both agar media.

Significance: The ability of enteric pathogens to enter the LTS phase on wheat grains and develop tolerance to ACP may pose a food safety risk in wheat-based products. The LTS phase cells should be considered in the design of ACP treatments for destroying pathogens such as *E. coli* O121 or *S. enterica* on wheat grains.

P3-69 Optimization of Nonthermal Plasma-Activated Water Processing Conditions for Inactivation of *Salmonella* Typhimurium

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❖ ***Developing Scientist Entrant***

Introduction: Plasma-activated water (PAW) is generated by exposing water to nonthermal air plasma at atmospheric pressure and has antimicrobial properties. The source of plasma, activation time, the volume of water, and generation approach affect the concentration of reactive species in PAW and thereby microbial inactivation efficacy.

Purpose: The purpose of this study was to optimize processing conditions of PAW for its inactivation efficiency against *Salmonella* Typhimurium using response surface methodology.

Methods: The Box-Behnken design (BBD) was used with 3 factors: volume of water, time for plasma activation, and the distance between the water surface and nozzle of the plasma jet. The effects of these parameters on pH, electrical conductivity (EC), oxidation-reduction potential (ORP), nitrate and nitrite concentration in PAW were statistically analyzed. A total of 15 BBD experiments were performed in duplicate to measure these responses and the microbial reduction of planktonic cells of *S.* Typhimurium incubated in PAW for 3 min. Statistical significance was determined at $P < 0.05$.

Results: The ORP value was significantly affected by the distance. The EC, pH, nitrite and nitrate concentrations in PAW were significantly affected by volume, time, distance, and the square of the distance. In addition, EC values were significantly affected by volume*time and nitrite and nitrate values were significantly affected by the square of time. An increase in time predicted a reduction in pH, increase in EC, nitrite and nitrate values. A decrease in distance predicted an increase in ORP, EC, nitrite and nitrate values. A reduction of 6.3 ± 0.3 log of *S.* Typhimurium was achieved when the time, distance, and volume were 15 min, 6.5 cm, and 250 mL, respectively.

Significance: The findings identify optimum processing conditions that can be useful for future research studies and in scaling up this technology for industrial applications.

P3-70 Evaluation of Food Safety Interventions in Low- and Middle-Income Countries (LMICs)

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Introduction: Current frameworks for evaluating food safety problems in low- and middle-income countries (LMICs) often lead to adoption of programs that ultimately fail because they do not adequately address many of the unique institutional and cultural features of LMICs.

Purpose: This study examines features of LMICs and suggests a framework for evaluating proposed interventions to maximize the probability of adoption and sustained success in a specified country.

Methods: Evaluations of proposed food safety interventions typically involve, at best, a pilot project (with pathogen testing) combined with a risk assessment, and (possibly) a cost-effectiveness analysis to yield expected benefits from widespread adoption of the proposed program. This study subsumes the standard approach in a broader framework that considers institutional constraints, culture, incentives for adoption, and risk tradeoffs. The model is constructed based on an examination of multiple literatures, including risk analysis, economics, consumer behavior, communications, and education.

Results: The resulting framework has the following broad attributes. First, there is an initial assessment of institutional capacity prior to intervention development. Intervention design and evaluation are then guided, in part, by a recursive process of examining cultural compatibility, incentive compatibility, risk tradeoffs, and newly obtained information about institutions. Finally, an implementation strategy is recommended based on similar factors.

Significance: The proposed framework is a first step towards formalizing what is generally understood by development specialists. Formal implementation will lead to better designed interventions, higher rates of sustainable adoption, and, ultimately, fewer foodborne illnesses in LMICs.

P3-71 Factors Affecting the Cross-Contamination of *Listeria monocytogenes* and *Salmonella enterica* on Bell Pepper during Chlorine Washing

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❖ ***Developing Scientist Entrant***

Introduction: Chlorine is commonly used to minimize microbial cross-contamination during washing. However, factors such as surface conditions and agitation during washing may affect the level of cross-contamination.

Purpose: This study evaluated the factors affecting the level of cross-contamination of *Listeria monocytogenes* and *Salmonella enterica* on the surface of bell pepper during chlorine washing.

Methods: Bell pepper surfaces were spot inoculated with 200 µL of *Listeria monocytogenes* and *Salmonella enterica* with high (~6 log) or low (~3 log) inoculum size and were washed together with non-inoculated bell peppers (intact or manually bruised) in water or 100 ppm of chlorine for 5 minutes. Washing was performed with or without agitation using a perforated pipe sitting on the bottom of the washing container and connected to an air blower. The tested pathogens were enumerated using the plating technique on respective selective agars.

Results: Cross-contamination up to 2.98 logs CFU/g of *Listeria monocytogenes* was observed in uninoculated bell peppers after washing with contaminated bell peppers in the chlorine solution. High inoculum samples resulted in a significantly higher ($P < 0.05$) level of cross-contamination of *L. monocytogenes* on non-inoculated bell peppers as compared to low inoculum size. Chlorine wash was able to prevent the cross-contamination of *Salmonella enterica*. For samples with a high level of inoculum, the surface bruise, as well as agitation during washing, didn't affect the level of *Listeria* cross-contamination. However, agitation during washing was a significant factor for low inoculum samples where *L. monocytogenes* contamination increased by 0.8 log CFU/g. Bruised samples inoculated with low inoculum size also resulted in greater cross-contamination (~2.4 log CFU/g) as compared to the intact samples (~1.5 log CFU/g).

Significance: Agitation during washing facilitates the dislodge of microorganisms from the produce surface. The use of sanitizer such as chlorine is effective in minimizing *Salmonella* cross-contamination during washing of fresh produce.

P3-72 Microbial Prevalence of *Listeria* in Fresh Produce and Virulence Gene Distribution in *L. monocytogenes*

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria,* which associated with high mortality rates, is one of the most common pathogenic bacteria contaminating fresh produce, but limited data are available on *Listeria* associated with urban agriculture.

Purpose: To understand *Listeria* contamination in fresh produce associated with urban agriculture and virulence genes distribution in *L. monocytogenes*.

Methods: A total of 432 fresh produce samples including leafy greens and root vegetables were collected from farmers markets and urban garden in Michigan and West Virginia in summer 2019. Vegetable rinse was pooled into 109 composite samples. *Listeria* was isolated by pre-enrichment in BLEB for 4 hours, followed by selective enrichment with acriflavine hydrochloride and nalidixic acid. Enriched samples were spread on PALCAM agar. Potential *Listeria* were identified by Gram staining and rapid L'mono differential agar and confirmed by PCR. *L. monocytogenes* was analyzed by serotype identification using PCR and genotyping using PFGE. Virulence genes were amplified by PCR in *L. monocytogenes* and subjected to Sanger sequencing.

Results: Out of 109 composite samples, 45 were positive for *Listeria*. Thirty-one samples were contaminated by *L. welshimeri*, 11 by *L. innocua*, and three by *L. monocytogenes*. Five *L. monocytogenes* isolates were further characterized. Serotypes 1/2a and 4b were identified in potatoes, radish, and romaine lettuce. Four unique PFGE patterns were identified. One romaine lettuce contained three different clones, two with unique PFGE patterns and one un-typeable. Virulence genes *iap, inl*B, *llx*S, *act*A, *prf*A, and *pts*A were detected in 4, 4, 3, 2, 2, and 1 isolates, respectively. One isolate from romaine lettuce and radish each carried 5 and 4 virulence genes, respectively. All remaining isolates had 3 or less genes tested.

Significance: The presence of *Listeria* spp., especially *L. monocytogenes,* in fresh produce indicates that fresh produce may pose serious food safety concern. Virulence genes in *L. monocytogenes* also suggest their potential to cause human disease.

P3-74 The Last Mile, Temperature Monitoring Solutions for Direct Perishable Shipments

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Introduction:

The volume of direct shipments of perishable products are increasing, especially during the COVID-19 pandemic, driving concerns for quality and safety related to temperature abuse.

Purpose:

With so many companies entering the perishable direct shipment market, we must minimize consumer illness while nurturing a culture of safety and quality; so it is essential to understand the options

Poster

when it comes to temperature monitoring.

Methods:

At IAFP 2019 in Kentucky, food safety leaders stated their concerns with perishable food deliveries, especially direct to consumers. How can we help consumers navigate "The Last Mile" so they are confident in the quality and safety of their home delivery of perishable food products? Here is the good news, these solutions already exist.

Vitsab International AB is a research and development company based in Malmo, Sweden and they collaborate with US FDA, WHO, and European Union Food Law to find and develop temperature monitoring solutions for specific applications. Vitsab also works with industry leading companies in the temperature monitoring business to customize the best solutions for specific temperature monitoring needs. Come peak behind the R&D curtain as Jeff Desrosiers, EVP with Vitsab International, presents a summary of years of research by reviewing the latest temperature monitoring solutions available from many of today's leading companies all geared towards validating "The Last Mile".

Results: The biggest challenges for temperature monitoring for consumers are ease of use, simplicity of understanding, and cost effectiveness. RFID, Wi-Fi Temperature Sensors, Data Loggers, Time Temperature Indicators; oh my? This detailed analysis of existing technologies and platforms provides insight into current solutions for temperature monitoring "The Last Mile" and what are your best options.

Significance:

Quality and safety of perishable food has always been a major driver behind regulation and consumer confidence. Since consumers are migrating towards home delivery as evident during the current pandemic, we can boldly move forward with existing temperature monitoring solutions.

P3-75 Efficacy of Dry Heat on the Inactivation of *Salmonella enterica* on Stainless Steel

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Introduction: *Salmonella* is an important enteropathogenic bacterium, causing serious foodborne illness, gastroenteritis. The pathogen can colonize the abiotic surfaces of food processing facilities, thereby increasing the subsequent risk of cross-contamination of finished products.

Purpose: This study examined the initial adhesion and desiccation survival of *S.* FUA1946 and *S.* ATCC13311 on stainless steel coupons (SSCs) and further evaluated the effectiveness of dry heat treatment for inactivating *Salmonella* on the SSCs.

Methods: The stainless steel coupons (type 304) were surface inoculated with 20 µL each of the overnight cultures of the individual strains and desiccated at 25°C and 33% RH. The viability was assessed for 5 days. This was followed by a heat treatment in closed aluminum-based thermal death time cells using a water bath at 70°C.

Results: Under desiccated conditions, populations of *S.* FUA1946 and *S.* ATCC13311 reduced by 0.26 ± 0.10 log CFU/cm^2 and 0.70 ± 0.11 log CFU/cm^2, respectively. The *S.* FUA1946 observed higher resilience to dry heat with a D_{70}-value of 43.02 ± 9.32 minutes, whereas *S.* ATCC13311 exhibited lower thermal tolerance with a low D_{70}-value of 6.65 ± 0.71 minutes and observed below the detection limit (1.3 log CFU/cm^2) after 13 minutes of the heat treatment. The differences in the strain type significantly affected the desiccation and thermal survival.

Significance: The survival of *S. enterica* strains in the low-a_w food processing environment is a major food safety concern and, numerous intervention strategies have been employed for its eradication. This study demonstrated the potential of dry heat for efficiently reducing the *Salmonella* populations on the stainless steel surfaces.

P3-76 Cyclic Temperature Abuse of Raw Poultry during Supply Chain Can Impact Food Safety and Shelf Life

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❖ ***Developing Scientist Entrant***

Introduction: During delivery, the doors of refrigerated trucks may be left open for an extended period leading to temperature abuse and potential food safety and spoilage concerns.

Purpose: The objective of this study was to investigate the effect of cyclic temperature abuse on the growth of *Salmonella* Typhimurium and aerobic plate count (APC) during simulated supply chain.

Methods: A total of 4 scenarios (24 h each) were tested in 3-independent replications as follows: Scenarios 1 and 2: 2 hours at 4°C followed by 2 hours at 25°C in sterile brain heart infusion (BHI) broth and chicken breast, respectively, Scenario 3: 24 hours at 25°C on chicken breast, and Scenario 4: 24 hours at 4°C on chicken breast. For all scenarios, *Salmonella* Typhimurium (35 µg/mL nalidixic acid resistant) was inoculated in sterile BHI broth and raw chicken breast to achieve approximately 10^4 CFU/mL target inoculum. Temperature probes were placed centrally in flasks and chicken breasts and both were placed in a programmable incubator. Uninoculated breast fillets were used to study spoilage. Sampling was conducted every 6 hours to determine *Salmonella* populations in inoculated chicken breast and broth and to analyze aerobic plate counts (APC) of uninoculated fillets. Data was analyzed and predictive equations were developed using polynomial regression.

Results: The growth of *Salmonella* in scenarios 1, 2, and 3 can be described by second order polynomial equations with $R^2 > 0.90$. The growth of *Salmonella* entered the log phase after 6 hours or 1.5 temperature cycles in broth and 12 hours or 2.5 temperature abuse cycles on chicken breast in Scenarios 1 and 2, respectively. Scenario 3 reached log phase after 6 hours or 1.5 temperature abuse cycles and Scenario 4 never reached log phase.

Significance: The study demonstrates the importance of cold-chain management during the delivery of raw poultry meat.

P3-77 Development of Bespoke Food Safety Culture Measurement Tool for a Low-Risk Food and Drink Manufacturer

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Introduction: Food safety culture (FSC) of a company relates to fundamental attitudes towards food safety (FS). Assessing FSC allows businesses to analyze the foundations of their FS management system. Due to great diversity within the food industry and to capture the complexity of a business' FSC, context, detail and bespoke assessment are required.

Purpose: To gather detailed management/operative-employee perspectives of current FS understanding and attitudes and obtain in-depth, qualitative understanding of factors influencing FSC, according to specific dimensions, to inform development of a bespoke FSC measurement tool.

Methods: In-depth interviews with management/operative employees explored perceptions associated with FSC dimensions and related themes specific to the business. Data gathered from interviews was used to influence questionnaire attitude-statements aiming to investigate perceptions identified in interview in greater depth and quality.

Results: Cumulatively, comprehensive analysis of management/operative-employee experience highlighted pride and satisfaction in employment, however lack of awareness of the term 'FSC' was identified. Many management-employees perceived FS to be paramount, whereas operative-employees

stated FS was important but not always a priority. Multiple management-employees suggested health and safety was more important than FS: "*health and safety will be number one priority.*" Management/operative-employees reacted positively to questions asked about feelings of empowerment in the business. Equally, management-employees were positive about perception of other's empowerment within the business: "*a lot of trust put in individuals.*" Operative-employees purported authority to ensure that food was safely produced "*no problem stopping a line if I see anything,*" "*everyone cares about their job.*"

Significance: Findings from this study will inform development of a bespoke FSC quantitative survey, enable tailoring to specific complexities of the business and allow management/operative-employee data to be analyzed separately as identified. Combining qualitative and quantitative data allows a greater understanding of the business' FSC and will be used to develop approaches to strengthen FSC in the business.

P3-78 Effect of Desiccation Stress on the Cross-Contamination of *Escherichia coli* O157:H7 from Food-Contact Surface to Food

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❖ ***Developing Scientist Entrant***

Introduction: Cross-contamination has been considered one of the major sources of foodborne illness. Although many studies on cross-contamination have been reported, the effect of desiccation stress prior to cross-contamination on transferring rate of bacterial cell is still unknown. If we know the difference in the transferring rate of bacteria between different exposure periods to desiccation stress, cross-contamination risks could be estimated more realistically.

Purpose: The objective of this study was to investigate the effect of desiccation stress on the cross-contamination of *Escherichia coli* O157:H7 by comparing transferring rate from food contact surface to real foods.

Methods: Pre-grown *E. coli* O157:H7 in TSB was inoculated onto different kinds of material piece (1× 1 cm) surface including stainless steel finished by #400, stainless steel finished by No. 2B, and polyethylene. The inoculated pieces were dried under safety cabinet for 60 min and then stored at 20°C and 45 ± 5 % relative humidity for up to 8 days. The inoculated pieces with different storage period were separately attached onto TSA plates and real food surfaces including salmon, beef, chicken, cheese, and cracker for 1 min. The numbers of *E. coli* O157:H7 on both the donor and recipient surfaces were determined.

Results: Regardless, the exposure period of desiccation stress (0-8 days) and the kinds of surface material, the numbers of *E. coli* O157:H7 on the donor surfaces (2-8 log CFU/cm^2) were overall equally transferred to the recipient TSA surfaces. However, there were significant differences in transferring rate among the kind of food. Higher transferring rate was demonstrated in fresh raw ingredients such as salmon, beef, and chicken meat.

Significance: The cross-contamination risk of *E. coli* O157:H7 to raw food ingredients would be estimated by the contamination levels of donor food-contact surface regardless the exposure to desiccation stress.

P3-79 Online Media Attention Devoted to Flour and Flour-related Food Safety in 2017–2020

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Introduction: The two seemingly unrelated activities, 1) flour consumption and 2) the use of online media, have soared simultaneously over COVID19 pandemic. However, consumers are not aware of the food safety risk associated with this raw food commodity, which may be contaminated foodborne pathogens, such as *Escherichia coli* and *Salmonella*. This study utilizes consumer's social media presence to investigate public perceptions of flour, flour usage, and flour borne risks in the US.

Purpose: Using the user-generated data in social media space, this study investigates how the U.S. public references flour and its uses, quantifies the share of media mentions about flour borne risks and/or risk mitigation, and determines if changes in interest in flour food safety is related to recall announcements.

Methods: Out of the various platforms assisting social media listening, Netbase was employed for data collection. Allowing authors to enter search terms related to flour and its related food diseases to the platform, Netbase provides social media analytics such as the number and changes of mentions, popular terms, or quantified sentiments circumscribed about the search terms from 2017 to 2020 (n = 179 weeks).

Results: Volume of flour-use interest rapidly increased in March 2020 under the stay-at-home order. The share of media devoted to flour-related food safety risks were extremely small albeit all the FDA's flour recalls captured by social media reactions. There is statistically significant negative correlation (P < 0.01) between net sentiment and chats on recalls. There are volume of mentions originating from food safety information or warning messages about flour-associated foodborne illnesses, "FDA's tips of not eating raw cookie dough, batter or other raw dough."

Significance: The findings provided insights of consumer social media response to flour use and food safety events associated with flour. More flour safety education programs may be desired to support consumers informed decision-making.

P3-80 Rapid Detection of *Listeria monocytogenes* Using Real-Time PCR Combined with Filtration and DNA Concentration after Short Enrichment in Enoki Mushrooms

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❖ ***Developing Scientist Entrant***

Introduction: To prevent *Listeria* outbreaks in Enoki mushrooms, development of rapid and sensitive detection method that can be used in the field is required.

Purpose: The aim of this study was to develop a method for rapid detection of *L. monocytogenes* in Enoki mushrooms by a real-time PCR combined with filtration and DNA concentration.

Methods: Enoki mushrooms purchased from local markets in Seoul, South Korea, were artificially inoculated with *L. monocytogenes*. ISO method (11290-1, 2017) was used to analyze Enoki mushrooms as a traditional culture-based method. A rapid detection method with short enrichment time was developed using real-time PCR, filtration and DNA concentration, and its limit of detection was compared with the conventional method.

Results: Short enrichment enabled the increase of pathogens present in Enoki mushrooms. Filtration-DNA concentration-real-time PCR method also allowed the detection of *L. monocytogenes* under 5 CFU/25 g in Enoki mushrooms within 6 h. Furthermore, when compared with the ISO method, the developed method required less time and showed the same limit of detection.

Significance: Real-time PCR method based on filtration and DNA concentration can sensitively detect pathogens as the conventional method, but rapidly, which increases field applicability.

P3-81 Contamination of *Escherichia fergusonii* and *E. coli* Strains Isolated from Fresh Leafy Vegetables Sold in Retail Market in Korea

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Introduction: Fresh vegetables are one of most favorable food and its consumption is increasing recently but also accounted for the most sharing attributed food to food poisonings with pathogenic *E. coli* as the most sharing pathogen in Korea.

Purpose: The purpose of this study is to investigate *E. coli* contamination level of leafy vegetables in retail market places and to seek prevention method by examining the contamination source.

Methods: The leafy vegetables such as lettuce were collected from retail market once in a month and *E. coli* contamination rate and level were investigated by distribution type. Quantitative analysis and qualitative analysis of pathogenic *E. coli* contaminated on leafy vegetables were performed by modifying the BAM. The multiplex PCR assay for 8 virulence genes and phylogenetic analysis by MLST were investigated.

Results: From April to September 2020, 55 lettuces, 9 spinaches, 4 cabbages, and 2 radishes, and 1 garlic chive sample sold in hypermarkets and local grocery stores were collected. *E. coli* was detected in 17 samples of a total of 71 leafy vegetables, showing a contamination rate of 23.9%. Contamination rate and average density of *E. coli* were 100%, 2.3 log CFU/g in garlic chive, 75.0%, 3.9 log CFU/g in Chinese cabbage, 22.2%, 3.1 log CFU/g in spinach, 20.0%, and 3.3 log CFU/g in lettuce, respectively. Meanwhile, a total of 37 strains were isolated from samples, and 6 *E. coli* and 31 *E. fergusonii* were identified. All strains did not have any virulence genes. Twenty out of 37 strains were classified into 15 different MLST sequence types. The phylogenetic tree showed that all strains were genetically separated from reference strains of *Escherichia/Shigella*.

Significance: *Escherichia* spp. contaminated on the leafy vegetables was mainly identified as *E. fergusonii* and these bacteria were known to be mainly derived from animal origin such as manure or compost. The microbiological quality control of the animal-derived soil amendment introduced is important.

P3-82 IoT-Blockchain Enabled Food Safety Decision Support System for the Manufactures and the Regulatory Authorities in the Dairy Sector in Sri Lanka

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Introduction: While there is a global trend to convert functionalities into industry 4.0 infrastructure which incorporates blockchain technology, artificial intelligence, and the internet of things (IoT) for tracking, tracing, and ensuring the safety of food, Sri Lanka still practices a system that is regulated by a manual process and that has features of industry 2.0. The existing system is incapable to address the widespread food fraud reported and a proper traceability system and risk management system based on the recommended risk-based inspection approach is required.

Purpose: The objective of the present work is to introduce an IoT-Blockchain enabled digital food safety decision support system to the dairy industry in Sri Lanka. The suitability of using blockchain technology for tracing and certifying the food origin and claim will be assessed.

Methods: The study will be conducted in three phases: identification of the software specifications and development of a software requirement specification document; development of the physical system focusing on business process reengineering; introduction of the system to the industry and generating a risk profile. The first phase would be accomplished via three stages: data collection, data analysis and data reporting. The output of the first phase will be the proposal for the second stage. The system developed in phase two will be implemented and validated in the third phase.

Results: A dynamic querying system and data models have been introduced via a NoSQL database system, comprising five modules: human resource management module, quality assurance module, tracking and tracing module, facility management module, process and unit operation management module. Data are transferred inter-system as JSON data. The process and unit operation management module acts as the smart-contract module.

Significance: When fully implemented, the proposed system will be a robust surveillance system for the food authorities, an administrative decision supporting system for the food manufacturers and support services, and an information sharing and complaining system for the consumers.

P3-83 Efficacy of Invisishield™ Modified Atmosphere Technology on the Viability of Microsporidia and *Salmonella* Newport

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Introduction: *Enterocytozoon intestinalis* is an intracellular pathogen responsible for gastrointestinal illness in humans and the spores are both environmentally resistant and difficult to inactivate in foods.

Purpose: The objective of this study is to determine the efficacy of InvisiShield™ Modified Atmosphere Technology system on the viability of *Encephalitozoon intestinalis* and *Salmonella* in tomatoes.

Methods: The skins of sliced tomatoes were spot inoculated with *Salmonella* Newport and microsporidia spores. Sliced tomatoes were sealed in a controlled environment containing ClO_2 or a regular plastic seal. The inoculated tomato skins were removed and processed on days 0, 1, 2, 5 and 7. Microsporidia spore viability was evaluated by *in vitro* assay using rabbit kidney (RK-13) cells and *S.* Newport plated onto XLT-4 agar.

Results: Chlorine dioxide treatment effectively reduced spore viability after one day of treatment (3-log reduction). Spore viability was completely inactivated following 24 hours of treatment with chlorine dioxide. A 4-log reduction of *Salmonella* was obtained after 1 hour treatment and non-detectable growth was achieved after day-2 treatment.

Significance: Low concentrations of chlorine dioxide in the enclosed tray can reduce *Salmonella* and microsporidia viability within 1-2 days without affecting the organoleptic properties of the sliced tomatoes.

P3-84 Quantitative and Qualitative Assessments on *Enterobacteriaceae*, Coliforms and Generic *Escherichia coli* on Fresh Vegetables Sold in Cambodian Fresh Produce Distribution Centers

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❖ ***Developing Scientist Entrant***

Introduction: Cambodia faces multiple foods safety challenges, such as lack of standards, incentives to follow hygienic food handling practices, sanitation infrastructure and regulatory oversight. Fresh vegetables are an essential part of Cambodian diets; however, vegetables are also associated with foodborne disease worldwide. Understanding how foodborne pathogens are transmitted along production chains is essential to developing means to control such contamination.

Purpose: The objective of this study was to conduct a quantitative and qualitative assessments on Enterobacteriaceae, coliforms and *E. coli* present on select vegetables (tomato, cucumber and lettuce).

Methods: Tomato (*n* = 145), cucumber (*n* = 132) and lettuce (*n* = 107) samples were collected from a produce distribution center in Battambang, Cambodia, monthly over a period of six months in both the dry and wet season. Samples were transported within 24 hours on ice to the laboratory for microbial analysis. Enterobacteriaceae, coliforms and *E. coli* were detected and quantified following methods of the Food and Drug Administration's Bacteriological Analytical Manual.

Results: Overall, the highest prevalence of Enterobacteriaceae ($P < 0.05$) was observed on lettuce (97.7%), followed by cucumber (85.9%) and tomato (65.6%). The highest prevalence of coliforms ($P < 0.05$) was observed on lettuce (96.7%), followed by cucumber (87.3%), and tomato (62.7%). For tomatoes, the prevalence of both Enterobacteriaceae and coliforms was higher during the wet season as compared to the dry season ($P < 0.05$). In contrast, the levels of Enterobacteriaceae and coliforms were greater in the dry season for cucumbers and lettuce, as compared to tomatoes ($P < 0.05$). Generic *E. coli* was most prevalent on lettuce (29%), followed by cucumber (9%) and tomato (1%) regardless of season.

Significance: These results provide primary data on foodborne pathogen carriage rates in vegetables sold in Cambodian vegetables distribution centers and can be used to identify common sources of contamination and interventions to limit such contamination.

P3-85 Nutritional, Physical–Chemical and Microbiological Characteristics of "Covilhete" – *Pastelaria Típica Portuguesa*

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Introduction: Covilhete is a tasty pastry based on flour, butter, water and salt, filled with a preparation of cooked minced meat. Given its gastronomic and economic value it is important to know the quality and safety of the product, thus contributing to the health and well-being of the consumer.

Purpose: The main objective of this study was to determine physical-chemical, nutritional and microbiological parameters in order to improve the product's characteristics in terms of food safety and quality.

Methods: Samples were collected from six batches from a local producer, prepared in 3 different times: raw, after cooking and after exposure. Microbiological parameters were determined, such as *Listeria monocytogenes*, *Staphylococcus aureus, Salmonella* spp. and, *Bacillus cereus, Escherichia coli, Clostridium perfringens, Enterobacteriaceae,* lactic acid bacteria (LAB), total viable counts (TVC) at 30°C and Molds/Yeasts. In addition to these analyses, after exposure, pH, water activity, fat, moisture, ash, protein and chloride content were determined.

Results: According to the data in the second time, samples have a positive level of acceptability, showing 100% acceptability for microorganisms such as LAB, Molds/Yeasts, *Staphylococcus aureus* and *Listeria monocytogenes* which remained in the last time, except for LAB presenting only 83% acceptability. Enterobacteriaceae had only 67% acceptability and *E. coli* is the one that is of most concern since after cooking it obtained an acceptability of 83%, reducing it to 50% after exposure. In relation to the values obtained for the protein content, it was 40.87%. Meanwhile, the percentage value of fat was 22.38%, a value contained in the parameters recommended by the WHO (15-30% fat).

Significance: Despite the acceptability levels of the final product, *E. coli* is considered a concern. After exposure, there is a reduction in acceptability, which may be due to poor application of good hygiene practices. Acknowledgements: This work was supported by the project UIDB/CVT/00772/2020 funded by the Fundação para a Ciência e Tecnologia (FCT).

P3-86 Isolation and Characterization of an Enteropathogen Growth Stimulating Factor from Bovine Tissues

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Introduction: In comparative studies using a highly selective medium for *Salmonella* and Shiga toxin-producing *E. coli* (STEC) enrichments of ground beef and spinach spiked with low levels of *E. coli* O157:H7 exhibited 50- to 100-fold increase in STEC recoveries in ground beef compared to spinach enrichments.

Purpose: Identify a soluble component supplied by ground beef which may be stimulating the growth of the pathogens.

Methods: Suspensions of ground beef were fractionated using ammonium sulfate precipitation and tested for activity. Activity was identified as stimulating *STEC* and *Salmonella* growth 5- to 10-fold over controls. Active fractions were affinity purified and analyzed by SDS-polyacrylamide gel electrophoresis (PAGE), with composition of protein bands determined by mass spectrometry analysis of tryptic digests. Candidate compounds were obtained from commercial sources to confirm activity and identity.

Results: The ammonium sulfate fractionated ground beef suspensions yielded preparations that markedly stimulated the growth rate and recovery of STEC and *Salmonella* spp. in both selective (PDX-STEC media) and non-selective (modified BPW broth) media by >100-fold and >10- fold, respectively. Affinity purification and SDS-PAGE identified three protein bands of 52-, 35- and 20-kD that each demonstrated growth stimulating activity. The mass spec analysis of the 52- and 35-kD proteins suggested they were 100 % homologous to the glycolytic protein: phosphoglucomutase (E.C. 5.4.2.2) and that the 35- and 20-kD proteins were degradation products of the 52-kD protein. Commercially prepared rabbit muscle phosphoglucomutase (PGM) used as a reference was shown to have demonstrable growth stimulating activity thereby confirming the identity of the proteins.

Significance: Mechanisms of growth stimulation by PGM may be through increasing bacterial fitness and environmental adaptability, thus allowing enhanced recovery and identification of pathogens from challenging samples.

P3-87 Surrogates for *Listeria monocytogenes* for High Pressure Processing Validation Studies

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Introduction: High pressure processing (HPP) is a non-thermal processing technique that uses pressure to treat packaged foods to eliminate spoilage and pathogenic organisms as well as degradative enzymes. HPP is recognized as a useful treatment to eliminate or reduce *Listeria monocytogenes* in to ready-to-eat (RTE) foods. When validating HPP processes for this pathogen, the use of a surrogate is highly recommended for safety. In addition, the surrogate should have growth rates and inactivation characteristics that are similar to *L. monocytogenes*. Identifying appropriate surrogates that meet these criteria will help increase the safety of HPP-treated RTE foods.

Purpose: This study aimed to find suitable surrogates for *L. monocytogenes* that can be used in HPP validation tests.

Methods: Five non-pathogenic species/strains (*L. innocua* 33090, *L. innocua* 51742, *Lactobacillus plantarum*, *Pediococcus acidilactici* and *Enterococcus faecium*) were screened for their pressure resistance. Overnight cultures were placed in polyethylene bags, vacuum packed and exposed to HPP in a 55L Hiperbaric system. Treatments included two pressure levels (450 and 550 MPa), and two holding times (3, 5 minutes). Bacterial enumeration was done using non-selective culture media and incubation conditions appropriate for each microorganism.

Results: A significant difference was found in the log reductions between surrogate candidates ($P < 0.05$). At 450 MPa (3 min), *L. innocua* ATCC 51745 had log reduction of 1.66 ± 0.18 log CFU/mL while *L. innocua* ATCC 33090 had a reduction of 2.69 ± 0.19 log CFU/mL. Inactivation increased with higher pressure and time. Reductions of other species ranged from 0.01 to 1.06 log CFU/mL in all treatments and were more resistant to HPP.

Significance: Food processors will be able to validate HPP processing parameters for efficacy against *L. monocytogenes* without compromising safety.

P3-88 Effects of Hydrodynamic Shear Stress and Equipment Surface on *Escherichia coli* O157:H7 Single- and Multi-Species Biofilm Formation

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Introduction: Bacterial Pathogens can form biofilms on food contact surfaces and subsequently contaminate food during processing. Non-pathogenic bacteria promote stronger biofilm formation of bacterial pathogens in multi-species biofilms.

Purpose: The aim of this study was to evaluate the single and multi-species biofilm formation of *E. coli* O157:H7 on different equipment surfaces at various hydrodynamic shear stresses.

Methods: Biofilms were grown on stainless steel (SS), PTFE and polycarbonate (PC) coupons at shear stresses of 0.013, 0.043 and 0.088 N/m^2 in a CDC bioreactor containing 10% trypticase soy broth that had been inoculated with *E. coli* O157:H7 alone or in combination with *R. insidiosa* for 48 h (N = 108). Bacterial populations in *E. coli* O157:H7 and *R. insidiosa* biofilms from surface coupons were removed by scrapping and determined by spiral plating on MacConkey agar with sorbitol and Tryptic soy agar, respectively.

Results: *E. coli* O157:H7 recovered from multi-species biofilms on PTFE (7.49 log CFU/cm^2) were significantly higher than SS (6.59 log CFU/cm^2). Overall, *E. coli* populations recovered from surfaces at 0.013 N/m^2 shear stress (6.55 log CFU/cm^2) were significantly lower than *E. coli* recovered at 0.043 N/m^2 and 0.088 N/m^2 (7.14 and 7.39 log CFU/cm^2, respectively). *R. insidiosa* populations in multispecies biofilms at all the shear stresses were lower on SS surface compared to PTFE and PC surfaces. *E. coli* O157:H7 recovered in multi-species biofilms at 0.013 N/m^2 shear stress (6.55 log CFU/cm^2) were significantly higher than the *E. coli* O157:H7 in single species biofilms (4.75 log CFU/cm^2). Similar trend of higher *E. coli* O157:H7 populations in multi-species biofilms was also observed at 0.043 N/m^2 and 0.088 N/m^2 shear stress.

Significance: Higher *E. coli* O157:H7 populations in multi-species biofilms may require additional interventions to remove pathogens from equipment surfaces. Lower shear stress and stainless steel surface will help in controlling bacterial biofilm formation.

P3-89 Predicting the Survival of *Listeria Monocytogenes* on Apples and Detection Using Ultraviolet Spectroscopy

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Introduction: Apples have been linked with listeriosis outbreaks, creating the need for better understanding of *Listeria monocytogenes* (*Lm*) survival on apples and improved detection methods.

Purpose: The objective was to assess the potential of non-invasive ultraviolet spectroscopy methods to detect *Lm* on apples. It is hypothesized that *Lm*-contaminated apples may exhibit distinguishable spectroscopic signatures.

Methods: Unwaxed Granny Smith and Red Delicious apples were spot-inoculated on the calyx and the equatorial surface with an avirulent *Lm* strain at 9-10 log CFU/apple. Drying (2-3 h) and storage (room temperature) up to 8 weeks followed. *Lm* was recovered from four apples through vortexing in sterile PBS at specific time points and enumerated on Modified Oxford Agar. Optical signatures (785 nm Raman and deep UV fluorescence) were collected using *Lm* cell suspensions at 6 and 8 log CFU/mL. Raman spectra measurements of ~5 s, using a Wasatch Photonics WP785, leveraged a 785 nm dual wavelength tunable laser for autofluorescence reduction. Deep UV (DUV) fluorescence excitation spectra were quantified using a DUV Raman PL200 microscope with a 248 nm pulsed laser that induces fluorescence emission.

Results: During drying the *Lm* CFU/apple decreased by <0.7 log. Subsequent reductions were noted by 0.9-1.3 log CFU/apple over 4 weeks for both varieties, and by 1.6-1.8 log CFU/apple after 8 weeks for Granny Smith. No noticeable differences were shown between samples from calyxes and equatorial regions. No significant optical signatures were detected in the 785 nm Raman spectra that can likely overcome the apple skin's autofluorescence and underlying Raman signatures. Conversely, differences were observed in the DUV fluorescence spectra due to *e.g.*, tryptophan in bacterial cells.

Significance: The emergence of listeriosis outbreaks via apples highlights the need for novel, non-destructive, early detection methods of *Lm* on apples. Ultraviolet spectroscopy shows potential for such detection by the industry.

P3-90 Exploring Food Handler Perceptions and Attitudes Towards Hand Hygiene Before and During Production

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Introduction: Handwashing, hand hygiene and personal hygiene are often implicated in food outbreak investigations. Consequently, understanding what hinders and supports handwashing in food manufacturing environments may prove invaluable to pinpoint bespoke interventional needs.

Purpose: To explore perceived food handler hand hygiene barriers and enablers in food manufacturing environments (before and during production) to inform intervention development.

Methods: A mixed-method (quantitative and qualitative) survey following the Global Food Safety Initiative's (GFSI) food safety culture dimensional framework, informed by existing company protocol/documentation, observational data and extensive interviews, was distributed among food handlers (n = 62) at food manufacturing sites (n = 3). A mixture of Likert scale statements and open-ended questions explored perceptions, attitudes and beliefs towards hand hygiene practices from different perspectives.

Results: The majority of food handlers indicated positive attitudes towards hand hygiene, confidence in handwashing ability and satisfaction with the hand hygiene resources available. However, in contrast to prior research, the presence of others (63% disagreement), hand sink locations/accessibility (79% disagreement), access to resources such as paper towel and soap (75% disagreement), being late for work (80% disagreement) or working under pressure during production (79% disagreement) did not influence handwashing behavior. Indeed, 37% replied that *"nothing"* prevented handwashing during production as it was *"not hard."* Nonetheless, despite having confidence in handwashing ability and glove-use at key production moments, only 50% believed their handwashing practices set a positive example for colleagues. Few (13%) agreed/somewhat agreed that additional hand hygiene training would be beneficial while 49% highlighted water temperature and sore hands from frequent handwashing (26% agreement) as potential barriers.

Significance: While confidence relating to hand hygiene execution (i.e., the intention) was high, self-belief (e.g., being a role-model) was mixed. As the GFSI posit that food safety culture is 'shared,' empowering food handlers using bespoke motivational training techniques to increase self-efficacy may lead to more potent, collective, hand hygiene improvements.

P3-91 Great Challenges Ahead for Global Food Safety Community: Practices in a Developing Country after Legislation

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Introduction: Food safety system in Pakistan is facing new challenges due to rapid population growth, globalization of food trade, poor sanitation, unhygienic practices and lack of consumer awareness. Increasing use of pesticides and insecticides in agriculture and toxic chemicals in food processing industries has raised many concerns to public health.

Purpose: The purpose of study was to assess and evaluate the current food safety practices adopted by various food establishments and food safety knowledge and awareness among food handlers.

Methods: In present study cross sectional Survey of 500 food establishments (fast food chains, dairy and milk shops, restaurants and hotels, sweets and bakers, bottled water and beverages, marriage halls and caterings, cafes and canteens and manufacturing industries) of 9 towns of Lahore capital which are under jurisdiction of Punjab Food Authority was carried out in collaboration of Department of Food Science and Human Nutrition, University of Veterinary and Animal Sciences Lahore Pakistan with Punjab Food Authority.

Results: Results of this study showed that food establishments have not properly adopted food safety practices. On part of management and personnel, situation at food establishments is highly pathetic: 36% food premises needs immediate improvement,75% needs major improvement, only 2% food establishments have showed good sign. Situation of personnel hygiene is also very pathetic:17% food establishments needs immediate improvement and 26% require major improvement and no establishment met the very good criteria. Almost 80% food establishments require immediate improvement because their maintenance and infrastructure. Sixty percent food establishments require urgent improvement in response to control of operation.

Significance: This study is quite helpful for food establishments to improve and adopt food safety practices. It has highlighted that appointment of competent and certified person in charge and food safety trainings are means of improving food safety culture.

P3-92 Thermal Inactivation Validation for *Salmonella enterica* in Chicken Feathers during Simulated Commercial Rendering

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Introduction: Thermal rendering of animal carcass inedible components (offal) produces useful ingredients and finished products for animal and human feed and food. Commercial rendering establishments must scientifically validate food safety hazards are effectively controlled according to the FDA Food Safety Modernization Act requirements.

Purpose: The purpose of this research was to model and validate *Salmonella* inactivation on chicken feathers during heating processes identical to those used in commercial U.S. rendering.

Methods: Chicken feathers free of excess scrap tissue or blood were inoculated with a blend of *Salmonella* serovars Typhimurium, Heidelberg, and Senftenberg to 8.0 ± 0.2 log CFU/g feathers. Galvanized steel vessels pre-heated to 87.8 and 93.3°C were loaded with 50-g aliquots of inoculated feathers. Immediately after loading, vessels were replaced into a heating bath. Sample-loaded vessels were capped and held for up to 18 min; surviving *Salmonella* were periodically enumerated on bismuth sulfite agar supplemented with 0.1% (w/v) sodium pyruvate to recover sub-lethally injured cells along with uninjured *Salmonella* cells (LOD: 1.0 log CFU/g). The experiment was replicated thrice and log-transformed microbiological data subjected to non-linear model fitting using GInaFiT v1.7 freeware. Inactivation curve parameters were compared between processing temperatures by use of ANOVA and for calculation of *D*-values at heating temperatures.

Results: *Salmonella* counts were affected by the interaction of heating temperature and time ($P \leq 0.0001$), but best fit a log-linear plus tail inactivation model type. *D*-values at 87.8 and 93.3°C were 1.31 ± 0.4 and 1.04 ± 0.78 min, respectively, and not different from one another ($P = 0.366$). The process *z*-value was 34.7°C. Regardless of heating temperature, *Salmonella* on feathers were reduced to non-detectable levels within 15 min of continuous heating.

Significance: *Salmonella* inactivation of ~7.0 log CFU/g was obtained on chicken feathers during simulated commercial rendering, aiding validation of effective hazard control to protect food safety of human and animal food or feed.

P3-93 Food Safety Knowledge, Training and Practice Among Ohio Food Pantry Volunteers

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Introduction: Food distribution organizations (FDOs), commonly known as food banks and food pantries, are subject to the many of the same food safety laws and regulations as food retailers or restaurants despite having significant differences in their operational structures, namely the utilization of volunteers in ensuring the safety of the food received and distributed.

Purpose: The purpose of this study was to assess food safety knowledge and practices among FDOs, particularly among volunteers, in a central Ohio county that encompasses urban, suburban and rural locations.

Methods: FDOs were purposively sampled from a compiled list of FDOs in Franklin County, Ohio based on location, service area population size and operational status. Managers of selected FDOs were invited in August and September 2020 by phone and email to participate in an in-person interview conducted during a site visit. Data were collected using a questionnaire adapted from a previous study conducted in North Carolina and via a walkthrough of the FDO operation. Data analysis was qualitative and focused on developing themes based on the framework of the questionnaire.

Results: Ten pantries serving between 120 and 600 households per week participated in the study. Pantry managers reported that volunteers perform some food-safety-related work, including evaluating shipments for safety and temperature control. However, only three pantries reported that providing training to their volunteers, and only two pantry managers were ServSafe certified. Importantly, food safety issues related to refrigeration of fresh cut produce and eggs were observed at two pantries.

Significance: Findings from this study provide insights into food safety knowledge among FDO volunteers and can be used to inform future outreach efforts aimed at preventing foodborne illness in the vulnerable populations that FDOs serve.

P3-94 Prompting Food Safety Culture Weekly Improvement in UK Food Manufacturing Companies Using Triangulation and Real-Time Technology

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Introduction: Food-related work behavior, other human factors, and food safety culture are known contributory risk factors in foodborne illness outbreaks. Food businesses need solutions to measure and strengthen their food safety culture in real time to enhance food safety performance.

Purpose: This project uses validated, real-time technology-enabled food safety culture measurement (Cultivate Pulse) to assess the impact of food safety culture improvement interventions in a weekly cyclic continuous improvement process.

Methods: This 12-month project involves ten UK food manufacturing businesses and a weekly improvement cycle, using Cultivate Pulse feedback to measure the organizational food safety culture via the concerted effort by all staff members moving through their routine operations. Food safety culture maturity levels are determined and reported back to the company weekly using the Cultivate Food Safety Maturity Model, and chief strengths and weaknesses elucidated with suggested target interventions for the following week(s). Triangulation encompasses real-time Cultivate Pulse technology, focus groups/interviews, and food safety document review. Interviews will be targeting management and leadership, focus group targeting frontline staff, to enhance engagement and explore intervention options. Food safety performance document review, for example internal and external audit records, company's reports, food safety management system procedures, etc., will contribute to triangulation and the understanding of company's food safety performance.

Results: Data are currently incomplete but summary of up-to-date findings will be provided.

Significance: To our knowledge, this is the first study using technology for companies to gain real-time insights into their food safety culture. Besides, triangulation uniquely employs validated, real-time technology to improve food safety culture in food manufacturing companies. Efficacy of weekly culture change compared to strategic culture change will be evaluated in their impact on company's adaptability.

P3-95 Knowledge and Current Practices Related to Agricultural Water Microbial Quality Among Kansas and Missouri Produce Growers

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❖ ***Developing Scientist Entrant***

Introduction: Kansas State University and University of Missouri Extension educators have been providing training and information on agricultural water microbial quality to help growers reduce risk. However, we recognized the need to determine the gap between produce safety best practices/Produce Safety Rule (PSR) requirements and Kansas and Missouri produce growers' knowledge and actions related to water quality.

Purpose: This survey aimed to better understand the current knowledge and practices of Kansas and Missouri growers related to agricultural water microbial quality. Information gathered helps determine future extension outputs and activities needed to adjust the practices of produce growers related to water quality.

Methods: Researchers developed the survey based on similar surveys and utilizing input from various experts in produce water quality and those working directly with produce growers in Kansas and Missouri. The IRB-approved survey contains 14 main questions about pre- and post-harvest water usage, water treatment, and microbial water testing. The survey was distributed to Kansas and Missouri produce growers attending produce safety-related events in late 2020 and early 2021 and was also disseminated to email lists of produce growers from both states.

Results: At the end of April 4th, a total of 72 growers completed the survey. Preliminary survey results indicate that 15.49% of the respondents tested their water more than once a year, while 35.21% of the participants have never tested their water. Approximately half (51.28%) of respondents indicated they use municipal water for post-harvest uses, while 6.41% indicated they use surface water post-harvest. Most (79.71%) of respondents indicated that they do not treat their post-harvest water.

Significance: Data suggest that further training and resources related to microbial water quality would help improve grower practices related to water quality and produce safety.

P3-96 Validation of a PCR Workflow for the Detection of *Campylobacter jejuni*, *C. coli* and *C. lari* in Raw and Ready-to-Cook Poultry Products

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Introduction: Gastroenteritis caused by *Campylobacter* is most commonly associated with the thermotolerant species *C. jejuni*, *C. coli* and *C. lari* and consumption of contaminated undercooked poultry products.

Purpose: The purpose of this study was to evaluate the Thermo Scientific™ SureTect™ Campylobacter jejuni, C. coli and C. lari PCR workflow (candidate method) for detection of *Campylobacter* from poultry matrices according to the AOAC® *Performance Tested Methods*℠ program.

Methods: An unpaired method comparison study evaluating performance with 375 g raw ground turkey and raw chicken thighs, 25 g ready-to-cook nuggets, 30 mL chicken carcass rinse and 4 x 4" turkey carcass sponges was conducted. The candidate method was compared to the following reference methods: USDA FSIS MLG 41.04 for raw meat and carcass sponges, and ISO 10272-1 for ready-to-cook products. Fifty-two target and 51 non-target isolates were tested in an inclusivity/exclusivity study. Method robustness and lot-to-lot stability studies were also completed.

Results: There were no statistically significant differences found in the matrix studies between the candidate and reference methods following probability of detection (POD) analysis. The candidate method detected presence of *Campylobacter* 2-3 days faster than the reference method. All 52 inclusivity and none of the 51 exclusivity isolates were detected by the PCR assay. Robustness testing demonstrated reliable performance with method deviations outside of the recommended parameters, and the real-time stability testing demonstrated that there were no statistically significant differences between kit lots.

Significance: The data shows that the candidate method workflow is a rapid and reliable alternative method for the detection of *Campylobacter* from raw and ready-to-cook poultry products.

P3-97 Suretect Salmonella Species PCR Assay *Official Methods of Analysis℠*

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Introduction: *Salmonella* is a rod-shaped Gram-negative bacterium that is one of the most frequent causes of food-related illnesses in the US. The CDC estimates that *Salmonella* bacteria cause around 1.35 million infections, 26,500 hospitalizations, and 420 deaths every year in the US. The detection of *Salmonella* before it can cause infection is crucial. The Thermo Scientific™ SureTect™ Salmonella species PCR Assay is a Real-Time PCR test for the detection of *Salmonella* from a broad range of foods and environmental surfaces.

Purpose: The aim of this study was to validate the SureTect method using the AOAC *Official Methods of Analysis℠ (OMA)* program in comparison to the reference method detailed in FDA/BAM Chapter 5.

Methods: The unpaired collaborative study followed the guidelines outlined in AOAC *Appendix J*. The food matrix selected for the study was cocoa powder. Cocoa products are a challenging matrix for PCR assays due to the presence of polyphenols, which can inhibit the PCR. Samples of 375 g were diluted 1-in-10 in non-selective pre-warmed BPW for the SureTect method. The study was conducted using a total of 13 analysts from 8 different laboratories. Twelve analysts were based in the US and one analyst was based in France.

Results: The study faced a number of challenges, chief of all being the COVID-19 pandemic, which meant a lot of the training was carried out remotely. Other challenges included the large sample size of cocoa powder for the SureTect samples. Despite these challenges, 12 valid data sets were obtained, with fractional positivity for the low inoculation level. The SureTect method statistically demonstrated accurate, reliable and reproducible results when compared to the FDA/BAM Chapter 5 reference method.

Significance: The *First Action OMA* status ensures the SureTect method produces accurate, reliable, and reproducible results for the detection of *Salmonella* in a broad range of foods and environmental surfaces.

P3-98 Compatibility Evaluation of a Residual DNA Removal Protocol with a Real-Time PCR Assay for the Detection of *Salmonella, Listeria, L. monocytogenes,* and *Cronobacter* in Various Food Matrices

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Introduction: The higher sensitivity of real-time pathogen PCR, while useful for food pathogen detection, has also led to detection of residual pathogen DNA that can be endemic to the production environment. This residual DNA can result in false presumptive signals that do not reflect the true food pathogen risk.

Poster

Purpose: The objective of this study was to evaluate the compatibility of a residual DNA removal BACTIVIAB™ PMAxx™ protocol (BV) with the GENE-UP®-based PCR assays: *Cronobacter* (CRO), *L. monocytogenes* (LMO2), *Listeria* spp. (LIS2), and *Salmonella* (SLM2) when specifically employed for detection in infant formula (CRO, 375 g), Mexican cheese (LMO2, 125 g), romaine lettuce (SLM2, 375 g), deli ham (LIS2, 125 g) and stainless steel (LIS2).

Methods: For every matrix trial, unpaired (n = 30) replicates were analyzed by the respective assay, and the appropriate reference method, BAM or MLG, (total N >300). For each trial, samples were tested at a fractional (n = 20), high (n = 5), and uninoculated (n = 5) levels (per the AOAC standards). Samples were processed as per the manufacturer recommended enrichment conditions. All presumptive results were confirmed by the pertinent reference culture methods (BAM or MLG). The performance data for each test/matrix trial was collected before and after the BV treatment and compared. Results for the respective trials were then compared to the reference methods by POD analysis both before and after the BV treatment.

Results: No statistically significant differences (95% CI) were observed between the reference and the candidate tests, and as expected BV treatment had no effect on the assay performance as indicated by POD values. Briefly, CRO [$dPOD_c$: 0.0-0.05; LCL: -0.43; UCL: 0.43], LIS2 [$dPOD_c$: 0.0-0.1; LCL: -0.43; UCL: 0.43], LMO2 [$dPOD_c$: 0.0-0.1; LCL: -0.43; UCL: 0.43], SLM2 [$dPOD_c$: 0.0; LCL: -0.43; UCL: 0.43].

Significance: These data indicate the compatibility of a residual DNA removal protocol with the tested food matrices on GENE-UP CRO, SLM2, LIS2, LMO2 assays.

P3-99 Performance Evaluation of Fluorescence Resonance Energy Transfer-based Real Time PCR for Detection of *Campylobacter* spp. in Poultry Rinsates

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Introduction: The incidence of Campylobacteriosis has been steadily rising in the US since 2011 (FoodNet), and despite the ongoing mitigation steps by the poultry industry, testing for raw poultry remains an effective strategy to surveil the incidence of *Campylobacter* sp. GENE-UP® *Campylobacter* sp. assay (CAM) is a real-time PCR assay that utilizes FRET hybridization chemistry to provide simultaneous multi-target detection.

Purpose: The purpose of this study was to evaluate the performance of CAM for the detection of *C. coli, C*. jejuni, and *C. lari* (target) in poultry rinsates.

Methods: For inclusivity, N = 20 strains from *C. coli, C. jejuni, C. lari* were grown in a selective broth for 24 h at 42°C under microaerophilic conditions and tested at 100 times the LOD. For exclusivity, N = 10 *Campylobacter* strains and N = 26 pertinent non-Campylobacter strains were grown for 24 h in non-selective broth. For analytical sensitivity, N = 30 *C. coli, C. jejuni*, *C. lari* strains were grown in non-selective broth for 24 h at 42°C; serial dilutions were then evaluated with CAM. Matrix verification samples (N = 52) were created by adding 30 mL of chicken rinse obtained from a poultry processing facility with 2X Bolton with supplement (1:1). Enrichments were spiked with either *C. coli, C. jejuni*, and *C. lari* at ≥10 CFU per test portion with each strain tested in 3-5 unique rinses and incubated at 42°C for 24 h. All presumptive results were confirmed by plating on CampyFood Agar, CEFEX and mCCDA per the manufacturer instructions.

Results: Inclusivity was 100%, and except few variants of *C. upsaliensis* and *C. fetus*, no exclusivity strains were detected. The analytical sensitivity of target strains was ~10^3 CFU/mL. The matrix verification trial indicated the CAM sensitivity, specificity and overall accuracy to be 100%.

Significance: These data indicate the efficacy of the GENE-UP CAM for the detection of *C. coli, C. jejuni*, and *C. lari* in poultry rinses.

P3-100 Performance Evaluation of a Residual DNA Removal Protocol with a Real-Time PCR Assay for Food Pathogen Detection in Diverse Food Matrices

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Introduction: Detection of residual pathogen DNA while using highly sensitive PCRs have led to false presumptive signals during the food pathogen testing. This issue has manifested itself as a distraction from identifying the true risk, and in turn have caused food processing delays and economic losses.

Purpose: The objective of this study was to evaluate the performance of a DNA removal BACTVIAB™ PMAxx™ protocol (BV) with the GENE-UP®-based PCR assays in a variety of food matrices.

Methods: The performance was evaluated using: 1) strains per real-time PCR assay for *Listeria* sp. (N = 9, LIS2), *L. monocytogenes* (N = 5, LMO2), and *Salmonella* spp. (N = 5, SLM2); 2) using food matrices: deli ham (N = 4; 125 g) and environmental swabs (N = 3) for LIS2, Mexican cheese (N = 4; 125 g) for LMO2, leafy produce (N = 5; 375 g), dairy powders (N = 5; 375 g) and dog treats (N = 6; 375 g) for SLM2. For strain trials, each organism was propagated in BHI to a known cell count, heat treated (95°C @15 min), serially diluted. For the matrix trials, uninoculated matrices were prepared using the assay instructions and mixed with the heat treated (95°C @ 15 min) suspension of known cell counts with one strain per matrix representing a simulated enrichment with serially varied amounts of dead cells/DNA. Both the serial dilutions of heated strains and the simulated matrices were tested on respective assays with and without the BV treatment.

Results: In all instances, for strain trial with N = 152 serial dilutions (64 on LIS2, 40 on SLM2, and 48 on LMO2) and with matrix trials representing N = 196 serial dilutions (84 on SLM2, 56 ea. on LIS2, LMO2), the PCR signal from heat treated suspensions were removed at ≤10^6 CFU/mL after the BV treatment.

Significance: These data indicate that the GENE-UP®- BV protocol can eliminate false signal from the residual pathogen DNA (up to 10^6 CFU/mL) during the food pathogen testing.

P3-101 Rapid Detection of *E. coli* O157:H7 in Poultry Matrices Using Shiga Toxin Gene Screen Loop-Mediated Isothermal Amplification (LAMP)-Bioluminescent Assay

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Introduction: Molecular methods based on the somatic antigen gene *rfb* O157 and/or the flagella gene *fliC* H7 are commonly used for detection of *E. coli* O157:H7. However, these methods may not accurately reflect the virulence of isolates. The virulence gene screen for *stx*1 and *stx*2 and *eae* may provide a better screening tool for detecting toxigenic *E. coli* O157:H7 and can be further confirmed with specific assays for O157 and/or H7 detection.

Purpose: To compare the Shiga toxin gene screen LAMP-Bioluminescent assay for detection of *E. coli* O157:H7 in poultry matrices to a H7 PCR method.

Methods: Detection of *E. coli* O157:H7 in 25 g each of mechanically separated chicken (n = 30), ground chicken (n = 30), and poultry parts (n = 30) inoculated with a toxigenic isolate of *E. coli* O157:H7 (ATCC 35150) was compared in a paired study using a LAMP and H7 PCR assay. All samples were culture confirmed.

Results: Detection of *E. coli* O157:H7 using the LAMP method and the H7 PCR method were in agreement. The POD analysis between the two methods did not show any significant difference at a 95% confidence interval for all matrices. Paired results showed a dPOD of zero with a 95% confidence interval of (-0.43, 0.43) for the non-inoculated and 5 CFU inoculation level samples and a dPOD of -0.1 for the 2 CFU level in mechanically separated chicken and ground chicken samples with a 95% confidence interval of (-0.31, 0.25). The two molecular methods were in agreement with the culture confirmation method.

Significance: The virulence gene screen LAMP-bioluminescent method provides a rapid and specific approach for the detection of toxigenic *E. coli* O157:H7. The method also provides a screening method for non-O157 STEC in poultry matrices. The presumptive positive results from the gene screen can be followed with specific assays for O157 or non-O157 STEC.

P3-102 Comparison of Viability qPCR and Culture-based Quantification in Challenge Studies

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❖ ***Developing Scientist Entrant***

Introduction: Validating food safety interventions can be expensive and time intensive using culture-based plate and count (PAC) methods. Quantitative Polymerase Chain Reaction (qPCR) may decrease turnaround time and costs associated with quantifying pathogens in challenge studies.

Purpose: Our goals were to assess DNA intercalating agents for suppression of dead cell DNA amplification and assess use of viability qPCR in challenge studies.

Methods: Experiments were conducted using *Listeria monocytogenes* (LM) and *Salmonella* Typhimurium (ST). DNA intercalating agents (Reagent D, Ethidium homodimer-2, SYTOX Blue, PMAxx, Live or Die NucFix Red, and PMAxx plus PMA Enhancer for Gram Negative Bacteria) were evaluated for efficacy in suppressing dead cell DNA amplification in qPCR using raw DNA, a 900:1 mix of dead to live cells in media, a 900:1 mix of dead to live cells in ground beef, and a 100,000:1 mix of dead to live cells in ground beef. Three biological replicates were tested in three independent trials (n = 9). The best intercalating agent was applied with qPCR in two challenge studies: 60°C heat treatment of inoculated ground beef and a 6% lauric arginate (LAE) dip of inoculated meat. Quantification results from qPCR and PAC were compared using ANOVA and Tukey pairwise tests ($P \leq 0.05$, C.I. = 95%).

Results: PMAxx most reduced dead cell signal without reducing live cell signal ($P \leq 0.05$, n = 9). In heat treatment experiments, qPCR and PAC yielded similar pretreatment cell estimates for both organisms, but qPCR overestimated final counts by 4.94 log CFU/g for ST and 3.31 for LM ($P \leq 0.05$, n = 9). In LAE experiments, pretreatment estimates for qPCR and PAC were similar for ST, but qPCR underestimated LM by 0.82 log CFU/cm^2 In LAE post-treatment experiments, qPCR underestimated final ST by 2.59 log CFU/cm^2; LM trended towards underestimation ($P \leq 0.05$, n = 12).

Significance: These results highlight the potential use of viability qPCR in challenge studies. More research is needed to address method limitations.

P3-103 AOAC Validation of RapidChek® Select™ *Salmonella* Test Method for the Detection of *Salmonella* Species in Raw Ground Pork

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Introduction: Raw meat and poultry are important vehicles of transmission of *Salmonella*. Control measures, like environmental monitoring, implemented along food production lines in meat and poultry abattoirs help reduce the levels of *Salmonella* in these matrices; however, microbiological testing of food product retains a key role in preventing foodborne salmonellosis.

Purpose: To validate the test method by demonstrating equivalent performance to the USDA MLG reference method for the detection of *Salmonella* spp. in raw ground pork.

Methods: Twenty-five gram (25 g) and three hundred and seventy-five gram (375 g) samples (test method) and twenty-five gram (25 g) (USDA MLG method) were inoculated with *Salmonella* Derby. For each method, 20 low-level inoculated, 5 high-level inoculated, and 5 negative control samples were tested. After inoculation, samples were held at 2-8°C for 72 hours. Test method samples were enriched in 225 mL primary media (25 g) or 1L (375 g), transferred to secondary media, evaluated with test strips and plated on selective agar. USDA MLG reference method samples were enriched in 75 mL mTSB, transferred to TT Hajna and mRV media, and plated on selective agar following the USDA MLG method Chapter 4.10.

Results: The test method resulted in 13 confirmed positives for the 25 g samples and 10 confirmed positive for the 375 g samples. The reference method (25 g) resulted in 4 confirmed positives. Probability of Detection (POD) analysis demonstrated a statistically significant difference at the 5% level in the number of positive samples detected by the test method (25 g and 375 g) compared to the USDA MLG method (25 g) for raw ground pork spiked with *Salmonella* Derby.

Significance: The RapidChek® SELECT *Salmonella* method offers a rapid and reliable tool for testing 25 g and 375 g samples of raw ground pork for *Salmonella* species.

P3-104 AOAC Validation of RapidChek® Select™ *Salmonella* Test Method for the Detection of *Salmonella* Species in Raw Pork Trim

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Introduction: Raw meat and poultry are important vehicles of transmission of *Salmonella*. Control measures, like environmental monitoring, implemented along food production lines in meat and poultry abattoirs help reduce the levels of *Salmonella* in these matrices; however, microbiological testing of food product retains a key role in preventing foodborne salmonellosis.

Purpose: To validate the test method by demonstrating equivalent performance to the USDA MLG reference method for the detection of *Salmonella* spp. in raw pork trim.

Methods: Twenty-five gram (25 g) and three hundred and seventy-five gram (375 g) samples (test method) and twenty-five gram (25 g) (USDA MLG method) were inoculated with *Salmonella* Typhimurium. For each method, 20 low-level inoculated, 5 high-level inoculated, and 5 negative control samples were tested. After inoculation, samples were held at 2-8°C for 72 hours. Test method samples were enriched in 225 mL of test method primary media plus supplement (25 g) or 1 L (375 g), 100 µl was transferred to 1 mL test method secondary media, enriched, evaluated with test strips and plated on selective agar. USDA MLG reference method samples were enriched in 75 mL mTSB, transferred to TT Hajna and mRV media, enriched and plated on selective agar following the USDA MLG method Chapter 4.10.

Results: Inoculated at 2.0 CFU/sample, the test method resulted in 14 confirmed positives for the 25 g samples versus 17 with the reference method (25 g). Inoculated at 0.9 CFU/sample, there were 12 confirmed positives for the 375 g test method samples versus 9 confirmed positives with the reference method (25 g). Probability of Detection (POD) analysis shows no statistically significant difference at the 5% level in the number of positive samples detected by the test method (25 g and 375 g) and the USDA MLG method (25 g) for raw pork trim spiked with *Salmonella* Typhimurium at low levels.

Significance: The RapidChek® SELECT *Salmonella* method offers a rapid and reliable tool for testing 25 g and 375 g samples of raw pork trim for *Salmonella* species.

P3-105 Colorimetric Sensing Arrays for Identification of *Salmonella* Viability in Foods

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Introduction: Foodborne pathogens of recent concern are those which typically do not produce exotoxins therefore, they are only virulent when viable. Current detection platforms are fairly rapid but are incapable of determining whether the detected pathogen is dead or alive.

Purpose: This project utilized colorimetric sensing arrays (CSAs) to monitor changes in volatile compounds in the headspace of enriched food products to correlate with bacterial viability.

Methods: A testing apparatus was set up in jar lids to enclose each CSA individually in plastic holders with a hole in the bottom to allow air exchange with samples beneath. Six samples were prepared for each trial and a scanner was used to collect images of the CSA every 30 min over 24 h. Trials utilized controls of buffered peptone water (BPW) media and inoculated *Salmonella enterica* serovar Typhimurium in BPW for each experiment and *Salmonella* inoculated BPW with foods (45 mL to 5 g); apple juice, ground chicken, ham, shredded cheddar and spinach. The CSA tickets were monitored over time using custom image segmentation routine to extract the average RGB color from each spot. We investigated Dynabeads® coated with anti-*Salmonella* antibodies

to reduce background signal in chicken and spinach samples.

Results: The CSAs exhibited color changes in response to BPW; several spots showed an additional color response in inoculated BPW and or food identifiable between 3.5-15 h depending on the starting *Salmonella* concentration (~$1x10^1$-$1x10^7$ CFU/mL) compared to controls with quicker response associated with the higher range of tested concentration. Pattern recognition techniques (support vector machine) were used as a detection algorithm to quantify the potential for predicting the presence of *Salmonella* from the color change responses. Data acquired with Dynabeads® showed specificity for *Salmonella* in foods with high background.

Significance: CSAs can be utilized to understand pathogen viability over time in food matrices.

P3-106 How to Use Next Generation Sequencing for Food Authenticity: A Ring Trial-based Evaluation

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Introduction: Next Generation Sequencing has been introduced for food authenticity by several laboratories. However, this high throughput DNA analysis method is new to the food sector and there is a need for validation and recognition of the method to be widely used for routine food authenticity analysis.

Purpose: A ring trial with ten laboratories was organized to evaluate the complete Thermo Fisher food authenticity workflow in a broad range of food samples. Three main target species groups were selected: meat, fish, and plants. A validation report is produced based on the results obtained by all the participants.

Methods: The food samples include both real samples and control samples prepared with mixtures of DNAs from several meat, fish, and plant species. The real food samples were selected based on the type of processing treatment used for their production. This included canned, dry, raw, liquid, and frozen food samples. A total of 72 samples were analyzed by each participant. The complete workflow tested include DNA extraction, library preparation with the SGS Allspecies ID kits, NGS run using the ION CHEF and S5 GeneStudio instruments and sequence data analysis with the SGS AllspeciesID software providing species identification.

Results: A total of 30 meat, fish and plant species ranging from 1% to 10% were correctly identified both in pure DNA or mixtures. The results obtained by each participant were compared showing a high reproducibility. For each of the real food products it was possible to identify the correct species even for canned foods. Once again the results obtianed for each participant were very much similar.

Significance: This is the first ring trial organized to evaluate a NGS workflow for meat, fish and plant species identification in food samples. This is a very important step towards the validation and global recognition of NGS as a very powerful and reliable tool for species identification that is one of the major food authenticity concerns.

P3-107 Microbial Type, Environmental Conditions, and Exposure Time Impact the Recovery of Microorganisms during Environmental Monitoring

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Introduction: Environmental monitoring (EM) programs are designed to prevent the transfer of pathogens between surfaces and food. However, limited knowledge exists regarding the impact of environmental conditions on microbial recovery during EM.

Purpose: To determine the impact of microorganism type, environmental conditions, and exposure time on EM performance.

Methods: One milliliter of 10^9 CFU/mL bacterial cocktail (*Listeria monocytogenes*, *Salmonella* Typhimurium) was inoculated onto three non-porous surfaces (polypropylene, stainless steel, neoprene) and allowed to dry. Surfaces were held in an environmental chamber for 24 or 72 h at 30°C/30%, 6°C/85%, and 30°C/85% relative humidity (RH). After the exposure period, the surfaces were swabbed with a polyurethane foam sponge pre-moistened with 10 mL of 1×PBS. Samples were plated onto selective agar. Experiments (n = 144) were replicated and analyzed in duplicate.

Results: The recovery of microorganisms varied significantly (P < 0.0001) from each other under similar conditions. Notably, the mean loss of *L. monocytogenes* differed significantly (P < 0.0001) at 6°C/85% RH (1.73 log CFU/mL) compared to 30°C/30% RH (2.99 log CFU/mL) and 30°C/85% (2.89 log CFU/mL). Similarly, the mean loss of *S.* Typhimurium at 6°C/85% RH (2.77 log CFU/mL) was significantly different (P < 0.0001) from 30°C/30% RH (4.73 log CFU/mL) and 30°C/85% RH (4.75 log CFU/mL). Over time, the *L. monocytogenes* mean loss at 24 h and 72 h was significantly different (P < 0.0073) with 2.25 log CFU/mL compared to 2.82 log CFU/mL, respectively. Similar results (P < 0.0001) for *S.* Typhimurium over time were observed with a mean loss of 3.14 log CFU/mL and 5.02 log CFU/mL at 24 h and 72 h, respectively.

Significance: Our results suggest that microbial recovery from environmental surfaces significantly varies by microorganism type, environmental conditions, and exposure time. These differences highlight the need to understand how EM test results are impacted by environmental conditions found in the food manufacturing industry.

P3-108 Detection of *Listeria monocytogenes* in 125-g Chocolate Liquor Samples Using the bioMérieux VIDAS® LIS, VIDAS® UP *Listeria*, GENE-UP® *Listeria* spp., GENE-UP® *Listeria monocytogenes* Assays and FDA BAM Methods

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Introduction: Chocolate liquor is a difficult matrix that can present detection issues for rapid screening methods. Very few matrix validation studies have evaluated the detection of *L. monocytogenes* in chocolate matrices or in larger 125-g composite samples.

Purpose: To determine if the detection of *L. monocytogenes* in 125-g samples of chocolate liquor by four rapid screening methods is comparable to the FDA BAM reference method in 25-g samples.

Methods: A spike containing 10% chocolate liquor and 90% NFDM powder was inoculated with *L. monocytogenes* at 9 CFU/g and stabilized at room temperature for two weeks. Chocolate liquor samples (25 g and 125 g) were inoculated with the stabilized spike at high = 2-5 CFU (N = 5) and fractional = 0.2-2 CFU (N = 20) levels. Inoculum levels were verified by MPN. Uninoculated controls (N = 5) were included. All samples (N = 30) were enriched in the medium specified for the rapid alternate or reference method and incubated following the minimum method parameters. Samples were screened by the four alternate assays or by the FDA BAM reference method. All sample enrichments were streaked to isolation media and confirmed following FDA BAM confirmation procedures.

Results: The proportion of fractional positives was 7/20 (POD 0.35) for VIDAS® LIS, 9/20 (POD 0.45) for VIDAS® UP *Listeria*, 6/20 (POD 0.30) for GENE-UP® *Listeria* spp., 6/20 (POD 0.30) for GENE-UP® *Listeria monocytogenes*. The difference in probability between methods was determined. No significant difference was observed between the alternate and reference methods. All confidence intervals (95%) contained 0. All five high level samples tested positive (POD = 1.00) and all five negative samples tested negative (POD = 0.00).

Significance: The four rapid method assays assessed in this study all provide a comparable recovery to the FDA BAM reference method when used to screen chocolate liquor for the presence of *Listeria* spp. in 125-g samples.

P3-109 A Method Comparison Study to Evaluate Recovery of *Bifidobacterium longum* from Pet Food Products

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Introduction: Initial studies demonstrated at least a one-log difference, between two different methods to recover *Bifidobacterium longum* from pet food products.

Purpose: The objective of this study was to compare two methods (an in-house method, and EN 15785 – Isolation and enumeration of *Bifidobacterium spp.*) to successfully recover a proprietary strain of *B. longum* from pet food products.

Methods: Pure probiotic powder (10^{10}-10^{11} CFU/g, n = 5), dried animal digest (DAD) spiked with probiotic powder (3 different levels – 10^{10}, 10^{8}, and 10^{6} CFU/g, n = 10 each), and sterile kibble spiked with DAD+probiotic powder (10^{6} CFU/g, n = 10) were produced by the Nestle Purina R&D Center (Aubigny, France). Each of these matrices were processed and enumerated as described in both methods. For precision, DAD+probiotic (10^{10} CFU/g, n = 20) were analyzed individually by two technicians over a four-day period, using the in-house method. Data for all samples from both methods were analyzed for relative trueness, accuracy profile, precision, and via a two-sample *t*-test (Minitab).

Results: For the relative trueness study, the in-house method provided a higher log recovery of *B. longum* from the probiotic powder (+1.43), DAD+probiotic (+1.11), and spiked kibble (+1.10) as compared the EN 15785 method. The accuracy profile study also demonstrated that the in-house method produced a higher log recovery (0.85 – 1.51) of *B. longum* from the three different spike levels of DAD+probiotic, than the EN 15785 method. The precision study demonstrated no significant difference (P > 0.05) between analysts for recovery of *B. longum* from DAD+probiotic. Overall, a significant difference (P < 0.05) in probiotic recovery was observed between the in-house and EN 15785 methods.

Significance: The in-house method produced higher recovery of *B. longum* from pet food products, compared to EN 15785. Data from this study will be shared with working groups (EN, ISO) for method development or revision.

P3-110 Performance Evaluation Real-Time PCR Assay for Detection of SARS-CoV-2 on Stainless Steel

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Introduction: SARS-CoV-2 has shown an ability for extended survival on stainless steel surfaces. While there is no credible evidence of surfaces aiding in the spread of the virus, especially via foodborne transmission, with the progression of the COVID19 outbreak, it became clear that testing for SARS-Cov-2 on surfaces can be an effective way to assess efficacy of cleaning, disinfection and environmental monitoring programs.

Purpose: The purpose of this study was to evaluate the performance of the veriPRO® SARS-CoV-2 Env test (I) on food contact surfaces using contaminated stainless steel (2" x 2"), in comparison to the 2019-nCoV CDC EUA Kit (II) as part of the AOAC RI™ ERV validation process.

Methods: Inclusivity and exclusivity were performed *in-silico* using 15,764 unique target strains, 8 near neighbors and 57 environmental background organisms. Matrix trials were performed with SARS-CoV-2 strain BEI NR-52281 with 30 unpaired samples where 5 replicates were evaluated at a high inoculation level ($2x10^{4}$ GU/test area), 20 at a low level ($2x10^{3}$ GU/test area), and 5 at an un-inoculated control level. Results for the candidate test (I) in the matrix trial were analyzed by POD statistical analysis.

Results: Candidate test (I) indicated a 99.97% inclusivity (N = 15,759), while no exclusivity strains were detected. No statistically significant differences were observed between the candidate test (I) and the reference test (II) at the high and non-inoculated levels [$dPOD_c$: 0.0; LCL: -0.43; UCL: 0.43], while at low inoculation, candidate test (I) outperformed the reference test (II) [$dPOD_c$: 0.55; LCL: 0.29; UCL: 0.74].

Significance: The data from this study, within the statistical certainty, demonstrates the equal or better performance of the candidate test (I) over the reference 2019-nCoV CDC EUA Kit (II) for testing on stainless steel surface.

P3-111 Changing the Paradigm of Bacterial Identification through Genome-based Identification System

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Introduction: An accurate and rapid identification of bacteria isolated from food or food processing environment is very important both for food quality assurance and for the tracing of outbreaks of bacterial pathogens within the food supply. There are several phenotypic, proteotypic and genotypic methodologies available for bacterial identification. Among them, mass spectrometry (MALDI-TOF MS) has been widely used because of its simplicity, reduced cost and rapid turnaround time. However, its ability to identify infrequently observed species is questionable due to a limited number of spectra in the database. Sequencing based genotypic methods (16S rRNA and Whole genome) is now considered the most powerful and accurate tool for bacterial identification and its database is extensively larger than MALDI-TOF.

Purpose: Develop a sequencing-based identification method for most accurate and definitive bacterial identification.

Methods: We, therefore, developed TrueBacID, a platform for accurate and precise bacterial identification system using sequence data from whole genome and/or other housekeeping genes including 16S rRNA. The system is designed to provide definitive species level identification in clinical, food safety and other industrial applications. It's database contains expert-curated and taxonomy-validated genome and gene sequence of Type- and Reference-strains and comprises largest number of species/sub-species, over 14,397 and 21,529, in its genome and 16S rRNA database, respectively.

Results: The performance has rigorously been validated in laboratory and clinical settings. In one study, it successfully identified all bacterial isolates (94% at species level) that completely failed identification by commercial MALDI-TOF MS systems. Additionally, it has also been benchmarked on publicly available datasets, demonstrating successful identification of >1,200 bacterial strains from an Intensive Care Unit of an US hospital with unprecedented accuracy and precision.

Significance: This cloud-based platform offers microbiologists around the world easy access to a sequencing-based "gold standard" of bacterial identification to ensure most accurate and definitive identification of bacterial isolates.

P3-112 Rapid Detection of *Salmonella enterica* in Fresh Produce by a Novel Microarray-based PathogenDx System

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Introduction: Consumption of fresh produce contaminated with *Salmonella enterica* may result in significant risk of foodborne illnesses. The diverse matrices pose great challenges for rapid *Salmonella* detection in fresh produce.

Purpose: The purpose of this ongoing study is to determine and work to improve the efficacy of PathogenDx microbial detection system in rapid detection of low *Salmonella* contamination in fresh produce.

Methods: Fresh produce (N = 96) including spinach, kale, arugula, Romaine lettuce, and Iceberg lettuce were spiked with *Salmonella enterica* Newport at two inoculation levels of 1 and 5 CFU/25 g sample. Spiked samples were enriched and concentrated by three methods prior to the microarray analysis. For the centrifugation and concentrator methods, spiked samples were enriched with 225 mL of universal pre-enrichment broth (UPB) for 3 h at 37°C. After enrichment, samples were concentrated by Innovaprep concentrator (100 mL/sample) or centrifugation (10 or 50 mL) at 10,000 rpm for 20 min. For the filtration procedure, 225 mL of PBS mixed with spiked sample was filtered through 0.45 µm filter. The filter was enriched in 10 mL UPB for 3 h at 37°C. Following concentration, samples (1 mL/test) were analyzed by microarray-based identification system to detect the presence of *Salmonella* (~ 6 h total

processing time). Samples were simultaneously analyzed by the FDA-BAM *Salmonella* detection procedure (~ 72 h total processing time).

Results: All samples spiked with 5 CFU of *Salmonella* were detected by the system following centrifugation of 10 mL volume or concentration by the concentrator pipette; and 87.5% by membrane filtration method. At 1 CFU/sample level, the system detected up to 75% of the spiked samples when concentrated by centrifugation, concentrator or membrane filtration. The FDA-BAM method that requires 24-h enrichment detected all spiked samples at 100%.

Significance: This novel approach may be used for rapid detection of *Salmonella* in fresh produce.

P3-113 Performance Evaluation of Loop-Mediated Isothermal Amplification (LAMP) – Bioluminescent Assay for *E. coli* O157 (including H7) Detection in Chicken Products, Environmental and Primary Production Samples

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Introduction: In 2020 Thailand became the world's fourth largest poultry exporter. Some export destination countries have a criterion for the absence of *E. coli* O157: H7, a toxin-producing strain that can cause hemorrhagic colitis. This organism may be transmitted to poultry at the primary production stage through contact with litter, feed or the environment. Rapid methods provide results significantly faster than the conventional methods enabling quicker action.

Purpose: To determine the performance of a loop-mediated isothermal amplification (LAMP) – bioluminescent assay for *E. coli* O157 (including H7) in chicken products, environmental and primary production samples compared to the ISO 16654 method.

Methods: A total of 120 samples (25 g each of cooked chicken with spice, cooked chicken, raw chicken, environmental swab and cloacal swab, *n* = 24 of each) were inoculated with different levels of *E. coli* O157: H7 ATCC 43888 (high: 6-9 CFU, *n* = 4 for each sample; medium: 3-5 CFU, *n* = 4 for each sample; low: 1-2 CFU; *n* = 8 for each sample; naturally contaminated; *n* = 8 for each sample). The samples were diluted 1/10 in buffered peptone water ISO (BPW ISO) or modified tryptone soy broth + novobiocin (mTSB+n) and incubated for 24 hours at 41.5°C. Enriched BPW ISO samples were tested using a LAMP assay while mTSB+n enriched samples were tested per ISO16654 method.

Results: Sensitivity for both the alternative method and the reference method was 100%, the false positive ratio and the relative trueness for the alternative method was 0% and 100%, respectively.

Significance: For all matrices evaluated, the LAMP assay was found to be equivalent to the reference method for the detection of *E. coli* O157. Hence, this study indicates that the alternative LAMP assay is a reliable method for rapid and specific detection of *E. coli* O157 from chicken products, environmental and primary production samples.

P3-114 Evaluation of the bioMérieux GENE-UP® *Listeria monocytogenes* (LMO 2) Real-Time PCR Assay for the Detection of *Listeria monocytogenes* in a Variety of Foods

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Introduction: The bioMérieux GENE-UP® *Listeria monocytogenes* (LMO 2) is a Fluorescence Resonance Energy Transfer (FRET) Technology & Melt Analysis based real-time PCR assay used for rapid and specific detection of *L. monocytogenes* from foods.

Purpose: The performance of this alternative method was compared to the Canadian culture-based reference method MFHPB-30 and evaluated according to Health Canada, Microbiological Methods Committee (MMC) guidelines for the relative validation of qualitative microbiological methods for consideration as a laboratory procedure (MFLP status).

Methods: Unpaired samples inoculated with *L. monocytogenes* were analyzed by the alternative and reference methods. Fruits and Vegetable-based products, Fish and Seafood products, Dairy Products, Ready-to-Eat Meat and Poultry and Multi-Ingredient Composite Foods were inoculated at three levels: 20 samples at 1-5 CFU/sample (L_1) likely to give fractional positive results (25-75%), 20 samples at a high level (L_2) at approximately 10 times L_1, and 5 un-inoculated samples. Alternative samples were enriched in LPT broth and tested after 22-24 h of incubation at 35 ± 1°C (for all foods except juices, which required 48 h). All analytical outcomes were culture confirmed by the reference method.

Results: Collectively, from the analysis of 1,350 unpaired samples, a probability of detection (POD) statistical model determined the alternative method met the criteria outlined by the MMC obtaining a relative sensitivity of 100%, relative specificity of 99.6%, a false positive rate of 0.4%, a false negative rate of 0% and test efficacy of 99.9%.

Significance: The bioMérieux GENE-UP® *Listeria monocytogenes* (LMO 2) assay is a suitable method for detecting *L. monocytogenes* in a variety of food matrices thereby significantly reducing reporting times over the reference method.

P3-115 Evaluation of a Loop-Mediated Isothermal Amplification (LAMP)-Bioluminescent Assay for Rapid Detection of *Cronobacter* in Chinese Powder Infant Formula (PIF) as Compared to the GB Method

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Introduction: GB method 4789.40-2016 uses DFI agar to screen enriched samples for *Cronobacter* (blue green colony) which takes 2-3 days to provide results. A rapid method for detection of *Cronobacter* will provide fast, accurate result compared to the culture method. In China, some powder infant formula (PIF) manufacturers also enumerate the non-green colonies (other *Enterobacteriaceae*) on DFI agar to determine the hygiene quality for process control. VRBG agar will provide a better enumeration of *Enterobacteriaceae* than the DFI agar.

Purpose: To verify the specificity and sensitivity of a LAMP-Bioluminescent assay for *Cronobacter* detection in PIF, ingredient and environmental swab samples compared to the GB method and utility of VRBG agar for detecting *Enterobacteriaceae* in environmental samples.

Methods: Three natural matrices, PIF (*n* = 10), ingredients (*n* = 40), environmental samples (*N* = 20) and artificially contaminated PIF samples (1 to 10 CFU/100 g, *n* = 30) were tested by the LAMP method and the GB 4789.40 method. The samples were enriched in BPW-ISO for 18 hours at 36°C and then tested with the LAMP assay and the GB method. In addition, presence of *Enterobacteriaceae* in environmental swab samples (*N* = 14) was compared using DFI and VRBG agar.

Results: The detection of *Cronobacter* by the LAMP method was in agreement with the GB method. Specificity and sensitivity of the LAMP method was 98.59% and 100%, respectively. For environmental samples, VRBG agar was better at detecting *Enterobacteriaceae* than DFI agar. Out of 14 environmental samples, VRBG agar detected *Enterobacteriaceae* in 11 samples, while DFI agar detected in only 8 samples.

Significance: The LAMP assay is a reliable alternative detection method for *Cronobacter* spp. in PIF, ingredients and environmental samples. The VRBG agar for *Enterobacteriaceae* provides better indication for hygiene quality than the DFI agar. Use of these two methods will provide a better solution for Chinese PIF producers than the current GB method.

P3-116 Sensitivity of BAM *Salmonella* Culture Method for the Recovery of *Salmonella* from Red, Yellow, White, and Sweet Onions

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Introduction: A multi-state foodborne outbreak, caused by *Salmonella* Newport, was reported during in July and August 2020 in the US and Canada. Red onions were suspected as the outbreak vehicle however, *S*. Newport was not found in the onions associated with the outbreak. Many onions, including yellow, white, and sweet onions were recalled.

Purpose: To determine the sensitivity of the BAM *Salmonella* culture method for use with onions and to investigate the inhibitory effect of onions on *Salmonella* growth.

Methods: To determine if compounds in onions inhibit the growth of *Salmonella*, we performed dilution to extinction of a *Salmonella* culture suspension in media with and without onions. If recovery of *Salmonella*, after 24 h incubation was less in the duplicate portions containing onions than in the duplicate portions of media without onions, then that would demonstrate the presence of inhibitors in the onions. Four varieties of onions (red, yellow, white and sweet) were tested. The test portion size was 25 g. We had 20 samples for each variety. The medium was tryptic soy broth (TSB)+K_2SO_3 (225 mL; BAM preenrichment). A serial dilution of a *Salmonella* culture suspension was added to comminuted onion/TSB+K_2SO_3, and solely TSB+K_2SO_3, respectively, and incubated at 35 ± 2°C for 24 ± 2 h, followed thereafter by the BAM culture method for isolation/confirmation.

Results: The BAM *Salmonella* culture method detected *Salmonella* at levels as low 1 CFU/onion and there was no inhibitory effect of onions on *Salmonella* growth in TSB+K_2SO_3.

Significance: This study provides important information for FDA outbreak investigations and promotes more research related to food matrix inhibitors for the detection of *Salmonella*.

P3-117 Evaluation of the GENE-UP® SLM (*Salmonella*) Assay: A Collaborative Study, OMA First Action 2020.02

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Introduction: Infection with nontyphoidal *Salmonella* bacteria is a leading cause of foodborne deaths and hospitalizations in the United States each year. *Salmonella* outbreaks associated with ground beef have persisted and in fact several recent large outbreaks have heightened the ongoing concerns of its safety. The bioMérieux GENE-UP® *Salmonella* (SLM) Kit is a real-time PCR Assay capable of detecting low levels of *Salmonella* even in the presence of high background product such as ground beef.

Purpose: To evaluate the interlaboratory performance of the candidate method against the USDA/FSIS MLG Ch. 4.10 reference method.

Methods: The candidate method was evaluated using 375-g unpaired test portions of raw ground beef in a multi-laboratory collaborative study consisting of fifteen collaborators from fourteen different laboratories. Each collaborator evaluated 12 replicates at three different levels of contamination: 0 CFU/test portion (un-inoculated), ~0.7 CFU/test portion (low) and ~2 CFU/test portion (high). Alternative confirmation using ASAP™ and CHROMID® *Salmonella* chromogenic agars was also performed.

Results: Statistical analysis was performed using the Probability of Detection model. The dLPODC values with a 95% confidence interval using either alternative or reference confirmation were: 0.00 (-0.03, 0.03) for the un-inoculated, -0.02 (-0.15, 0.12) for the low, and 0.02 (-0.03, 0.09) for the high contamination levels. The results demonstrate that there was no statistically significant difference in performance between the candidate and reference method for this evaluation.

Significance: These data supported the certification of the candidate method as an AOAC Official Method of Analysis℠ (OMA), First Action status.

P3-118 A Multiplex High-Resolution Melt Curve Real-Time PCR Assay for the Detection of ESBL-Producing *E. coli* O157:H7 in Foods

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne outbreaks associated with *Escherichia coli* O157:H7 have been increasing in the U.S. Incidences of β-lactamase-encoding genes in *E. coli* O157:H7 isolated from food products have also been reported.

Purpose: This study aimed to develop a multiplex HRM real-time PCR for the simultaneous detection of virulence and extended spectrum β-lactamase (ESBL) genes in *E. coli* O157:H7 in food samples.

Methods: A multiplex real-time PCR assay was developed for the simultaneous detection of four virulence (*eae*A, *uid*A, stx_1, stx_2) and one ESBL (bla_{CTX-M}) genes in *E. coli* O157:H7. The assay specificity and accuracy were, respectively, validated by using genomic DNA from gram-positive and gram-negative bacteria and Shiga toxin-producing *E. coli* (STEC) non-O157 and O157:H7 strains, and genomic DNA from bacteria previously reported to have the bla_{CTX-M} ESBL gene. Ground beef and boneless chicken were spiked with a cocktail of *E. coli* O157:H7 and ESBL-producing *E. coli* (1:1) at concentrations of 10, 100 and 1000 CFU/mL, and the detection limit of the assay was determined.

Results: Among 45 non-STEC DNA samples tested, 38 were negative for virulence and ESBL genes and seven were positive for *uid*A. The assay was able to detect bla_{CTX-M} in 16 out of 19 processed DNA samples that were previously confirmed to be positive for this gene. All four virulence genes were detected in all eight O157 STEC strains tested, while at least three or more virulence genes were detected in all 50 non-O157 STEC strains tested. A detection limit of 10 CFU/325 g was achieved within 6-8 h of enrichment for the food samples tested.

Significance: The multiplex HRM real-time PCR assay can be used as a rapid, specific and cost-effective tool for simultaneous detection of virulence and ESBL genes in *E. coli* O157:H7 that are present in low concentrations in food samples and unable to be detected by current culture methods.

P3-119 Selectivity of Culture Media for Lactic Acid Bacteria and Staphylococci Enumeration in Raw Milk Cheeses

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Introduction: Lactic acid bacteria (LAB) is a relevant group in raw milk cheeses, responsible for sensorial aspects. Staphylococci can indicate hygiene in production and some strains can produce enterotoxins, a hazard linked to cheeses.

Purpose: To assess the autochthonous microbiota of raw milk cheeses interference on the enumeration protocols for LAB and staphylococci.

Methods: Raw milk cheeses (n = 55) produced in Serro region, Brazil, were plated for LAB (five protocols, based on MRS, M17 and Petrifilm™ LAB, 30 or 37°C for 24, 48 and 72 h) and staphylococci (two protocols, based on Baird Parker agar and Petrifilm™ STX, 37°C for 24 and 48 h). Colonies were characterized as LAB and staphylococci based on Gram staining and production of catalase, coagulase and DNAse. Staphylococci colonies were also characterized as typical or atypical. Frequencies of colonies confirmed as LAB and staphylococci were compared by Chi-Square ($P < 0.05$) and counts were compared by ANOVA ($P < 0.05$).

Poster

Results: A total of 2,569 colonies were obtained from LAB protocols, and 1,901 were confirmed as LAB: Petrifilm™ LAB presented the best confirmation performance (82.9%), significantly higher when compared to M17 (67.4%) and MRS (72.9 to 74.2%) ($P < 0.05$). For staphylococci, 1,164 colonies were obtained and 340 were typical: typical colonies from Petrifilm™ STX were the most confirmed as staphylococci (74.1 to 90.8%), at significantly higher frequencies when compared to Baird Parker (34.8 to 61.9%) ($P < 0.05$). LAB counts varied from 6.3 to 6.8 log CFU/g among protocols ($P > 0.05$); staphylococci counts in Baird Parker (4.5 to 4.8 log CFU/g) were significantly higher when compared to Petrifilm™ STX (3.5 to 3.6 log CFU/g) ($P < 0.05$).

Significance: Petrifilm™ LAB and STX presented better selectivity for LAB and staphylococci in raw milk cheeses, resulting in more reliable counts.

Acknowledgments: CNPq, CAPES, FAPEMIG and 3M.

P3-120 Improvement of Automated VIDAS® L M X Assay for "Next Day" Detection of *Listeria monocytogenes* in Foods and Environmental Samples

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Introduction: Rapid and sensitive detection of *Listeria monocytogenes* is essential for food industry to reduce delays, accelerate decision-making and ensure safe food supply.

Purpose: Studies were conducted to validate a reagent formulation change to the automated VIDAS® *L. monocytogenes* Xpress (LMX) assay which allows a 20-minutes reduction assay run time.

Methods: The immunological test is based on an automated Enzyme Linked Fluorescent Assay (ELFA) targeting specific *Listeria monocytogenes* antigens. The change involves replacing the two-step conjugate reaction to a single incubation step. The performance of both protocols was compared through different studies carried out with pure strains and food matrices. Samples were enriched for 26-30 h at 37 ± 1°C, heated 5 minutes at 95-100°C and then tested with the VIDAS® instrument. A specific enrichment protocol was used for raw milk cheeses. Positive samples were confirmed by streaking enrichment broths onto a chromogenic selective agar.

Results: Inclusivity and exclusivity were both 100% using 25 *L. monocytogenes* strains and 20 non-target strains (broths inoculated with around 10 and 10^5 CFU/225 mL, respectively) with the modified assay. The sensitivity level of the new immunoassay was evaluated by testing several dilutions of each target strain and was comparable to that of the reference test. The specificity level of the methods was also compared on 209 food samples (25 g): 45 meat, 43 composite, 40 seafood, 40 vegetable and 41 dairy products. Eight naturally contaminated samples were confirmed positive and 201 samples were found negative by both methods. There was no statistical difference in results between the modified method and the USDA/FSIS reference method for deli ham (0.2-10 CFU/125 g) using dPOD analysis. Based on these data, the modification of the improved detection method was approved by AFNOR Certification (BIO-12/27-02/10 certificate), AOAC Research Institute (*Performance Tested Methods*℠ No. 091103) and AOAC INTERNATIONAL (Official Method of Analysis No. 2013.11).

Significance: The reduction of the run time of the automated immunological assay (60 minutes instead of 80) provides a faster, sensitive and convenient method allowing a presumptive result within 27 hours of sample preparation.

P3-121 Development of a Recombinase Polymerase Amplification Combined with Lateral Flow Dipstick Assay for Equipment-Free Detection of *Vibrio vulnificus* in Oysters

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Introduction: *Vibrio vulnificus* (Vv) inhabits estuarine waters around the world and can cause severe infection in humans by eating contaminated raw or undercooked oysters. In addition, Vv can cause life-threatening infection through an open wound of recreational beach swimmers or people who encounter contaminated marine water. Currently, methods for detecting and quantification of Vv require lengthy time and equipment.

Purpose: The objective is to develop a simple and rapid method, which can be operated without well-trained operators and equipment, by detecting the specific DNA sequences of Vv using the replication amplification polymerase (RPA) combined with lateral flow dipstick (LFD).

Methods: Optimization of the incubation time and temperature for RPA LFD was determined using newly designed primers and probes for Vv. The detection limits of bacterial DNA as well as colony forming unit (CFU) were evaluated using serially diluted samples. Specificity of RPA LFD was assessed by the employment of other foodborne bacterial DNA samples. Sensitivity of RPA LFD was evaluated by comparison with PCR, Real-time PCR and DNA hybridization methods, approved by the FDA's bacteriological analytical method.

Results: RPA LFD could detect 100 fg of DNA and 20 CFUs of Vv from both direct culture and inoculated oysters within 20 minutes (One-way ANOVA, $P < 0.05$). RPA LFD showed the test and control positive band with the incubation temperature from 30 to 45°C. The test was highly specific to Vv, not responsive to other foodborne pathogenic bacteria. As compared to the detection limits of PCR, Real-time PCR and DNA hybridization methods (1 ng, 10 fg, and 100 CFU by most probable number, respectively), our results suggest the RPA LFD assay we developed would be one of the sensitive methods for detecting *V. vulnificus* in oysters.

Significance: The technique developed can be useful to the oyster and other shellfish industries for enhancing food safety.

P3-122 AOAC PTM Certified 112001 Solus One *E. coli* O157 ELISA Immunoassay as a Competitive Alternative to Molecular Platforms in the Detection of *Escherichia coli* O157:H7 in the Raw Beef Supply Chain

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Introduction: The rapid and accurate detection of *Escherichia coli* O157:H7 in the raw beef supply chain is important for food safety. Rapid time-to-result detection systems for this pathogen are dominated by molecular based assays; but to date have not been challenged by an ELISA-based system.

Purpose: Development of a simple ELISA-based immunoassay certified to AOAC PTM standards, that can detect *Escherichia coli* O157:H7 from 25–375 g raw beef samples through a single enrichment step with a negative or presumptive positive result within 10-14 hours.

Methods: AOAC PTM inclusivity, exclusivity, robustness, product consistency, stability and method comparison studies comparing test to reference protocols were carried out. Twenty-five to 375 g raw beef samples either un-inoculated or artificially inoculated with *E. coli* O157:H7 strains; were enriched for 10 hours at 41.5°C in ISO BPW containing the Solus selective supplement. All enriched test and reference method samples were confirmed according to USDA MLG 5C.00 protocols.

Results: PTM validation studies indicated all 50 target and 40 non-target organisms were detected or correctly excluded. Robustness, product consistency and stability studies demonstrated no POD statistically significant differences. Method comparison study POD analysis indicated statistically significant differences at $P < 0.05$ (0.04–0.58 and 0.03–0.88 confidence interval of respective dPOD's for ground beef samples with more positive results being detected in favor of the candidate method. The test was granted PTM status, certificate no. 112001.

Significance: Successful development of an AOAC PTM certified immunoassay with concurrent improvements in sensitivity and specificity that challenges molecular based assays for rapid time-to-results in the detection of *Escherichia coli* O157 within raw beef samples that does not require the specialized skills required for molecular methods.

P3-123 The Use of Specific Swine Detection Methods to Ensure Halal Authenticity

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Introduction: In the past few decades, Halal meat has had growing sales with Muslim communities totaling nearly 25% of the world population. The qualification of Halal, permitted as per Islamic Shari'ah, addresses attributes that refer to the method of production and establishes that products must be free of any prohibited ingredients, such as pork, animals slaughtered improperly and other intoxicants.

Purpose: To ensure Halal authenticity, food safety enforcement authorities perform controls at each stage of the agrifood chain, and Halal entities are responsible of certifying goods apt for consumption by Muslims through coherent measures and adequate analytical monitoring.

Methods: RapidFinder™ Halal ID kit has been used to analyze a total of 898 samples including raw meat products, processed foods, food additives and condiments and animal feed. A highly sensitive analytical method with a limit of detection of 0.0005% based on real-time PCR was applied.

Results: Analytical surveillance confirmed that 87% of the Halal products were free of pork. Overall, 120 samples contained traces of pork, including food products and animal feed. The results enabled the users to implement additional control measures when needed to ensure the pork-free composition

Significance: The present study highlights the effectiveness of implementing analytical surveillance to ensure the authenticity of food products by minimizing accidental contamination.

P3-124 The Use of Specific Animal DNA Detection Methods to Ensure Vegan Authenticity

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Introduction: More and more consumers are choosing a vegan or vegetarian lifestyle, whether for ethical, environmental, health or religious reasons.

Purpose: In the food industry, binding legal definitions of the terms 'vegan' and 'vegetarian' have not been adopted yet at European level. As a result, there are no standardized criteria for a reliable labelling of vegan and vegetarian products which can lead to incorrect labelling by the producers themselves. This legal gap causes uncertainty between consumers and producers.

Methods: Detection of animal DNA presence in food samples via molecular techniques is crucial to improve the traceability and control in the food supply chain, as well as a necessary quality control for handling and cleaning processes in production lines. Furthermore, DNA analysis is more sensitive and more specific than similar strategies, such as protein-based analysis. RapidFinder™ Vegan ID kit has been used to analyze a total of 83 samples (food, feed, vegetable protein hydrolysate, wine, etc.) using a highly sensitive analytical method (limit of detection > 0.01%).

Results: The results confirmed the absence of animal DNA in 100% of vegan products, and animal DNA was only detected in samples containing poultry feathers and viscera intended for animal feed production. The results enabled the users to implement additional control measures when needed to ensure animal DNA-free composition.

Significance: The present study highlights the importance of implementing specific and sensitive analytical surveillance methods to ensure the authenticity of vegan products.

P3-125 One Year of Next Generation Sequencing (NGS) Data Collection for Food Analysis: Overview of Meat- and Fish-based Samples

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Introduction: DNA analysis has gained its importance in the service of food authenticity and safety. Next Generation Sequencing (NGS) has been introduced in this sector as a powerful and robust DNA-based method for species identification in food products.

Purpose: NGS requires the development of the correct workflow to ensure results reliability and to make the most of the advantages of this high throughput technique.

Methods: For the analysis of meat and fish complex food products we developed a new workflow that uses SGS All Species ID kits and software together with Ion Torrent™ platform. At SGS this technology has been successfully implemented into the testing laboratory and we are proving this solution to our clients.

Results: Here we will present the data collected from a full year of routine analysis. In 2020 we tested a total of 2,100 meat samples and 1,058 fish samples with the mentioned workflow. The samples belong to all kinds of food categories, including highly processed products such as canned food. The results showed a total of 3,278 meat species and 1,565 fish species identified, being the most common species *Sus scrofa* and *Gadus morhua,* respectively. Also, the workflow was applied with an overall successful rate of 99.6% meaning that only in 12 samples the DNA extraction was not successful.

Significance: The results show that the referred workflow can be implemented for routine analysis contributing for food authenticity and safety.

P3-126 Evaluation of the bioMérieux GENE-up® *Salmonella 2* (SLM 2) Real-Time PCR Assay for *Salmonella* in a Variety of Foods

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Introduction: The bioMérieux GENE-UP® *Salmonella* 2 (SLM2) is a Fluorescence Resonance Energy Transfer (FRET) Technology & Melt Analysis based real-time PCR system used for rapid and specific detection of *Salmonella* species from foods and environmental samples.

Purpose: The performance of this alternative method was compared to the Canadian culture-based reference method MFHPB-20 and evaluated according to Health Canada, Microbiological Methods Committee (MMC) guidelines for the relative validation of qualitative microbiological methods for consideration as a laboratory procedure (MFLP status).

Methods: Unpaired samples inoculated with *Salmonella* spp. were analyzed by the alternative and reference methods. Fruits and Vegetable-based products, Raw Poultry products, Dairy Products, Ready-to-Eat Meat and Poultry, and Eggs and Egg-derivatives were inoculated at three levels: 20 samples at a level (L_1;<1-5 CFU/25 g) likely to give fractional positive results (25-75%), 20 samples at a high level (L_2) at approximately 10 times L_1, and 5 un-inoculated samples. Alternative samples were enriched in BPW broth and tested after 18-26 h of incubation at 41.5 ± 1°C. All analytical outcomes were culture confirmed by the reference method

Results: Collectively, from the analysis of 1,350 unpaired samples, a probability of detection (POD) statistical model determined the alternative method met the criteria outlined by the MMC obtaining a relative sensitivity of 100%, relative specificity of 99%, a false positive rate of 1%, a false negative rate of 0% and test efficacy of 99.9%.

Significance: This bioMérieux GENE-UP® *Salmonella* 2 (SLM2) assay is a suitable method for detecting *Salmonella* spp. in a variety of food matrices thereby significantly reducing reporting times over the reference method.

P3-127 Evaluation of the Biomérieux *VIDAS® Listeria monocytogenes* (LMX) Enzyme-based Immunoassay for the Detection of *Listeria monocytogenes* in a Variety of Foods

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Introduction: The bioMérieux VIDAS® *Listeria monocytogenes* (LMX) is an enzyme immunoassay for use on the VIDAS® family of instruments for the rapid and specific detection of *Listeria monocytogenes* in foods using the ELFA method (Enzyme Linked Fluorescent Assay).
Purpose: The performance of this alternative method was compared to the Canadian culture-based reference method MFHPB-30 and evaluated according to Health Canada, Microbiological Methods Committee (MMC) guidelines for the relative validation of qualitative microbiological methods for consideration as a laboratory procedure (MFLP status).
Methods: Unpaired samples inoculated with *L. monocytogenes* were analyzed by the alternative and reference methods. Fruits and Vegetable-based products, Fish and Seafood products, Dairy Products, Ready-to-Eat Meat and Poultry and Multi-Ingredient Composite Foods were inoculated at three levels: 20 samples at a level (L_1) likely to give fractional positive results (25-75%), 20 samples at a high level (L_2) at approximately 10 times L_1, and 5 un-inoculated samples. Alternative samples were enriched in LMX broth and tested after 26-48 h of incubation at 37 ± 1°C. All analytical outcomes were culture confirmed by the reference method.
Results: Collectively, from the analysis of 1,350 unpaired samples, a probability of detection (POD) statistical model determined the alternative method met the criteria outlined by the MMC obtaining a relative sensitivity of 100%, relative specificity of 100%, a false positive rate of 0%, a false negative rate of 0% and test efficacy of 100%.
Significance: The bioMérieux VIDAS® *Listeria monocytogenes* (LMX) assay is a suitable method for detecting *Listeria monocytogenes* in a variety of food matrices thereby significantly reducing reporting times over the reference method.

P3-128 Comparison between Standard Plate Count Agar and a Ready-to-Use Aerobic Count Method Using Laboratory Pasteurized Count of Raw Milk Samples

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Introduction: Lab Pasteurized Count (LPC) is an aerobic count of raw milk after water bath pasteurization. LPC is not a regulated procedure; but it is used as a farm sanitary indicator and predictor of processed milk shelf life. Dairy cooperatives need comparative data to standard plate count (SPC) when adopting simplified aerobic count methods.
Purpose: Evaluate LPC samples using a ready-to-use aerobic plate method compared to SPC.
Methods: Eurofin-DQCI (Mound View, MN) supplied refrigerated (0-4.5°C) raw milk samples 48-72 h from collection. They were processed 63 ± 1°C for 30 min and immediately transferred to ice bath. SPC Agar (Difco) using pour plate method was performed along with Peel Plate AC (PP-AC, Charm Sciences, Inc., Lawrence, MA). One milliliter of undiluted and 1:10 diluted samples in Butterfields buffer (Weber Scientific, Hamilton, NJ) were plated in duplicate and incubated 32 ± 1°C for 48 ± 3 h. Paired statistical analysis (LaBudde) was applied. Mean log average differences are considered significant when their upper 95% confidence level (UCL) > 0.5 or lower confidence level (LCL) < -0.5.
Results: In Sep. 2020, *N* = 60 samples demonstrated no significant differences. *N* = 24 samples at 1:10 dilution in countable range had a slight PP-AC positive bias with mean log difference 0.17 with LCL = -0.04 and UCL = 0.39. *N* = 52 samples with any count at 1:10 dilution, the normal procedure followed by dairy cooperatives, had similar positive bias mean log difference 0.23 with LCL = 0.11 and UCL = 0.35. *N* = 32 undiluted samples in the countable range had a SPC slight positive bias with mean log difference -0.17 with LCL = -0.26 and UCL -0.08. The 1:10 dilution results are similar to *N* = 40 samples evaluated in the summer of 2019 with mean log difference 0.04 with LCL = -0.02 and UCL = 0.10.
Significance: Dairy cooperatives may consider this comparative data when determining if simplified methods are suitable for their purpose.

P3-129 Development of a Pretreatment Method to Improve the Detection Efficiency of Real-Time PCR for *Listeria monocytogenes* in Meat and Processed Meat

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Introduction: The presence of *Listeria monocytogenes* in meat and processed meat can be a problem in the meat industry, and thus, it is important to detect *L. monocytogenes* before the products are distributed. However, the food matrix in meat and processed meat can be an inhibitor in the process of detecting *L. monocytogenes* with real-time PCR.
Purpose: The objective of this study was to a method reducing the food matrix during DNA extraction method, which results in improved detection efficiency of real-time PCR for *L. monocytogenes* in meat and processed meat.
Methods: *L. monocytogenes* was inoculated in the meat or processed meat at 2 log CFU/g. 225 mL of Listeria enrichment broth containing 0.1% pyruvate and 0.1% ferric citrate were added to the sample bag containing 25 g of meat or processed meat (*n* = 6), and it was enriched at 30°C for 12 h. The enriched media were treated as follows; 1) after centrifugation of the enriched media, washing the cell pellets with PBS or 70% ethanol, followed by DNA extraction, 2) before centrifugation of the enriched media, filtering the enriched media with the filter paper, followed by DNA extraction. The extracted DNA samples were analyzed with real-time PCR.
Results: Real-time PCR analysis showed that positive rates of *L. monocytogenes* in ham, sausage, pork belly, and pork tenderloin were higher ($P < 0.05$) in the washed samples with PBS after enrichment than non-washing samples. In addition, the PCR positive rate for *L. monocytogenes* was higher ($P < 0.05$) in the samples filtered with filter paper before centrifugation of the in the enriched culture media than sterilized gauze, especially for high fat samples.
Significance: Filtering the enriched sample with filter paper before centrifugation or washing the cell pellet after centrifugation of the enriched media improves DNA purity, thus improving the detection efficiency of real-time PCR for *L. monocytogenes* in meat and processed meat.

P3-130 Hygiena BAX® System Salquant™ (SalQuant) AOAC Validation for Comminuted Chicken and Turkey

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Introduction: An affordable, rapid and accurate enumeration method is needed to provide the poultry industry with the ability to quantify *Salmonella*. *Salmonella* enumeration provides producers with a tool to adjust processing practices that will improve food safety for consumers.
Purpose: SalQuant is a method that uses the cycle threshold values (CT) obtained from a BAX® System Real-Time PCR Assay for *Salmonella* to estimate load by using a linear-fit-equation. This method was compared to the U.S. Department of Agriculture Food Safety and Inspection Service *Microbiology Laboratory Guidebook* 2.05 MPN method (USDA-FSIS-MLG-MPN) in raw comminuted chicken and turkey (325 g) for enumeration of *Salmonella.*
Methods: Five samples from each matrix were inoculated with *Salmonella* at 3 different inoculation levels, with five samples left non-inoculated (*n* = 20 per matrix). After 48-72 hours cold stress, 1,625 mL of BPW was added and samples hand massaged. For SalQuant, 30 mL of sample homogenate was combined with 30 mL of prewarmed BAX® MP media with antibiotics. Samples were incubated at 42 ± 1°C and sampled at 8 hours, placed back in incubator for a 24 hours prevalence testing timepoint. Results were analyzed and compared to a USDA-FSIS-MLG-MPN using the AOAC-RI PTM quantitative worksheet and JMP v. 14.

Results: The mean differences (candidate log mean – reference log mean) and 90% confidence intervals between SalQuant vs. USDA-FSIS-MLG-MPN for comminuted chicken at the low, medium and high inoculation levels were -0.051 (-0.450, 0.348), -0.404 (-0.811, 0.002) and -0.127 (-0.526, 0.273) and comminuted turkey were -0.033 (-0.382, 0.316), -0.237 (-0.834, 0.360) and 0.363 (-0.206, 0.931), respectively.

Significance: This rapid PCR enumeration method allows the user to detect a log CFU/g of *Salmonella* in raw comminuted chicken and turkey (325 g) within 10 hours. This method will give processors a more rapid tool to ensure that *Salmonella* is at a level that does not adversely affect human health.

P3-131 Understanding Conditions That Affect Recovery of Non-Enveloped Virus from Aqueous Solution Using Magnetic Ionic Liquids

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❖ ***Developing Scientist Entrant***

Introduction: Isolation and identification of pathogens from food samples is critical for controlling foodborne illness. However, foodborne viruses are difficult to isolate due to low concentration and uneven distribution. Magnetic ionic liquids (MILs) are a diverse class of hydrophobic solvents that can be easily separated from aqueous solutions and have previously proven effective for capture of bacterial pathogens.

Purpose: In this study, we demonstrated and optimized the capture and purification of bacteriophage MS2, a human norovirus surrogate, using MILs.

Methods: MS2 was diluted into 0.1% peptone water to 10^5 PFU/mL and extracted using cobalt-, manganese-, or nickel-based MILs. The MILs were added to the MS2 suspension, vortexed for 30 seconds to capture the bacteriophage, and separated using a magnet. The supernatant was then removed, and captured MS2 was eluted into Luria broth by vortexing for 120 seconds. MS2 RNA was then purified from each fraction via phenol-chloroform extraction and quantified via RT-qPCR. Three trials were conducted for each condition to determine average recovery efficiency.

Results: Cobalt- and manganese-based MILs showed recovery efficiencies of 12.5 ± 4.6% and 13.8 ± 3.8%, respectively, significantly ($P < 0.05$) outperforming nickel (2.55 ± 0.54%). Cobalt was used for further optimization assays, which included varying solution pH, increasing MIL volume, and adding surfactants. Although using pH 9 buffer initially led to greater recovery efficiency compared to peptone water (22.3 ± 6.5% versus 9.84 ± 6.3%), results became irregular over time, possibly due to capsid degradation from high pH. Increasing MIL volume led to decreased recovery efficiency, likely due to reduced droplet dispersion, and adding surfactants gave negligible increase in recovery efficiency.

Significance: Our results indicate MILs have potential as a capture reagent for foodborne virus detection, with room for further optimization. This informs future work on pre-analytical sample processing, one of the major hurdles to sensitive detection of viruses in foods.

P3-132 A Productivity Analysis of the 3M™ Petrifilm™ Plate Reader Advanced

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Introduction: Artificial intelligence (AI) systems have enabled businesses to see gains in productivity and profitability, increasing output of manual tasks allowing technicians to perform more value-added activities to better utilize their skills. In food safety testing labs, automated colony enumeration systems utilizing AI can replace traditional manual counting by technicians, which can be time consuming and tedious.

Purpose: To compare time required for manual and automated reader counting of 3M™ Petrifilm™ Aerobic Count Plates and 3M™ Petrifilm™ Rapid Aerobic Count Plates using the 3M™ Petrifilm™ Plate Reader Advanced.

Methods: Six samples ($n = 6$) of skim milk were diluted to achieve 1:10, 1:100 and 1:1000 dilution. Two sets of the diluted samples were spiked with *Staphylococcus aureus* (ATCC 6538) at a low (37 CFU/mL), medium (100 CFU/mL) and high (275 CFU/mL) inoculum level. One set of the diluted samples was used to inoculate 75 plates for each dilution. Following incubation, the plates were separated into stacks of 25 plates each, one stack of 25 for each of the three technicians for manual counting. The Protocol was repeated for each laboratory participating in the study.

Results: The automated reader on average reduced the time to enumerate the aerobic count plates by 81% across the 3 inoculum levels. The average colony count based on 2,475 aerobic count plates was 118 colonies/plate. Similarly, the automated reader on average reduced the time to count rapid aerobic count plates by 77%. The average colony count based on 2,475 rapid aerobic count plates was 110 colonies/plate. Plates containing only the high inoculum level showed a 91% and 94% reduction in time-to-enumerate, respectively, for the aerobic and rapid aerobic count plates.

Significance: The use of automated counting technology utilizing AI provides results that are equivalent to a trained microbiologist and decreases the time to count, which in turn increases laboratory productivity.

P3-133 Evaluation of Automated Plate Reading as Compared to Human Interpretation for *Enterobacteriaceae*, Coliform, *E. coli* and Yeast and Molds in Food Matrices

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Introduction: Manual colony enumeration of indicator microorganisms is tedious, error prone, and inefficient. The use of more agile and automated counting methods can increase laboratory productivity as well as enable accurate results and better record keeping.

Purpose: To compare enumeration of *Enterobacteriaceae*, Coliform, *E. coli* and Yeast and Molds on sample-ready 3M™ Petrifilm™ Plates with an artificial intelligence (AI) enabled automated reader, with manual counting.

Methods: Ice cream, salad dressing, and orange juice artificially contaminated with two levels (about 80 and 780 CFU/g) of *E. coli* ATCC 8539, *S. aureus* ATCC 25923, or *C. albicans* ATCC 10231 and naturally contaminated hamburger and fresh cheese (n = 20-24 samples for each matrix) were evaluated. Samples were diluted 1:10 fold and plated on respective sample-ready plates. After incubation, plates were enumerated manually and with an automated reader. A paired t-test was conducted to determinate statistical differences between interpretations ($P < 0.05$).

Results: There was no statistical difference between the human and automated counting for all the sample-ready plates tested. P-values obtained for *Enterobacteriaceae* (ice cream, meat, and cheese), *E. coli* (salad dressing), Coliforms (meat) and yeast and mold (juice) plates were 0.075, 0.50, 0.115, 0.58, 0.83 and 0.06, respectively.

Significance: The 3M Petrifilm Plate Reader Advanced enabled reliable and more productive enumeration of indicator organisms compared to manual enumeration.

P3-134 Rapid Detection of *Salmonella* spp. and *Listeria monocytogenes* in Food Dressings with Loop-Mediated Isothermal Amplification (LAMP)-Bioluminescent Assays

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Introduction: Changes in lifestyle, such as lack of time for food preparation, has increased demand for ready-to-eat foods. The industry of dips, sauces and mayonnaise has met the market demand with several products. Despite the processing of the ingredients and their intrinsic characteristics, they can be sources of foodborne pathogens such as *Salmonella* and *Listeria monocytogenes.*

Purpose: To determine the specificity, sensitivity and accuracy of specific LAMP-bioluminescent assay for detection of *Salmonella* and *L. monocytogenes* in food dressings as compared to culture confirmation with respective ISO methods.

Methods: In our study, 5 matrices (salad dressing, mayonnaise, light mayonnaise, spiced-mayonnaise-based dressing, and ketchup) with a total of 400 samples (200 for *Salmonella* spp. and 200 for *Listeria monocytogenes*), were evaluated. Samples were uninoculated (n = 100 for each target) and artificially

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contaminated with the target and an interferent organism (*n* = 100 for *S.* Typhimurium ATCC 14028 (1-5 CFU) and *Proteus mirabilis* WDCM 00023 (100-500 CFU) per 25 g, and *n* = 100 for *L. monocytogenes* ATCC 19115 (1-5 CFU) and *P. mirabilis* WDCM 00023 (100-500 CFU2) per 25 g). Samples were diluted in 225 mL of BPW ISO for *Salmonella* and Demi-Fraser broth for *L. monocytogenes*, incubated at 37°C for 24 h and tested with respective Lamp assays. All enrichments were culture confirmed by respective ISO methods. Probability of Detection (POD), sensitivity and specificity of the assays were determined.

Results: Based on POD analysis, there was no significant difference between the LAMP assays and culture confirmation. The sensitivity, specificity, accuracy of the LAMP assays were 100% with no false positives or false negatives.

Significance: The alternative LAMP-bioluminescent method enabled reliable and rapid detection of *Salmonella* spp. and *L. monocytogenes* in food dressings.

P3-135 Detection of *Listeria* in Sprout Irrigation Water and Environmental Surface Samples Using the *Listeria* Canary® Zephyr Assay

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Introduction: *Listeria* species are environmentally ubiquitous and can cause listeriosis through consumption of contaminated food. Their ability to survive at extreme conditions poses a high risk in food industries. The CANARY® Zephyr is an immuno-based biosensor platform that allows for rapid and sensitive detection of foodborne pathogens including *Listeria* spp.

Purpose: The purpose of this study was to evaluate the performance of the new *Listeria* assay for its inclusivity, exclusivity, and sensitivity.

Methods: Overnight cultures of *Listeria* strains (*n* = 50) were diluted to 100 times of the limit of detection (LOD) for testing (10^4 CFU/mL). Thirty-four non-target strains were cultured and tested without dilution. Matrix studies were conducted in sprout irrigation water and various environmental surfaces including stainless steel, rubber, and plastic (1" × 1" test area). Each matrix was inoculated with diluted *Listeria* cultures at a low-level expected to yield fractional positive results and a high-level expected to yield all positive results. Water samples (25 mL) were enriched using 225 mL *Listeria* Enrichment Media at 37°C for 24 ± 2 hours. Similarly, environmental surfaces were sampled with swabs and enriched in 10 mL *Listeria* Enrichment Media. Samples were prepared and tested following the assay user guide. Assay performance were compared to the FDA reference method and all presumptive results were culture confirmed following FDA guideline.

Results: All 50 *Listeria* isolates were detected, and all 34 non-target strains returned negative. All presumptive results were confirmed by culture. No statistically significant differences were observed between the candidate and the reference method for sprout irrigation water and environmental surfaces by probability of detection (POD) analysis.

Significance: The new assay demonstrated equivalent performance to the FDA BAM reference method. Thus, it provides an alternative method for rapid and reliable detection of *Listeria* in irrigation water and environmental surface samples.

P3-136 Magnetic Separation and Luminescence-based Detection of Bacteria in Agricultural Samples Using Engineered Bacteriophage

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Introduction: Rapid separation and detection of pathogens and their bacterial indicators in agricultural matrices can be accomplished using genetic engineering and synthetic biology to synthesize bacteriophages that pragmatically bind to magnetic nanoparticles and express high quantities of reporter enzymes.

Purpose: The study investigates synthetic biology to improve the outlook of phage-based biosensing by combining bacterial separation and detection into a single platform.

Methods: The phages (T4, RB69) were genetically engineered using CRISPR-Cas9 to allow expression of binding moieties (binding peptides, unnatural amino acids) on their capsid proteins for direct conjugation to magnetic nanoparticles. The magnetized phages allowed rapid separation of *E. coli* from 100 mL water samples. Genes for a cellulose-binding luciferase were inserted into the phages for simple concentration of reporter enzymes following phage infection of target bacteria. In order to optimize reporter expression levels, a promoter optimization study was conducted across five early, five middle, and five late promoters.

Results: Phages were bound to magnetic nanoparticles using two modification strategies, 1) azide-containing unnatural amino acids on the capsid, and 2) silica-binding peptides displayed on the capsid. The phages did bind to the nanoparticles and maintained a dispersed suspension. Phages with binding moieties demonstrated a significantly higher detection signal verses those without, indicating effective binding of the phages to nanoparticles. Although there was a significant decrease in signal when a large background of competing bacteria were introduced, the sensor was able to detect 7 CFU/100 mL.

Significance: Rapid methods to detect pathogens and bacterial indicators in process water, agricultural water, or other liquid agricultural samples can allow more effective responses. Phage-based detection, which has often been hampered by high limits of detection, were engineered to pragmatically and cost effectively bind magnetic nanoparticles, allowing low detection limits from regulatory appropriate sample sizes.

P3-137 Universal Sample Preparation and Cell-Free Paper Sensor for Detection of Foodborne Pathogens in Food Matrices

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Introduction: Rapid, low cost cell-free paper sensors can overcome limitations of culture counting and PCR that require long wait times (24-48 h), expert personnel, and centralized labs.

Purpose: We developed a diagnostic platform that uses magnetic separation and molecular diagnostics to detect *E. coli* O157:H7 in complex sample matrices in 1.5-2 h.

Methods: For the system, bacteria are isolated from sample matrix using magnetic beads conjugated to Fc-mannose binding lectin (FcMBL-MB) followed by thermal extraction of nucleic acids and recombinase polymerase amplification (RPA) of target dsDNA sequences. Target sequences recognized by complimentary guide RNA activate Cas12a to cleave ssDNA reporter probes that produce a fluorescent signal (Specific High-Sensitivity Enzymatic Reporter UnLOCKing or SHERLOCK). To validate molecular diagnostic design, dilutions of dsDNA target sequences underwent RPA and SHERLOCK alone. Next, target *E. coli* O157:H7 and non-target *E. coli* O127:H6 were diluted in general test water, lysed, and measured after RPA and SHERLOCK. *E. coli* O157:H7 diluted in general test water with and without FcMBL-MB concentration were measured using SHERLOCK. For all tests $n \geq 3$ and student's t-test ($\alpha \leq 0.05$) was used to determine limit of detection (LOD)

Results: The LOD for synthetic dsDNA concentration after RPA and SHERLOCK was 7.1 aM (~single molecule/µL) with affinity for 7 Shiga toxin-producing strains (stx1 and stx2) of *E. coli*. LOD for E. *coli* O157:H7 was 10^3 CFU/mL using RPA and SHERLOCK in the presence of non-target bacteria. LOD for *E. coli* O157:H7 in general test water for the full system including MB-FcMBL concentration, RPA and SHERLOCK was 10^0-10^1 CFU/mL or 50-500 CFU in 50 mL.

Significance: FcMBL-MB, which has affinity for diverse bacteria strains, facilitates "universal" sample preparation followed by specific, sensitive molecular diagnostics. This enables rapid, easy-to-use cell-free paper biosensors in resource-limited settings for food and water safety.

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P3-138 Comparison of Selected Preenrichment Media and Rapid Screening Methods in Detection of *Salmonella* in Spent Sprout Irrigation Water Samples

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Introduction: Detection of *Salmonella* in spent sprout irrigation water (SSIW) samples is of great importance, highlighted by the number of outbreaks associated with sprouts both in the United States and around the world.

Purpose: This study aimed to compare different preenrichment broth and rapid screening methods for the ability to detect *Salmonella* in SSIW samples from broccoli, mung bean, and alfalfa sprouts.

Methods: SSIW samples from each sprout variety were inoculated with *Salmonella enterica* serotypes Cubana and Enteritidis, respectively, ranging from 0.7 to 1.5 CFU per 375 mL SSIW test portion. Twenty SSIW test portions were pre-enriched using universal preenrichment broth (UPB, 1:3 sample-to-broth ratio) or lactose broth (LB, 1:9 sample-to-broth ratio). The BAM *Salmonella* culture method was followed thereafter. Five selected rapid methods were used in the paired study for comparison. Results were statistically analyzed using Fisher's Exact Test for pairwise comparisons. A logistic regression method was used to detect interactions between *Salmonella* serotype and SSIW variety.

Results: The microbial background varied between the sprout variety and sample batch, with microbial count spanning from 1.5 to 6.9 log CFU/mL of SSIW. The difference in the detection rate for *Salmonella* between LB and UPB was significant ($P = 0.02$) after controlling the effect of SSIW sample + serovar. The detection rate for *Salmonella* with selected rapid methods was comparable to culture method in low APC background samples. In samples with a high APC background, however, a drastic difference in performance was observed between the rapid methods when compared to culture method and with different preenrichment broth (UPB >> LB).

Significance: This study validated UPB as a better preenrichment broth than LB for the isolation and detection of *Salmonella enterica* from SSIW. UPB may be a preenrichment medium that is optimal for both rapid screening and culture methods.

P3-139 Method Comparison of Clear SafetyTM (CS) *Listeria* 3-in-1 Assay, Multiplex PCR and Whole-Genome Sequencing for Performing Speciation/Subtyping Analysis of *Listeria* Species

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Introduction: Food manufacturers, during investigation of environmental pathogens, often face challenges to determine the nature of persistence (e.g. transient vs. resident) of the organisms found. Subtyping methods that can accurately identify and track foodborne bacterial pathogens not only aid in the investigation of environmental pathogens but are also critical to ensuring food safety. Such tools can also enable a better understanding of the sanitary state of the facility's environment over time, enhance root-cause analysis investigation, and increase the effectiveness of implemented corrective actions.

Purpose: A method comparison study for speciation/subtyping analysis of *Listeria* spp. was performed between CS *Listeria* 3-in-1 assay and both Conagra Brands' internal multiplex PCR (mPCR) and Whole-Genome Sequencing (WGS) methods.

Methods: A total of 45 environmental swab samples determined presumptive positive for *Listeria* spp. were speciated/strain typed using CS, mPCR and WGS methods. The CS *Listeria* 3-in-1 assay was able to speciate and strain characterize each sample simultaneously within a single test. Additionally, mPCR and WGS methods were performed separately on each sample by testing directly out of the primary enrichment using mPCR to speciate and WGS-subtyping analysis on purified *Listeria* isolates.

Results: *Listeria innocua*, *L. monocytogenes* and *L. welshimeri* were detected out of the enrichment samples, with the latter two species identified the most. Also, it was determined there was a one persistent strain of both *L. innocua* and *L. welshimeri* and two persistent strain types of *L. monocytogenes*. Overall, there was 86% correlation ($P<0.05$) for speciation and 100% correlation for subtyping analysis between CS *Listeria* 3-in-1 assay, mPCR, and WGS methods.

Significance: CS *Listeria* 3-in-1 assay offers a rapid (~12-hour detection vs. weeks) and more simplified option for simultaneous speciation/subtyping analysis helping manufacturers design strategies to quickly mitigate food safety risks.

P3-140 Detection of *Listeria monocytogenes* in Mixed Environmental Sponge Swab Enrichment Cultures Using Hygiena™ BAX® PCR and Real-Time PCR Assays versus USDA MLG and FDA BAM Reference Methods

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Introduction: Environmental testing for *Listeria* is a vital food safety program. Individual sponge swabs are collected from multiple sites to take action when screening is positive, but this requires more time and cost. Combining multiple samples post-enrichment could address both limitations, while allowing traceback for a positive mixed set.

Purpose: To assess the impact on *L. monocytogenes* detection when enrichments from pathogen-inoculated sponges are mixed with enrichments from four competitor only inoculated sponges.

Methods: Sponge swabs hydrated with DE neutralizing broth were co-inoculated at a fractional level with approximately 1.0 CFU *L. monocytogenes* and approximately 10 CFU *Enterococcus faecium* (competitor) ($n = 20$). Additional sponges ($n = 80$) were inoculated with 10 CFU *E. faecium* and enrichments from each were mixed with the co-inoculated enrichments in a 1:5 ratio. High-spiked (5 CFU/sponge, $n = 5$) and negative (uninoculated, $n = 5$) sponges were also included. Inoculated sponges were refrigerated 18 h to simulate shipping conditions prior to enrichment and screened using four alternate PCR assays or FDA BAM and USDA reference methods. All sample enrichments were culturally confirmed.

Results: Individual reference sponges demonstrated comparable recovery to the five-sponge mixed enrichments when assayed by all PCR methods. The proportion of fractional positives was 15/20 (POD = 0.75) for individual FDA enrichments, 16/20 (POD = 0.80) for USDA individual enrichments, 12/20 (POD = 0.60) for *Listeria* and *L. monocytogenes* PCR mixed enrichments, 17/20 (POD = 0.85) for real-time *Listeria* PCR mixed enrichments, and 18/20 (POD = 0.90) for real-time *L. monocytogenes* PCR mixed enrichments. The difference in probability (dPOD) between individual reference sponge enrichments and secondary mixed sponge enrichments assayed by the PCR methods indicated no significant difference since the 95% confidence intervals contained 0 in all cases. All five high inoculated sponges tested positive (POD 1.00) and all five negative sponges tested negative (POD 0.00).

Significance: Five-sponge mixed enrichments provide a comparable recovery with no statistical difference to testing sponges individually.

P3-141 Evaluation of a STEC Detection Real-Time PCR Method Workflow Vs. the ISO/TS 13136:2012 Reference Method

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are causative agents of severe gastrointestinal disease and may lead to hemolytic uremic syndrome. The sources of these infections are most commonly foods that are intended to be eaten raw or part-cooked (including meat, vegetables and fruits).

Purpose: The purpose of this study was to evaluate the performance of the SureTect™ Escherichia coli O157:H7 and STEC PCR Assays for screening and identification of STEC from two different food categories with large sample sizes (375 g) to improve sampling plans for: (i) meat (excluding poultry), (ii) vegetables and fruits.

Methods: A panel of samples from various origins were tested according to the technical guidelines of the ISO 16140-2 sensitivity study. A minimum of 90 samples were tested for each category, to meet the ISO 16140-2 requirements for the sensitivity and relative level of detection (RLOD) studies. All samples were enriched using a short protocol (8 hours for meat samples, 10 hours for vegetables and fruit samples) and a long protocol (24 hours) prior to running the SureTect PCR workflow. The samples were also tested after a 72-hours cold storage hold post-enrichment. Positive PCR results were confirmed using a combination of culture and molecular techniques. As part of the inclusivity and exclusivity studies, 50 strains and 30 non-target strains were tested.

Results: The number of positive deviations was significantly higher than the negative deviations for the sensitivity study. This indicates a skew in performance in favor of the alternative method for the meat and vegetables categories. For RLOD study, similar results were observed between the two methods with RLOD values below the acceptability limits. For inclusivity and exclusivity studies, all strains provided expected results; this demonstrates 100 % correct specificity and selectivity with the tested pure strains.

Significance: The data indicates that the SureTect method is a suitable alternative to the ISO/TS 13136:2012 method for 375 g meat (excluding poultry) and vegetables and fruits testing.

P3-142 Validation of a PCR Workflow for the Detection and Confirmation of *Escherichia coli* O157:H7 and the *E. coli* STEC Serotypes O26, O45, O103, O111, O121 and O145 from Fresh Raw Spinach, Fresh Baby Leaves, Fresh Cut Tomatoes, Frozen Raw Beef, Raw Beef Trim and Beef Carcass Sponges

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Introduction: Shiga toxin-producing *E. coli* (STEC) strains cause an estimated 265,000 plus illnesses annually in the United States transmitted through contaminated food, predominantly leafy greens and raw beef.

Purpose: The purpose of this study was to evaluate the Thermo Scientific™ SureTect™ Escherichia coli O157:H7 and STEC Screening PCR and SureTect™ Escherichia coli STEC Identification PCR workflow for detection and confirmation of *E. coli* O157:H7 and STEC serotypes O26, O45, O103, O111, O121 and O145 from selected matrices according to the AOAC® *Performance Tested Methods*℠ program.

Methods: A matrix study consisting of 375 g Fresh Raw Spinach, 375 g Raw Beef Trim, Beef Carcass Sponges, 25 g Fresh Cut Tomatoes, 25 g Frozen Ground Beef and 25 g Fresh Baby Leaves was conducted according to AOAC Appendix J. The candidate method was compared against FDA BAM Chapter 4A for Fresh Raw Spinach, USDA FSIS MLG 5C.00 for Raw Beef Trim and Carcass Sponges, and ISO/TS 13136:2012 for Fresh Cut Tomatoes, Fresh Baby Leaves and Frozen Raw Ground Beef. Product stability, robustness and two inclusivity/exclusivity studies were completed. Inclusivity/exclusivity studies included the standard detection study as outlined in AOAC Appendix J and a confirmation study based on ISO16140-6:2019, which included 162 inclusivity and 105 exclusivity isolates tested from specific selective and non-selective agars.

Results: Matrix studies showed no statistically significant differences between the candidate and reference methods through probability of detection (POD), all confidence intervals met the AOAC requirement of straddling 0. Both inclusivity/exclusivity studies successfully detected or excluded all isolates and product stability and robustness testing demonstrated no statistically significant differences between kit lots or method deviations, respectively.

Significance: The data shows that both assays constitute a rapid and reliable alternative workflow for the detection and confirmation of *E. coli* O157:H7 and stipulated STEC serotypes from selected vegetable and meat matrices.

P3-143 Crowdsourcing and Machine Learning Approaches for Extracting Information Indicating Potential Foodborne Outbreak from Social Media

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Introduction: Foodborne outbreak is a serious but preventable public health challenge. The current outbreak detection method usually leads to a significant delay between the first infection and when action is taken to inform the public about the event of an outbreak. In recent years, social media (e.g., Twitter) has been employed as a new tool to identify unreported foodborne illnesses.

Purpose: However, there is a huge gap between identification of sporadic illnesses and detection of a potential outbreak. Also, valuable information, such as people, time, location, and food that indicates foodborne illnesses should be extracted from the social media text.

Methods: In this work, a joint learning approach is developed to identify unreported foodborne illnesses and extract foodborne illness-related entities from text data on the social media platform Twitter. Labelling is the first and critical step in building the machine learning models, in which we deploy human labelers using crowdsourcing. We ask annotators to do two tasks: (i) rate a set of tweets by their relevancy to a foodborne illness incident and (ii) label out specific elements. The inter-rater agreement metric is computed to develop a majority-confirmed relevancy label for each tweet.

Results: Unlike previous methods, our model leverages the mutually beneficial relationships between relevance prediction and entity extraction. Experiment results show that the accuracy of relevance prediction is 0.8153, and the F1 score of entity extraction is 0.6134.

Significance: The key elements such as people, time, location, and food detected from sentences indicating foodborne illnesses will be used as critical information for analysis of potential outbreaks in future studies.

P3-144 Text Mining of Social Media Posts for Identifying Potential Food Safety Issues on Farmers' Markets in Illinois

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Introduction: Farmers' markets (FMs) are growing as significant venues for the sale of local produces such as fresh fruits and vegetables. However, FMs can also encounter food safety issues due to the nature of its open environment, lack of refrigeration units and sanitation facilities, and less strict inspection requirements.

Purpose: In aim to better understand the food safety concerns in Illinois FMs from consumer perspective, we employed social media platforms Yelp and Twitter as the source of data and used text mining to build models that can automatically identify consumer responses on food safety aspect. In addition to food safety, other aspects such as quality, availability, and environment were also considered in classification model.

Methods: Naïve Bayes (NB), Support Vector Machines (SVM), Logistic Regressions (LR), k-Nearest Neighbor (k-NN), and Random Forests (RF) methods were used in building the classification model.

Results: SVM was identified as the optimal model with highest accuracy in correct classification on both Twitter (0.68) data and Yelp data (0.75). Based on the SVM coefficients of the trained models, the most important features/words used in the classification were identified. As for food safety, the important features discovered from the two datasets were different. On Twitter, words like 'safety', 'coli', 'health', 'recall', 'illness', 'train', 'tip', and 'foodborne' were commonly mentioned, indicating people intend to talk about issues of foodborne outbreaks. Also, most of the relevant tweets were focused on food safety practices on farmers market, instead of complaints of people getting sick after visiting farmers' market. On Yelp, people tend to comment on the hygiene conditions of a farmers' market with words like 'clean', 'messy', 'safety', 'gross', 'rotten' being used as the critical features.

Significance: The findings could help local public health department know about the hygiene status or potential food safety issues of a farmers' market

based on consumer reviews.

P3-145 Foodborne Illness of Hepatitis A Virus by Lettuce Consumption

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Introduction: Because there are cases of hepatitis A virus contamination in lettuce and lettuce consumed fresh, consumption of lettuce may cause hepatitis A virus foodborne illness. Thus, the risk of hepatitis A virus by lettuce consumption needs to be evaluated.
Purpose: This study evaluated the risk of hepatitis A virus foodborne illness by lettuce consumption in Korea.
Methods: One hundred lettuce (iceberg) samples were collected from markets to evaluate the prevalence of hepatitis A virus. The lettuce samples inoculated with hepatitis A virus, and viral titers were enumerated during storage at 4°C−25°C. The hepatitis A virus titers were fitted to the Baranyi model to calculate the shoulder period (h) and death rate (log PFU/g/h). These kinetic parameters were then fitted to a polynomial model as a function of temperature. Distribution temperature and time, and consumption data for lettuce were surveyed. A dose-response model for hepatitis A was also searched. The simulation model in @Risk with these data estimated the probability of hepatitis A virus foodborne illness.
Results: Hepatitis A virus was detected 1 sample, and thus, initial contamination level was estimated by the Beta distribution (2, 156) to be -6.2 log PFU/g. The developed predictive models showed that hepatitis A virus titers decreased under the investigated conditions with Uniform distribution (0.325, 1.643) for time and Pert distribution (10, 18, 25) for temperature. The LogLogistic distribution [RiskPert (1.8200, 1.8200, 335.00, RiskTruncate (0,236.8))] showed that average consumption amount for lettuce was 2.76 g at 1.59% of frequency. The Beta-Poisson model $[1-(1+Dose/186.4)^{-0.373}]$ was appropriate to evaluate the dose response for hepatitis A virus. The simulation showed that the probability of hepatitis A virus foodborne illness by lettuce consumption was 6.79×10^{-10} per person per day.
Significance: The results indicate that the risk of hepatitis A virus by lettuce consumption is low in Korea.

P3-146 Risk Assessment of Hepatitis A Virus Foodborne Illness by Fermented Shellfish Consumption

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❖ ***Developing Scientist Entrant***

Introduction: Hepatitis A virus (HAV) infection is the most serious viral infection. Cases for HAV has increased dramatically in Korea, and fermented shellfish was recently disclosed as a main vector. Thus, the risk of HAV foodborne illness by fermented shellfish consumption needs to be evaluated.
Purpose: This study estimated the risk of HAV outbreaks by consumption of fermented shellfish in Korea.
Methods: The prevalence data for HAV in fermented shellfish was collected by investigating fermented shellfish. HAV was inoculated into 25 g of fermented shellfish samples at 7.4 log PFU/g, and the fermented shellfish samples were stored at -20-25°C. HAV in the fermented shellfish was enumerated by a plaque assay with Frhk-4 cells. The predictive models to describe the kinetic behavior of HAV in fermented shellfish were developed. The consumption frequency and amounts for fermented shellfish were surveyed. With the collected data, a risk assessment simulation was conducted to estimate the probabilities of foodborne illness by intake of fermented shellfish, using @RISK software.
Results: Of 136 samples, 44 samples were contaminated with HAV, and thus, the initial contamination level (-3.7 log PFU/g) of HAV estimated by RiskBeta (45, 93). The developed predictive model showed that the HAV counts decreased as temperature increased. The daily consumption amount fitted by the Exponential distribution [Expon (8.1789, Shift (0.69235)] was 8.86 g per person with 0.13% of consumption frequency. Beta-Poisson model $[1-(1+Dose/4.4\times10^{186.4})^{-0.373}]$ was selected for the dose-response of HAV. The simulation with the data showed that the probability of HAV foodborne illness by fermented shellfish consumption was 6.56×10^{-11}/person/day, and only for those who consumed fermented shellfish as the population, the probability was 8.11×10^{-8}/person/day.
Significance: This result indicates that the risk of HAV foodborne illness by fermented shellfish seems low because of low consumption frequency in Korea, but those who consumed fermented shellfish has significantly increased risk.

P3-147 Quantitative Microbial Risk Assessment for Norovirus Foodborne Illness by Kimchi Consumption

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Introduction: Kimchi is one of the most consumed foods in Korea. Norovirus sometimes infects humans through fresh agricultural products and foods. Thus, norovirus could be contaminated in kimchi.
Purpose: This study investigated the prevalence of norovirus in kimchi and analyzed the risk of norovirus foodborne illness by kimchi consumption in Korea.
Methods: One hundred kimchi samples were collected from markets and analyzed to detect norovirus by PCR. To examine the growth pattern of norovirus, murine norovirus inoculated in kimchi was enumerated during storage at 4 - 25°C. The viral titers were used to develop predictive models, which describe the kinetic behaviors of norovirus. Market display time and temperature, and transportation time and temperature were collected, and consumption data were surveyed. A dose-response model was searched through literatures. With these data, the probability of norovirus foodborne illness was estimated by a simulation in @RISK.
Results: Of 100 samples, norovirus was not detected in kimchi, and thus, the Beta distribution (1, 101) estimated the initial contamination level of norovirus. The developed predictive model showed that the norovirus titers decreased as temperature increased. Market display temperature and time were fitted with the Uniform distribution (6.7, 13.0) and the Uniform distribution (0, 24), respectively. Temperature and time during transportation were fitted with the Pert distribution (10, 18, 25) and the Uniform distribution (0.325, 1.643), respectively. Average consumption amount was 84.7 g, calculated by the Gamma distribution [Gamma(1.1538,72.654,Shift(0.85475))] at 68.2% of frequency. ${}_1F_1$ hypergeometric dose response model $[Risk=1-(1+\eta CV)^{-r}]$ (η, 2.55×10^{-3}; CV, dose; r, 0.086) was selected as an appropriate model. The simulation with all data showed that the probability of the foodborne illness was 1.6×10^{-9}/person/day.
Significance: The results indicate that the risk of norovirus foodborne illness by kimchi consumption is low in Korea.

P3-148 Risk of Norovirus Foodborne Illness by Raw Radish (*Raphanus sativus*) Consumption in Korea

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Introduction: Norovirus is known to cause foodborne illness, and the foodborne illness by Raw radish (*Raphanus sativus*) consumption often occurred in Korea.
Purpose: The object of this study was to estimate the risk of norovirus foodborne illness by raw radish consumption in Korea.
Methods: One hundred seven raw radish samples were collected from markets to detect norovirus with PCR. To evaluate the fates of norovirus in the radish, predictive models were developed with the viral plaque counts at 4-25°C. The time and temperature for market display, transportation, and home storage were collected, and the consumption patterns were surveyed. A dose-response model was searched in literatures. With these data, a simulation model was prepared, and the probability of norovirus foodborne illness/person/day was estimated by Monte Carlo simulation in @RISK.
Results: Of 107 radish samples, norovirus was not detected and thus, the initial contamination level of norovirus was estimated to be – 6.7 log PFU/g, using Beta distribution (1,108). The developed predictive model showed that the norovirus titers decreased as temperature increased at 4-25°C. Market display temperature and time were fitted with Uniform distribution (22,24) and Uniform distribution (0,24), respectively. Temperature and time during

transportation were fitted with Pert distribution (10,18,25) and Uniform distribution (0.325, 1.643), respectively. Home storage time was fitted with Uniform distribution (0,336) and the temperature was fitted with LogLogistic distribution (-29.283,33.227,26.666,RiskTruncate(-5,20)), respectively. Average consumption amount was calculated by Lognormal distribution (61.845,87.271, Shift(-2.1124)) at 25% of frequency. ${}_1F_1$ hypergeometric model [Risk=1-(1+ηCV)$^{-r}$](η, 2.55×10^{-3}; CV, dose; r, 0.086) was used as the dose response model for norovirus. A simulation with these data showed that the probability of the foodborne illness by radish consumption was 4.73×10^{-13}/person/day.

Significance: The results indicate that the probability of norovirus foodborne illness by raw radish consumption is low in Korea.

P3-149 Evaluation of Microbial Quality and Safety of Sushi Sold in Ontario Using Combined Culture and Molecular Methods

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Introduction: Previous studies and outbreaks have implicated sushi as a risk for foodborne illness. Microbiological risks need to be assessed to support efforts in preventing outbreaks.

Purpose: To evaluate the microbiological quality and safety of sushi using a combination of culture and molecular methods.

Methods: Samples were purchased from 101 restaurants and 72 grocery stores from 40 cities/towns across Ontario over a 2-year period using convenience sampling, and tested for a variety of pathogenic or indicator organisms using official analytical methods. Overall bacterial composition was also analyzed based on the 16S rRNA gene using MiSeq. *L. monocytogenes* isolates were subtyped using multiple locus variable number of tandem repeats analysis (MLVA).

Results: Among the 520 samples tested using conventional methods, *L. monocytogenes* (<2 log CFU/g) was detected in 16 (3.1%) samples, *V. cholerae* in 6 (1.3%) samples, *S. aureus* in 4 (0.8%) samples, *B. cereus* in 14 (2.7%) samples, and *E. coli* in 7 (1.3%) samples. *L. monocytogenes*, *S. aureus* and *E. coli* were found predominantly in restaurant-sourced samples. Restaurant samples also showed 0.7 log CFU/g higher average total aerobic colony counts (ACC) compared to grocery store samples ($P < 0.0001$). The 16 *L. monocytogenes* isolates resulted in 7 MLVA genotypes with 8 isolates forming a predominant cluster (genotype). Four isolates of the main genotype were associated with samples taken within 3 days from 4 distinct restaurants in the same city, indicating a potential common origin. Unpaired MiSeq analysis of 100 samples revealed the presence of presumptive *Salmonella* and *Campylobacter* in 11 and 8 samples, respectively, with 2 samples containing both *Salmonella* and *Campylobacter*, which were undetected through the conventional targeted testing approach.

Significance: The surveillance data on microbiological quality of sushi sold in Ontario can be used to support efforts for mitigating risks associated with growing consumption of sushi.

P3-150 Quantitative Risk of *Staphylococcus aureus* Foodborne Illness by Home Meal Replacement (HMR) Foods

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❖ ***Developing Scientist Entrant***

Introduction: Home meal replacement (HMR) foods are processed food for immediate consumption, and thus, they might be susceptible to contamination with the foodborne pathogen such as *Staphylococcus aureus.*

Purpose: The objective of this study was to assess the risk of *S. aureus* foodborne illness in HMR foods.

Methods: HMR food samples collected from markets were analyzed to detect *S. aureus.* Temperature and time data for distribution and display were collected. Primary models were developed with the Baranyi model with *S. aureus* cell counts, and maximum specific growth rate (μ_{max}; log CFU/g/h) and lag phase duration (*LPD*; h) were calculated. The kinetic parameters were analyzed with a secondary model (Polynomial model). Consumption amount and frequency of HMR foods, and a dose-response model were also surveyed. With all collected data, a simulation model was prepared in @Risk, and the probability of foodborne illness for *S. aureus* by HMR food consumption was estimated by Monte Carlo simulation.

Results: One hundred forty-two samples were analyzed, and one sample was positive for of *S. aureus.* Thus, the initial contamination level was estimated to be -3.3 log CFU/g by the Beta distribution. The developed primary models showed that *S. aureus* cell counts increased as storage temperature increased. The secondary models for μ_{max} and *LPD* were appropriate with 0.973 and 0.979 of R^2, respectively. Weibull distribution for the consumption amount showed that average amount is 297.42 g at 0.8% of frequency. The Exponential dose-response model [risk=1-exp(-r×dose), ($r=7.64\times10^{-8}$)] was appropriate for *S. aureus* foodborne illness. Eventually, the simulation showed that the probability of *S. aureus* foodborne illness by consumption of HMR food was 1.0×10^{-9}/person/day.

Significance: This result should be useful in evaluating the risk of *S. aureus* foodborne illness by HMR food consumption.

P3-151 Risk Model of Hand-to-Food Norovirus Transmission in School Cafeterias: Evaluating the Impact of Share Tables and Intervention Strategies on Student Exposure and Dose Response

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❖ ***Developing Scientist Entrant***

Introduction: Share tables (ST) allow students to share unwanted food items with other students in school cafeterias, making them a viable alternative to reduce food waste and insecurity. The relative risk of hand-food norovirus cross-contamination from a shedding student due to the implementation of share tables is likely small, but food safety concerns from stakeholders may hinder their implementation.

Purpose: This study aims to evaluate the increased risk of norovirus transmission in school cafeterias due to the implementation of share tables and determine the effectiveness of intervention strategies, suggested by stakeholders, at moderating risks.

Methods: A QMRA was built to simulate one week of students passing through a school cafeteria service**.** Hand-food cross-contamination is simulated in 4 different modules: (1) selection of food from service line, (2) consumption of food, (3) placement of food in share table and (4) selection of food from share table. The model examines 12 difference scenarios, including washing of fruit, wrapping fruit items, increased handwashing, exclusion of ill students, among others A dose response model was adapted into the model to assess illness prevalence due exposure to contaminated fruit. A nominal range sensitivity analysis was conducted to assess the impact of inputs as well as to verify model accuracy.

Results: Through 200 one-week iterations the most effective intervention strategies were i) handwashing station (56% decrease), ii) wrapping fruit, and iii) implementation of wash bucket, 56, 48 and 15% reduction in illness prevalence, respectively. Other interventions decreased the illness prevalence slightly but not as significantly. The most impactful inputs were those related to initial contamination and cross contamination. Feces in hands (3.12), Norovirus in feces (2.93), and transfer probabilities (1.2-1.9).

Significance: When finished, stakeholders could modify this model for their own system. Giving them the ability to assess the risk associated to their system.

Poster

P3-152 Development of a Novel Dose-Response Modeling Approach to Incorporate Salmonella Enterica Heterogeneity based on Gene Expression Data

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❖ ***Developing Scientist Entrant***

Introduction: Estimating microbial dose-response is one of the most important aspects of a food safety risk assessment. Dose-response models provide us with the probability estimate of a specific response (such as infection) as a result of ingesting a specific dose of pathogens. In recent years, these models are increasingly being explored with gene expression data.

Purpose: The purpose of this study was to propose a model that considers the weights of expression of *Salmonella enterica* genes that could be associated with a host response.

Methods: Machine learning techniques such as Elastic Net regularization can help identify major genes/gene groups. A novel Elastic Net-based weighted Poisson regression approach was employed to identify Salmonella genes significant to a dose-response irrespective of the serovar. Complete *Salmonella* dose-response data for multiple serovars were obtained from prior human feeding trials and outbreaks. The best-fit Elastic Net model was obtained by 10-fold cross validation.

Results: The best-fit Elastic Net model (alpha = 0.300; lambda-penalty = 28.1061) identified 33 gene expression-dose interaction terms that significantly added to the predictability of the model. Of these, 9 genes associated with *Salmonella* metabolism and virulence were found to be significant by the best-fit Poisson regression model ($P < 0.05$). In general, we found that genes coding for bacterial metabolism had the greatest impact on the probability of illness given exposure to *Salmonella enterica.* The subsample analysis using human feeding trial data or outbreak data alone yielded insignificant results, with the best-fit model (alpha = 0.800; lambda-penalty = 27.9430) selecting zero significant covariates.

Significance: This method could redefine dose-response relationships for initial infection from relative proportions of significant genes from a microbial genetic dataset, which would help in refining the endpoint and risk estimations.

P3-153 Application of Advanced Data Analytics to Analyze Effects of Salmonella Gene Expression on Changes in Stress Response

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Introduction: In recent years, the widespread application of whole genome sequencing for foodborne pathogen surveillance and epidemiology has ushered in the era of "big-data" in food safety. The past few years have also seen a rise in the development of approaches to incorporate WGS data in predicting the severity and risk of disease, although this function is in its infancy.

Purpose: The purpose of this study is to analyze *Salmonella* gene expression under environmental, food processing-related, and infectivity-related stress, and develop models to predict the genetic patterns indicative of *Salmonella* stress response.

Methods: Proteomic and transcriptomic datasets describing the gene expression profiles under non-stress and various stress conditions were obtained from a thorough literature search. The effect of different stress factors on the gene expression profiles were modeled on Python v.3.9.1. The predictive performance, interpretability, and training time of a number of linear and non-linear machine learning classification techniques in predicting the expression patterns of *Salmonella* genes under various stress conditions were compared in order to identify the best performing model. The models tested included logistic regression, support vector machine, random forest, and gradient-boosted trees. Model evaluation was performed by analyzing the area under the receiving operating characteristic (ROC) curve.

Results: The literature survey identified six studies and datasets describing the differences in gene expression under various stress conditions and simulated host infectivity conditions representative of pathogenicity. The predictive models were successful in identifying phenotypically important *Salmonella* genes indicative of increased virulence and survival, including genes from the *Salmonella* pathogenicity islands, type-3 secretion system and *rpoS* genes.

Significance: The results of this study would help us identify recurrent co-existent genetic patterns indicative of *Salmonella* stress response, that would be useful in refining current predictive models describing *Salmonella* survivability through the food chain and risk and severity of infection in human hosts.

P2-154 Withdrawn

Poster

P3-155 Quantitative Transfer of *E. coli* (Non-Pathogenic) from Wheat into Milling Fractions and Equipment during Lab Scale Milling

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Introduction: Multiple recalls involving Shiga toxin-producing - *E. coli* (STEC) - contaminated wheat flour occurred in recent years. Cross-contamination is a major cause of food adulteration with pathogens leading to foodborne illnesses.

Purpose: Quantify *E. coli* cells transferred into mill fractions from contaminated wheat and milling equipment during lab-scale milling to develop a predictive model for larger scale operations.

Methods: Wheat grains were tempered to 16% moisture content for 24 h. Inoculated wheat batches (20 batches; 900 g/batch) was tempered using a 4-strain *E. coli* inoculum (~3 log CFU/g) while non-inoculated wheats (20 batches; 900 g/batch) were tempered using sterile water. Inoculated wheats were milled first followed by non-inoculated batches using a Chopin lab-scale roller mill. Mill surfaces ($n = 34$) were swabbed after each run and mill fractions were aseptically sampled. *E. coli* counts were enumerated by plating. *E. coli* counts of the flour fractions were plotted against wheat quantity milled and a best fit model describing the trends was selected.

Results: *E. coli* counts were higher ($P < 0.05$) in the break, and reduction rolls, feeders, sifter, and hopper surfaces (76 CFU/cm^2) of the mill. *E. coli* counts of the flour fractions increased (3.4-3.9 log CFU/g) with increasing amounts of inoculated wheat milled. The *E. coli* counts of surfaces (0 – 1 CFU/cm^2) and flour fractions (<0.5 log CFU/g) declined at the end of the non-inoculated wheat milling run. Flour fractions (break, sizing, reduction, and straight-grade) produced were positive for *E. coli* ($n = 20$). The models describing the *E. coli* counts of the flour fractions during the inoculated ($R^2 = 0.54$; se = 0.42) and non-inoculated ($R^2 = 0.82$; se = 0.23) runs had good fit.

Significance: Results from the study could provide useful information to mill personnel in improving control of *E. coli* contamination during milling.

P3-156 Development of Risk Assessment Model to Predict the Occurrence of Late Blowing Defect in Gouda Cheese and Evaluate the Intervention Strategies

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❖ ***Developing Scientist Entrant***

Introduction: Late blowing defect (LBD) is an important spoilage issue in semi-hard cheese with the outgrowth of *Clostridium tyrobutyricum* spores

during cheese ripening considered to be the primary cause. While previous studies have explored the microbial and physicochemical factors impacting the defect, a risk assessment tool is lacking for better managing the risk.

Purpose: This study aimed to develop a stochastic predictive model to estimate the occurrence of LBD in Gouda cheese and evaluate different prevention strategies.

Methods: The spore concentration distribution of butyric acid bacteria (BAB) in bulk tank milk was obtained from 8 dairy farms over 12 months. The level of *C. tyrobutyricum* from raw milk to the end of ripening was simulated based on Gouda brined for 2 days in saturated brine at 8°C and ripened at 13°C. Predicted *C. tyrobutyricum* concentrations during ripening and estimated threshold levels for LBD were used to predict occurrence of LBD.

Results: The model predicted that 9.2% (±1.7%) of Gouda cheese (with mean cheese pH of 5.39) produced with raw milk with a mean BAB spore count of 132 MPN/L showed LBD by day 60. For approximately 27% of cheeses, LBD was predicted to occur between days 60 and 90, which is consistent with observations from the literature and the cheese industry. Sensitivity analysis suggested the spore count in raw milk is the most important variable. The implementation of microfiltration or bactofugation of raw milk (98% efficiency of spore removal), delayed LBD occurrence beyond the first 60 days of ripening.

Significance: This model represents a tool for predicting the levels of *C. tyrobutyricum* in Gouda cheese and provides a framework for systematic assessment of strategies to mitigate the occurrence of LBD. The sensitivity analysis identified key knowledge gap in developing a more accurate model.

P3-157 Identifying the Genotypic and Phenotypic Intra Species Variability of *S. enterica* Isolated from Food and Human Cases in Central Region of Mexico

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Introduction: The variability in the behavior of pathogens strains in food and their virulence could have implications for food consumers' risk level.

Purpose: To evaluate the genotypic print (GP) and phenotypic print (PP) variability of *S. enterica* strains isolated from food (FI) and human cases (HI) in the central region of Mexico (CRM).

Methods: A total of 284 FI (chicken, tomato, mango) and 38 HI from CRM were characterized according to their GP (presence of 13 virulence genes), and PP (antimicrobial resistance to 14 antibiotics, growth rate (μ), and detection time (DT) at acidic conditions (pH4 and pH5), and biofilm formation capacity through the time (24 h, 48 h, 72 h). Multiple factor analysis (MFA) and hierarchical analysis (HA) were performed to group the isolates using GP and PP.

Results: Seven genetic profiles (virulotypes) were found, being virulotype 2 the most abundant in FI (68.7%) and HI (60.5%). Regarding PP, 54.9% and 26.3% of FI and HI were multidrug-resistant, respectively. At pH 4, most HI (81.6%) and one-third of FI did not grow. Among the isolates high variability on the ability to grow under acidic conditions [(μpH4 (0.003-0.038 OD600nm/h), μpH5 (0.016-0.066 OD600nm/h), DTpH4 (13.2-26.3 h), DTpH5 (6.1-17.3 h) and biofilm formation (0.109-2.08 OD595nm) were observed; in general, the HI had lower ability to grow at pH 4 and 5, and to form biofilms than FI. The antimicrobial resistance and the behavior at acid conditions were the characteristics that had a significant effect on the MFA. The isolates were grouped in six clusters (C1:13.4%, C2:20.5%, C3:21.4%, C4:16.1%, C5:19.6%, C6:9.0%); the majority of HI belong to C4 (57.9%), and FI to C3 (23.9%).

Significance: A huge intra-species variability of GP and PP of *S. enterica* was found; this evidence could refine the exposure assessment and hazard characterization.

P3-158 Modeling Growth of *Bacillus cereus* from Spores during Cooling of a Beef/Rice Combination Product

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Introduction: *Bacillus cereus* is frequently implicated in foodborne outbreaks associated with the consumption of temperature-abused, non-meat multi-component products such as rice that is added to beef. The main contributing factor leading to outbreaks is the fact that these products are cooked in large quantities and then, inadequately chilled or stored at room temperatures for an extended period prior to consumption.

Purpose: The objective of this study was to quantify *B. cereus* growth from spores in a cooked beef and rice product at several isothermal conditions (between 13 to 46°C).

Methods: Samples (5 g) were inoculated with a cocktail of four strains of heat-shocked (80°C/10 min) *B. cereus* spores to obtain approximately 2 log CFU/g levels and subsequently, stored at isothermal conditions. Three trials were performed. The number of viable *B. cereus* cells was determined at appropriate intervals by plating on mannitol egg yolk polymyxin agar and incubating for 24 h at 30°C. Gompertz equation was used to fit curves to the growth data; the Gompertz A, B, C and M parameters were then used to calculate lag-phase duration (LPD), exponential growth rate (EGR), generation time (GT), and maximum population-density (MPD) values.

Results: The data indicated that the growth kinetics of *B. cereus* were dependent on temperature, particularly in regard to exponential growth rates (EGR) and lag-phase durations (LPD). The EGR at 13 and 46°C were 0.06 and 0.80 (h^{-1}), respectively. While no lag was detected at 46°C, it was 48.22 h at 13°C.

Significance: The dynamic model developed using the modified Ratkowsky square-root model will assist processors to predict potential *B. cereus* behavior in cooked multicomponent products during chilling or stored at improper temperatures or with the disposition of product subject to cooling deviations as well as to determine compliance with regulatory performance standards.

P3-159 Predictive Modeling for Food Source Attribution of *Listeria monocytogenes* in Fresh Fruits and Vegetables

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Introduction: In the United States, the consumption of fresh fruits and vegetables has increased in recent years as consumers seek to make healthier lifestyle choices. However, the number of multistate outbreaks associated with fresh produce has increased concomitantly. *Listeria monocytogenes* have been associated with several foods recalls and listeriosis.

Purpose: This study was aimed at developing a model for the source attribution of listeriosis cases to different fresh fruits and vegetables using genomic data and machine learning approaches.

Methods: Eligible datasets of whole-genome sequences (WGS) of *L. monocytogenes* isolates and associated metadata within the U.S. were collected from the National Center for Biotechnology Information (NCBI) Pathogen Detection database. Different fresh fruits and vegetables considered in our analyses included apples, avocado, peaches, lettuce, potato salad, spinach, and cut raw vegetables. Source attribution was performed using a predictive machine learning approach in R (v.3.6.2).

Results: A random forest algorithm trained on the variations in the core genes of the food sources dataset predicted the occurrence of human listeriosis with an accuracy of 89%. The application of this model to the individual source datasets indicated a balanced accuracy of 0.95, 0.50, 1.00, 0.92, 0.85, 0.95, and 0.93 for apples, avocado, peaches, lettuce, potato salad, spinach, and cut raw vegetables, respectively. A number of genes significantly associated with *L. monocytogenes* adaptability, adhesion, colonization, and pathogenicity were identified.

Significance: *L. monocytogenes* isolates extracted from apples, peaches, and spinach were highly predicted to cause listeriosis. This indicates that WGS coupled with machine learning-based predictive modeling will greatly improve our capability to track *L. monocytogenes* and other foodborne pathogens from any food source. Thus, as we obtain more epidemiological information, it will be possible to strengthen the link between epidemiologic and WGS data using this approach.

P3-160 Hydroponic/Aquaponic Farming Food Safety Risk Identification by Food Safety Practice Survey and NGS Microbial Community Analysis

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❖ ***Developing Scientist Entrant***

Introduction: Hydroponic/aquaponic farming presents different food safety risks from traditional agriculture that are under-examined.
Purpose: This study investigated the microbiomes of hydroponic/aquaponic systems and linked the results to food safety practices.
Methods: A hydroponic farm and an aquaponic farm were visited for a food safety practice survey and sampling. A total of 3-6 produce types (25 grams each) were collected from each farm. The same or similar produce varieties were also obtained from grocery stores or lab hydroponic systems. The nutrition solutions (250 mL) were collected from 2-3 spots of each farm or lab hydroponic system cycle. A total of 20 fresh produce samples and 12 nutrition solution samples were plated for the total bacterial load. Environmental samples of hands, shoes, and tools were swabbed. All the fresh produce, nutrition solution, and swab samples were sequenced for the bacterial microbiome using the full-length 16s-ITS-23s on the PacBio system. The bacterial sequences were further analyzed with the amplicon sequence variant (ASV) method and compared to the Athena bacterial genome database and marked to the strain level.
Results: The hydroponic farm's microgreens showed similar bacterial loads (7.3-8.6 log CFU/g) and bacterial communities to the lab-grown microgreens. The farm's nutrition solutions had significantly higher bacterial loads but lower community diversity than those from the lab system. The lettuce samples from the aquaponic farm had bacterial loads (3.7-4.0 log CFU/g) similar to the lab-grown lettuce but significantly lower than the grocery store lettuce ($P < 0.01$), while the dominating bacteria varied. The potential risks at both farms are associated with hygiene practices.
Significance: This novel next-generation sequencing (NGS) approach of combining 16s-ITS-23s sequencing with ASV analysis to identify strain level bacteria is a cost-effective, high throughput method for pathogen risk identification. This study provided insight into potential food safety hazards in hydroponic/ aquaponic farming systems without targeting specific foodborne pathogens.

P3-161 Withdrawn

P3-162 Dynamics of *Listeria monocytogenes* Low Population in Fresh-Cut Papaya during Storage at Different Temperatures

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Introduction: Fresh-cut fruits have been involved in outbreaks caused by *Listeria monocytogenes* worldwide. The growth of low populations of this pathogen in fresh-cut papaya during storage remains unclear
Purpose: To assess the growth dynamics of *L. monocytogenes* cells in fresh-cut papaya during storage at different temperatures after inoculation with 1-4 cells
Methods: *L. monocytogenes* C1-387 was standardized at 10^3 CFU/mL and serially diluted in a 96-well microplate (two-fold dilution each new column) to achieve an inoculum of 1-4 cells, confirmed by plating. Fruits were disinfected (200 ppm sodium hypochlorite solution; 15 min, aseptically peeled, and cut into 3 cm^3 pieces. Samples were inoculated with 1-4 *L. monocytogenes* cells and distributed in polypropylene containers, and stored at 4, 8, 12 and 16°C for 1, 3, 5, 7 or 10 days. After inoculation and storage, samples were hand-homogenized for 30 s in a 0.85% (w/v) sodium chloride solution, and plated on supplement Listeria Selective Agar. Following incubation at 37°C for 24 h, cells were enumerated (detection limit: 0.6 log CFU/g). All analyses were performed three times in triplicate. Relative/cumulative distribution of *L. monocytogenes* levels in fresh-cut papaya was calculated for each evaluated temperature.
Results: In fresh-cut papaya stored at 4 and 8°C, *L. monocytogenes* population was around 2 log CFU/g after 10 and 7 days of storage, respectively. The same cumulative distribution was achieved at day 1 when the product was stored at 12°C, while it reached ~3.7 log CFU/g at day 10. When fresh-cut papaya was stored at 16°C, the *L. monocytogenes* population was ~3.6 log CFU/g and ~4.5 log CFU/g in day 3 and 10, respectively
Significance: The growth dynamics of low population *L. monocytogenes* in fresh-cut papaya are temperature dependent, where the population increases with increasing temperature. Findings indicate 4°C as the ideal temperature to control *L. monocytogenes* proliferation when fresh-cut papaya is stored for 10 days.

P3-163 Efficacy of Celery Powder As an Antimicrobial Against Listeria monocytogenes in Prepacked Deli-Style Turkey Breast Under Refrigeration and Temperature Abuse Conditions

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Introduction: *Listeria monocytogenes* can be a post-processing contaminant of ready-to-eat foods including deli meats. Deli meats are among the highest risk foods resulting in listeriosis as they do not undergo additional cooking steps prior to consumption.
Purpose: To evaluate the efficacy of commercial celery powder as an antimicrobial against *L. monocytogenes* in prepacked deli-style turkey breast stored under ideal and temperature abuse conditions.
Methods: Oven-roasted turkey breast samples (25 g) from two manufacturers containing celery as a nitrite source were surface inoculated with approximately 5 log CFU (4 x 10^3 CFU/g) *L. monocytogenes* 10403S. Inoculated samples were vacuum packed, stored at 4, 7, 10, and 15°C for 21 d. Both inoculated and uninoculated (negative controls) samples were enumerated on days 0 through 21 post-inoculation. A total of 25 mL PBS was added to a sample followed by stomaching at 260 rpm for one min. Serial dilutions were plated on MOX, incubated at 30°C for 48 h reported as log CFU/g of deli meat. Three independent replications were conducted for each experiment.
Results: To date, growth curves have been completed for 4, 7, and 15°C. The average recovery on d 0 was 4.6 log CFU/g. No significant growth observed after 14 d for samples stored at 4°C ($P > 0.05$) and ~4.3 log CFU/g recovered on 21 d. About ~1.1 log CFU increase observed when incubated at 7°C for 21 d ($P > 0.05$). There was an average 2.9-log increase at day 7 when incubated at 15°C. Overall ~6.7 and ~7.2 logs CFU/g were observed at day 14 and 21, respectively, representing an overall ~3 log-CFU/g increase at 15°C for 21 d. *(Data acquisition underway for 10°C.)*
Significance: This study is evaluating the use and effectiveness of antimicrobials used by deli meat processors against *L. monocytogenes* in deli products. These data underscore the importance of maintaining refrigeration temperatures to complement the efficacy of antimicrobials.

Poster

P3-164 Modelling the Inactivation and Determination of Fluence (UV-C Dose) Required for Incremental Inactivation of Several Strains of *Cronobacter* spp. Suspensions in Phosphate Buffered Saline

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Introduction: *Cronobacter* spp. are Gram-negative pathogens associated with infant infections as a result of consumption of contaminated reconstituted infant formula. Application of non-thermal technologies such as UV radiation to inactivate pathogens in foods requires an understanding of its UV sensitivities.

Purpose: Determine the ultraviolet-C (UV-C) sensitivity of *Cronobacter* spp. using a standard collimated beam UV-C device.

Methods: Thirteen strains of *Cronobacter* spp. which includes ten *C. sakazakii*, two *C. muytjensii*, one *C. dublenensis*, and a 5-strain cocktail of *C. sakazakii* were used in the study. Five mL of stationary phase bacterial suspension (~10^7 CFU/mL) in PBS with an A254 of <1 cm^{-1} were exposed to 2, 4, 6, 8 and 10 mJ/cm^2 of UV-C radiation using a standard collimated beam device housing a low pressure mercury lamp emitting UV-C radiation at 254 nm. Surviving *Cronobacter* spp. populations (CFU/mL) were determine by serial dilution and plating and transformed to log-reduction and plotted against UV dose. For inactivation kinetics modelling, three models (Log linear model, Weibull model and Double Weibull model) were applied using GinaFit tool and reported goodness of fit (R^2), 4D values (dose required for 4-log reduction of target microbe) were calculated.

Results: For all the *Cronobacter* spp. the best fitting models were non-linear Weibull and/or double Weibull models with R^2 0.95. The 4D values for individual microbial strains ranged from a minimum 6.5 mJ/cm^2 to a maximum 10 mJ/cm^2. *C. sakazakii* cocktail, had a 4D value of 9.4 mJ/cm^2 (R^2 = 0.9691) which was in the range of individual strains.

Significance: The calculated 4D values will be useful to estimate the target doses required for 5-log reduction of *C. sakazakii* in different beverages by UV-C irradiation.

P3-165 Improved Risk Assessment Model for Determining Robust Sanitation Programs and Other Preventive Controls

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Introduction: Risk-based preventive controls mandated by FSMA are also critical requirements of a facility's food safety plan. However, there is a justifiable basis for recommending an improved risk assessment model since the conventional CODEX-based risk model may be insufficient for determining robust sanitation and other preventive controls.

Purpose: To propose a useful improved model for evaluating risks posed by some *"reasonably foreseeable hazards"* and for justifying controls that are compliant with food safety and sanitation regulations and standards.

Methods: The improved risk assessment model was developed using the following:

- The CODEX-based Risk model was acknowledged as the starting point, i.e., *Likelihood* of hazard occurring *x Severity* of hazard consequence to public health = *Risk Class*;
- *Detectability* element borrowed from the industry standards was incorporated into the model, i.e., *Risk Class / Detectability* of hazard before it causes harm to public = *Risk Priority;*
- *Controllability* element was also included, i.e., *Risk Priority / Controllability* of hazard or the ease with which the hazard can be managed = *Risk Level* = improved risk model.

The improved model was used to evaluate the key food safety hazards encountered within six generic steps of an RTE Sandwich preparation process, and derived risk outcomes were compared with CODEX-based risk outcomes.

Results: Comparative analysis revealed that, by adopting the improved model, at least three sanitation-related risk outcomes may be further mitigated by integrating environmental monitoring and robust pre-requisite programs. Hence, by incorporating detectability and controllability elements into the upgraded model, a 50% improvement in accountability of risk-based controls was noticed.

Significance: The improved risk model should aid in better determining sanitation and other preventive controls for CODEX or FDA food safety plans. Understanding the use and benefits of detection systems, and the development of robust control programs, should be realized through application of this model.

P3-166 Assessing the Growth of *Listeria monocytogenes* in Salmon with or without the Competition of Background Microflora – a One-Step Kinetic Analysis

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Introduction: With the presence of natural microbiota, competition may occur between *L. monocytogenes* and the natural microbiota. The predictive models developed for describing the growth of *L. monocytogenes* in sterile foods may be not accurate for the shelf-life prediction and risk assessment in real world.

Purpose: This study aimed at developing mathematical models to predict the growth behavior of *L. monocytogenes* with or without the presence of natural microbiota in salmon.

Methods: At temperatures between 4 and 35°C, the growth data of *L. monocytogenes* in sterile salmon were collected to develop the non-competitive growth model, while the growth data of both *L. monocytogenes* and background microflora in raw salmon were analyzed simultaneously to develop the competitive model. Validations for the developed models were further performed under isothermal conditions.

Results: In raw salmon, background microflora exhibited an inhibiting effect on the growth of *L. monocytogenes*. The lag time of *L. monocytogenes* in raw salmon was extended by 2.849 times and its specific growth rate was decreased by 78.7% due to the existence of the native microbiota. The relatively low value of RMSE (0.3 log CFU/g) was obtained, demonstrating that the non-competitive and competitive models were accurate to describe the growth of *L. monocytogenes* in sterile salmon and the interaction between *L. monocytogenes* and the native microflora in raw salmon, respectively. The validation results indicated that the non-competitive and competitive models were accurate. Overall, the residual errors of predictions of these two models both followed a Laplace distribution. The RMSE values of the models were both 0.4 log CFU/g and more than 79% of prediction errors were within normal experimental errors (± 0.5 log CFU/g).

Significance: The results can be used for the risk assessments of *L. monocytogenes* and microbiological shelf-life prediction of salmon in real world.

P3-167 Kill Step Management Combining Real-Time Data Collection and Cloud-based Analytical Services

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Introduction: Process validation is a well-established approach to estimate the lethality of industrial processes against pathogenic microorganisms. To go further into process control, new methodology can be evaluated to get real time process lethality evaluation. Combining literature knowledge, laboratory work and industrial scale data, such methodologies can help industrials keeping their product safe in real-time while improving their production

practices.

Purpose: Objective of the study was to use a combination of lab and industrial scale data to predict the efficiency of an industrial sterilization system on *Salmonella* in a cocoa product.

Methods: Lab thermal death time (TDT) study was conducted on the cocoa product at three temperatures (90-100-110°C). For each temperature, five exposure times and two assays per condition were performed. Parallel, Novolyze platform recorded heat penetration data onto the industrial system. Industrial data combined with *D*- and *z*-values allowed to evaluate microbial lethality of the sterilization step on *Salmonella* using linear model described by the Alliance for Innovation & operational Excellence. Log reduction achieved by the industrial system was evaluated for 87 batches using this method.

Results: Lab study allowed to calculate D_{90}(14 ± 4 min), D_{100}(8 ± 1 min), D_{110}(8 ± 1min) and z(81 ± 44°C) values. Isothermal evaluation showed that a minimum 35 min at 106°C was necessary to achieve a minimum 5.2-log reduction. Dynamic evaluation (i.e., considering whole process, not only CCP) showed that a total time of 55 min above 90°C allowed to achieve a minimum 5.2-log reduction.

Significance: The method allows to get real-time monitoring of the process by combination of lab-scale experimental data and industrial-scale real condition data. Such application brings industrial processors flexibility, allowing them to adjust treatment parameters in real time to maintain product safety while rising their productivity rate and reducing their production costs and environmental impact.

P3-168 Application of a Monte Carlo Simulation Model to Evaluate the Effectiveness of Different Interventions in Reducing the Spoilage of Pasteurized Fluid Milk Due to Post-Pasteurization Contamination

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Introduction: Monte Carlo (MC) simulations can be used to evaluate the factors that can affect the final shelf life of fluid milk and help industry with improved decision making on selection and implementation of strategies that reduce fluid milk contamination with Gram-negative bacteria introduced as post-pasteurization contamination (PPC). We thus used a MC simulation model to run "what-if scenarios" to assess different interventions processing plants can implement to reduce PPC.

Purpose: To quantitatively describe the impact of different interventions on the reduction of fluid milk spoilage due to PPC by using a Monte Carlo simulation model and "what-if scenarios."

Methods: A MC simulation model was developed to predict spoilage of pasteurized fluid milk packaged in ½ gallon containers due to PPC. The MC simulation was used to run "what-if" scenarios to predict the impact different interventions on fluid milk shelf life. The four interventions tested were: (i) reducing frequency of contaminated samples, (ii) reducing the initial contamination concentration, (iii) implementing "seek and destroy" to eliminating specific spoilage organism niches, and (iv) improved storage temperature control.

Results: "What-if" analyses indicated that interventions that reduce the frequency of PPC would have a large effect on reducing fluid milk spoilage due to PPC. "What-if" simulations that decreased the frequency of PPC contamination from 100% to 10% predicted that only 3.33% of half-gallons of milk would be spoiled by day 7 and 5.31% of half-gallons by day 10 (>20,000 CFU/mL), compared to the baseline of 33.94% and 53.71% on day 7 and day 10, respectively. This translates to an extension of shelf life by more than 7 days.

Significance: This study provides a road map of how "what-if" analyses can be used to help industry make decisions on selecting the most appropriate strategies for extending product shelf life and reducing food waste.

P3-169 Meta-Analysis of Almond Pasteurization Validations

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❖ ***Developing Scientist Entrant***

Introduction: Since 2007, the Almond Board of California (ABC) has managed a mandatory program requiring a minimum 4-log reduction of *Salmonella* for California almonds sold in North America. The resulting collection of 300+ validation reports may be the largest collection of data on industry practices for preventive control validations.

Purpose: The objective of this study was to analyze the meta-data collected from validation reports to identify the efficacy of ABC validation requirements, industry practices, and different inactivation technologies on *Salmonella*/surrogate reductions and process reproducibility. This information can be used to improve almond process validation methodologies.

Methods: Reports were provided by ABC and systematically reviewed to extract categorical information (e.g., process type, year of report, validation approach), quantitative information (e.g., surrogate survivor counts, process duration), and qualitative information (e.g., issues with extracting relevant information). Where possible, *Salmonella*/surrogate survivor data were statistically analyzed to estimate biological variability and likelihood of achieving >95% prediction interval for >4-log reductions. No company-identifying or proprietary information was collected or analyzed.

Results: Reports were organized according to processing technology, including blanching (40), dry roast (62), oil roast (116), propylene oxide (30), and steam/moist heat/other (75). Based on preliminary dry roasting results (10 studies), most studies successfully achieved >4-log reductions of *Salmonella*/surrogate. Only surrogate studies with the most limited data collection (3 replications with 3 samples) failed to achieve the 95% prediction interval for >4-log reductions. All results from approved validation studies were nominally >4-log reductions; however, data limitations in some studies constrained the ability to draw statistical conclusions about 95% confidence prediction intervals being above 4-log reductions.

Significance: This study is yielding recommendations to improve validation requirements and methodologies, further improving almond safety and industry practices. The quantification of reproducibility of these industry-scale treatments may be used to inform the expected levels of reproducibility in similar industries.

P3-170 Mechanistic Dose-Response Model for *Campylobacter jejuni* Infection Probability

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❖ ***Developing Scientist Entrant***

Introduction: Although understanding the dose-response relationship between ingested pathogenic bacteria and infection probability is a key factor for appropriate risk assessment of foodborne pathogens, the current approaches for dose-response modeling of low-dose exposures of pathogens rely on assumptions and extrapolations. The Key Events Dose-Response Framework has been proposed as an alternative approach for evaluating low-dose exposures that is a mechanistic approach to predict infection/illness probability from pathogen exposures.

Purpose: The objective was to estimate the cell invasion probability of *Campylobacter jejuni* assumed as an infection probability with mechanistic approach considering food type and host age based on reported human body's digestion behaviors, and to compare the prediction with a reported dose-response relationship.

Methods: The key events for infection mechanism were defined in this study as follows: (i) pathogen reduction in the stomach; (ii) transfer to the small intestine from the stomach; (iii) pathogen invasion into small intestinal epithelial cells. Mathematical models for the key events were developed with the Bayesian estimation for describing the digestion processes and the pathogen behaviors. Combining all the key events models, the dose-response relationship of the cell invasion probability assumed as infection probability.

Results: The infection probabilities of young and elderly individuals consuming liquid food, as well as elderly individuals consuming solid food were estimated similar to the dose-response relationships with bovine milk in literature. The 95% prediction bands of the developed model covered the reported

dose-response relationship. However, there was 1.2 log CFU difference in the prediction dose in the case of young individuals consuming solid food, which resulted in discrepancy with the reported dose-response relationship.

Significance: Considering food types and individual age, the developed mechanistic dose-response model successfully described the actual dose-response relationship for *C. jejuni*'s infection probability. The KEDRF has potential as an alternative approach for dose-response modeling of foodborne pathogens.

P3-171 Growth of Shiga Toxin-producing *E. coli* (STEC) and Generic *E. coli* in Ground Pork at 10°C, 25°C, and 40°C

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❖ ***Developing Scientist Entrant***

Introduction: Pork products are a potential source of Shiga toxin-producing *Escherichia coli* (STEC). Generic *E. coli* could simulate the growth behavior of STEC under the various product and process conditions and be used as a potential surrogate for STEC.

Purpose: The purpose of this study was to compare the growth of STEC and generic *E. coli* in raw pork products at processing (10°C), room (25°C), and sublethal (40°C) temperature.

Methods: *E. coli* ATCC25922 and STEC were inoculated in fresh ground pork (80% lean, 20% fat) to a final concentration of 2-3 log CFU/g. A 5-gram inoculated pouches (two replications) of each treatment temperature (40°C, 25°C and, 10°C) were submerged in water baths for 25 to 300 hours. Cells were recovered on MacConkey agar. Growth curves were plotted for each temperature profile at different times. Lag phase duration (LPD), Specific growth rate (SGR), and maximum population density (MPD) were obtained from each growth curve using ComBase.

Results: At room and sublethal temperatures, no lag phase was observed for any *E. coli* suggesting a significant portion of the product could experience growth during potential temperature abuse at home and foodservice. Generic E. coli grow faster at 25, and 40°C than STEC in the pork sample (SGR: 0.30 ± 0.027 vs. 0.165 ± 0.013 ln/h; 0.47 ± 0.030 vs. 0.38 ± 0.02 ln/h). Longer LPD and lesser SGR were observed at abusing storage temperature (10°C) for STEC than that of generic E. coli (86.0 ± 9.45 h vs. 193 ± 18.26 h; SGR: 0.01 ± 0.003 vs. 0.05 ± 0.009 ln/h). These results indicate that *E. coli* ATCC25922 may be potentially used as a surrogate for STEC's growth in pork products.

Significance: These resources will provide pork industries with better strategies to improve raw pork products' safety and mitigate the risk of STEC transmission through this commodity.

P3-172 Evaluation of Invisishield™ Technology to Reduce Human Norovirus and Hepatitis A Virus on Tomatoes Using the Antimicrobial Chlorine Dioxide

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Introduction: Chlorine dioxide (ClO_2) is a promising antimicrobial with various food applications, one of those being antimicrobial packaging systems. The ability of this technology to inactivate foodborne viruses has not been studied.

Purpose: To evaluate a novel ClO_2 – based antimicrobial packaging system (InvisiShield™) for its efficacy against human norovirus (HNV) and hepatitis A virus (HAV).

Methods: Grape tomatoes or blueberries were placed in polypropylene trays and selectively inoculated with 5.6 log HNV Genome Equivalent Copies (GEC; 20% stool suspension) or 6.2 log HAV GEC (cell culture lysate). Trays were heat sealed with a three-phase polymer film consisting of a base, channeling agent, and the ClO_2 active (treatment); or control (no active) film and stored at 7°C for 0, 24, 48 h, and 7 days. At each timepoint, the product was collected and processed for virus concentration using the sequential steps of elution, polyethylene glycol precipitation, and RNA extraction. Viruses in extracts were quantified using RNase-RT-qPCR.

Results: Log reductions (LR) in HNV GEC for tomatoes were 2.2 ± 1.3, 2.9 ± 0.7, and 3.6 ± 0.3, after 24, 48 hours and 7 days, respectively. HAV GEC LR for tomatoes were 0.4 ± 0.2, 1.0 ± 0.1, and 2.1 ± 0.7, after 24, 48 hours and 7 days, respectively. LR in HNV GEC for blueberries were 1.4 ± 0.7, 1.7 ± 0.5, and 2.7 ± 0.2, after 24, 48 hours and 7 days, respectively. HAV GEC LR for blueberries were 0.1 ± 0.2, 1.2 ± 0.4, and 3.2 ± 0.2, after 24, 48 hours and 7 days, respectively. Position of the fruit in the tray did not affect inactivation ($P > 0.05$).

Significance: This novel ClO_2-based antimicrobial packaging system effectively reduced concentrations of HNV and HAV on grape tomatoes and blueberries after even one day, with efficacy improving over 7 days refrigerated storage. This technology shows promise in reducing foodborne viruses on fresh produce items.

P3-173 Development of TiO2-Containing Antimicrobial Packaging Trigged by Pulsed Light to Reduce Microbial Contaminants

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Introduction: Food packaging provides physical protection for foods. However, food packaging materials usually don't possess antimicrobial properties and even worse they may be contaminated with spoilage and pathogenic microorganisms, which could cause cross contamination, short shelf life and health risk.

Purpose: The objective of this study was to develop a packaging material containing nano scale TiO_2 and the antimicrobial activities could be activated by pulsed ultraviolet light (PL) and could be maintained after food is packaged.

Methods: Polylactic acid (PLA) powder was mixed with TiO_2 powder in methylene chloride. The mixture was homogenized to evenly distribute TiO_2 in the PLA polymer. The ratios of TiO_2/PLA were 0.5, 0.2, 0.1, 0.01, 0.005 g/g. Ten mL of each TiO_2/PLA solution was evenly distributed to a Teflon Petri dish followed by air-drying at room temperature to obtain antimicrobial films. A laboratory-scale pulsed light (PL) treatment system (1.04 J/cm^2 per second) was used to activate the film samples. The antimicrobial activities against *E. coli* and *L. innocua* inoculated on the film surface were evaluated following inoculation-PL treatment and PL treatment-inoculation.

Results: The antimicrobial activity of the film was activated by normal fluorescent light and enhanced by pulsed UV light. The populations of *L. innocua* and *E. coli* on PLA-TiO_2 antimicrobial films were reduced by 96% and 98.5% under normal light conditions, respectively, while pulsed light treatment for 5 seconds significantly ($P > 0.05$) increased the antimaterial effect and achieved over 99.9% reduction of the bacteria. Light-activated PLA-TiO_2 antimicrobial films maintained antibacterial activity and had lower bacterial populations on the surface than that without light-activated films.

Significance: The results demonstrate that the developed antimicrobial packaging material can be used to fabricate food containers or packaging films with pathogen-free surface. Further studies will be conducted with real food samples.

P3-174 Insights into Hand Hygiene Practices of Food Handlers in Convenience Stores – A Video Camera-based Observation Study

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Introduction: Proper hand hygiene in food handling settings is critical to food safety. Observational studies investigating hand hygiene practices in convenience stores are scarce compared to other food handling settings.
Purpose: This study used video observations in convenience stores to evaluate food handlers' hand hygiene compliance, frequency, and soiling of hands.
Methods: Three convenience stores from the same chain were chosen for sampling. Menu items were primarily ready to eat sandwiches and salads. 4K resolution action cameras were installed above a single handwashing sink in each location and a second camera overlooking the food prep area was installed at two locations. Cameras recorded a single shift of 4K video at 30 frames per second, resulting in an average of 5.9 h of footage per camera. Two trained evaluators independently analyzed footage for (a) evidence of visible soil on hands prior to a handwash attempt; (b) handwashing compliance (according to FDA Food Code); and (c) frequency of handwashes.
Results: A Cohen's-kappa of 0.84 was achieved by evaluators reviewing footage. A total of 126 attempted handwashing events were captured from a total of 29.6 h of recorded video, with 26% (n = 32) of attempts correct per FDA Food Code. The main cause of handwashing compliance failure was not washing hands for at least 10 seconds. Average length of handwashing attempts varied greatly amongst stores, ranging from 22.47 to 40.15 seconds. No evidence of visible soil on hands was observed amongst any of the 126 handwash attempts. Data analysis to characterize handwashing triggers and hand soiling is currently underway for footage obtained of food prep area.
Significance: This study highlights the application video observation to capture insights into hand hygiene behaviors amongst food handlers in larger food-handling settings. Insights generated from this study can be used as benchmarks for future hand hygiene intervention studies.

P3-175 Automated Hand Hygiene Monitoring Systems Reveal Insights into Behaviors of Food Handlers in Two Restaurant Types

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Introduction: Hand sanitizers can be used by restaurant food handlers only after performing a handwash per the FDA Food Code. Product usage characteristics by food employees when hand sanitizers are made available are not well understood.
Purpose: This study was performed to learn about hand hygiene product usage in two restaurant types.
Methods: Remote monitoring soap and sanitizer dispensers, placed side-by-side at handwash sinks used by food handlers, were installed in four casual dining (Chain A; 697 days) and three quick service restaurants (Chain B; 217 days). Data curation resulted in 253,835 total events captured (209,848 soap, 31,815 sanitizer, and 12,172 regimen [defined as soap followed by sanitize at the same sink within 60 seconds]). Dispenses were aggregated by year, and statistical analysis was performed on event type by hour with a Poisson generalized linear mixed model (GLMM) with random effects for Store and Year, and fixed effects for Event Type and Restaurant Type.
Results: Soap (2.98 per hour), sanitizer (1.58 per hour), and regimen use (1.38 per hour) were statistically different within restaurant types ($P \leq 0.004$), indicating a preference for soap use. When event types were pooled, casual dining restaurants had higher event rates (2.84 per hour) than quick service (2.07 per hour) ($P \leq 0.004$). Time of day impacted product use, with 12:00 to 19:00 having highest usage. No seasonal effect on product use was identified. Analysis of data by sink location (e.g., raw vs. ready to eat preparation area) is ongoing.
Significance: Soap was the most frequently used hand hygiene method by food employees in both restaurant types, even when hand sanitizers are available. Regimen use, despite being the preferred hand hygiene method by both chains, was lower than expected. This data provides robust baseline benchmarks for future hand hygiene intervention studies in these settings.

P3-176 Street Foods in Southwest Nigeria: FOOD Safety, Culture, Health, and Governance

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Introduction: Prepared meals for sale in streets, stalls, open spaces, or market squares are ubiquitous around southwest Nigeria and throughout the world as well. Street foods are immersed in cultural, diversity and economic significance, health implications, and diverse regulatory or governing conditions both in developing and developed countries.
Purpose: Key issues to be observed in this presentation is to understand and identify food safety conditions, Cultural perceptions of street food, the economic impact of street foods, as well as health and regulatory parameters of street food in southwest Nigeria. This presentation exposes how street food business based on the combination of tradition and innovation can offer a competitive advantage and strengthen a strong connection to southwest Nigeria using variables of food safety, culture, economy, health, and governance.
Methods: Utilizing a quantitative research methodology approach, this presentation surveyed 120 vendors and consumers of street food in the 6 South West Nigeria states; Ekiti, Lagos, Ogun, Ondo, Osun, and Oyo. The survey was carried out to determine the characteristics of street food activities based on the elements of the research topics (food safety, culture, economy, health, and governance).
Results: The results provided from this study guide decisions on street food policies, provide educational indicators to improve the level of street food operations, as well as to provide a level of comparisons of street food activities in south west Nigeria against global operations.
Significance: Based on the responses made by responders to this research survey, the relationship between food safety, culture, economy, health, and governance and street vended foods in Nigeria was comprehended as a tool to promote and enhance the identity of street vended foods in south west Nigeria.

P3-177 Microbiological Survey of Cantaloupe Contact Surfaces in the Retail Environment

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Introduction: Cantaloupes have commonly been implicated in outbreaks of foodborne pathogens. Extensive research has been conducted to investigate the prevalence of *Listeria* in fresh produce production settings, but little has been done in the retail environment.
Purpose: The goal of this study was to determine the microbial quality and presence of *Listeria* spp. on whole and fresh cut cantaloupe contact surfaces in retail environments.
Methods: A total of 333 swab samples distributed across 13 sites were taken from 10 different stores of two retail partners. Swabbing sites were determined by the flow of whole and fresh cut cantaloupes in retail environments. Surfaces were categorized into four groups: base, perimeter, transport and fresh cut. A total of 13 sites were sampled (4 base, 3 perimeter, 2 transport, and 4 fresh cut). Samples were processed for the presence of *Listeria* and enumerated for aerobic plate count (APC), coliforms and *E. coli*.
Results: The average APC and coliform counts varied between surface types and ranged from 3.2 to 6.9 and <1.7 to 5.0 log CFU/cm^2, respectively. All samples collected were below the limit of detection (<1.7 log CFU/cm^2) for generic *E. coli*. For whole cantaloupe surfaces, base surfaces had significantly ($P \leq 0.05$) higher APC, coliform counts and *Listeria* prevalence. Foam surfaces had significantly ($P \leq 0.05$) higher APC and *Listeria* prevalence compared to the other whole cantaloupe contact surfaces, with all foam samples collected being positive for *Listeria* (19/19). For fresh cut cantaloupe contact surfaces,

drying racks had significantly ($P \leq 0.05$) higher APC, coliform counts and *Listeria* prevalence compared to the other fresh cut surfaces.

Significance: The results of this study suggest that the type of cantaloupe contact surfaces should be considered in retail settings in order to avoid the harborage of *Listeria* spp. Along with surface type, sanitation practices may also influence the microbial quality and prevalence of *Listeria*.

P3-178 Employees' Knowledge Associated with Food Allergy Management for Independent Ethnic Restaurants

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Introduction: More than 30% of all identified food allergic reactions were attributable to foods from commercial foodservice operations. Especially, ethnic restaurants were considered as high-risk dining places because of potential risks related to cross-contacts and hidden allergens in sauces.

Purpose: The purpose of this study is to examine factors affecting employees' knowledge associated with food allergy management for the ethnic restaurant.

Methods: A total of 275 respondents who have experience working at least for 6 months at the ethnic restaurant participated in the survey through M-Turk. Descriptive statistics, independent *t*-test and One-way ANOVA were used for data analysis to investigate whether or not there is statistically significant differences in mean depending on individual and organizational factors.

Results: The results revealed that employees' food allergy knowledge varies based on several factors such as job position (server, cook, and manager) ($P < 0.001$), food allergic reaction experience ($P < 0.001$), food safety certification ($P < 0.01$), and operation type (offered allergen-free menu items) ($P < 0.001$).

Significance: These findings will be valuable when developing food allergy training program to ensure that proper food handling practices for customers with food allergies.

P3-179 Cross-Contamination of Kitchen Surfaces, Utensils, and Hands of Volunteers Following Meal Preparation Lacking a Hand Hygiene Intervention

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Introduction: In the FDA Food Code, hand antiseptics cannot be used in lieu of soap and water for hand hygiene. Scientific evidence has demonstrated equivalency of some hand antiseptics to soap and water for bacterial removal from hands, but the majority of these studies did not consider food soils, nor did they establish a baseline for bacterial contamination on hands. Without a baseline, it is difficult to determine the efficacy of hand hygiene interventions.

Purpose: To characterize the baseline bacterial load on hands of volunteers and high touch kitchen surfaces after meal preparation of artificially inoculated product(s) in a research kitchen.

Methods: Chicken tenderloins and ground beef were inoculated with a GFP tagged *Escherichia coli* DH5α strain at 7-10 log CFU/meat item. Twenty volunteers with food handling experience were recruited and were instructed to follow recipes to prepare 20 beef tacos, followed by 10 chicken salads. Volunteers were told to physically handle raw meat, and not to wash their hands, wear gloves, or use ABHS during meal preparation. The first five surfaces touched after handling of the inoculated meat were swabbed, and whole hand sampling via the "glove juice" method was performed after meal preparation. The concentration of DH5α was determined for surfaces and hands by plating serial dilutions on TSB containing kanamycin.

Results: Following meal preparation, hand rinsates averaged 4.0 ± 1.3 log CFU per sample ($n = 20$, 100% positive). The average DH5α concentration on contaminated environmental surfaces was 1.8 ± 1.5 log CFU per item, with 18 unique surface types showing evidence of contamination ($n = 200$, 75% positive).

Significance: These data provide a baseline upon which to compare the efficacy of individual and combined hand hygiene interventions in food handling settings in future studies.

P3-180 Masks as a Potential Source of Cross-Contamination during Food Preparation

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Introduction: During the COVID-19 pandemic, mask-wearing has become a common practice in the food service industry. However, this practice could potentially result in cross-contamination if masks are contaminated by adjusting without changing between tasks.

Purpose: The objective of this study was to determine if masks served as vehicles for cross-contamination between tasks of handling contaminated chicken and chopping lettuce.

Methods: Chicken breasts were inoculated with a nonpathogenic surrogate panel of *E. coli* (ATCC MP-26). After inoculation and attachment, chicken breasts were sliced for 1, 5, or 10 min while touching a 90-cm^2 area on duplicate, single-use surgical masks on mannequins every 1 min (simulating mask adjustment). One mask was enumerated on *E. coli*/Coliform Petrifilm™ immediately, and the second mask was used as a contamination source (touching each minute) while chopping 250 g of lettuce for 5 min. The second mask and 10 g of lettuce were enumerated and log CFU/cm^2 or g of *E. coli* were calculated. Transfer of *E. coli* from contaminated chicken to mask, and from contaminated mask to lettuce were compared based on touching frequency and inoculation level. Experiments were conducted with high and low inoculations (ca. 6 and 4 log CFU/mL, respectively) and three replications were performed for each ($n = 18$).

Results: Masks touched while slicing both the high- and low-inoculated chicken showed significant contamination for the 1-min (3.2 ± 0.8 and 1.4 ± 0.3 log CFU/cm^2), 5-min (4.8 ± 0.2 and 2.6 ± 0.2 log CFU/cm^2), and 10-min (4.6 ± 0.5 and 2.8 ± 0.4 log CFU/cm^2) scenarios ($P < 0.05$). Inoculation level had no significant effect on mask contamination ($P > 0.05$). Lettuce was contaminated with 2.0 ± 1.0 log CFU/g *E. coli* from the masks. Neither touching time nor inoculation level had significant effects ($P > 0.05$).

Significance: Data indicate contamination occurred from chicken to lettuce via contaminated masks, suggesting masks can serve as vehicles for cross-contamination during food preparation.

P3-181 An Empirically Derived Measure of Food Safety Culture Amongst Restaurant Food Workers

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Introduction: Considered an emerging risk factor for foodborne illness, food safety culture (FSC) remains a nascent concept in the restaurant industry. Given the potential value of understanding the FSC of restaurants to food safety, it is important to be able to assess FSC.

Purpose: This study aimed to validate the components associated with FSC and identify a tool that could be used to asses workers' beliefs of FSC within their restaurant.

Methods: We surveyed restaurant food workers for their level of agreement with twenty-eight statements about food safety within their restaurant using a Likert-type scale (1 – Strongly Disagree to 5 – Strongly Agree). We then used factor analysis and structural equation modeling to identify the associated components and their interrelationship.

Results: We received 578 surveys from 331 restaurants in eight heath department jurisdictions across the United States. From these surveys, we iden-

tified four primary components of FSC: Leadership (mean – 4.28, SD – 0.69); Employee Commitment (mean – 4.49, SD – 0.62); Resources (mean – 4.69, SD – 0.57); and Management Commitment (mean – 3.94, SD – 1.05). We identified one higher order component, Workers' Beliefs of FSC (mean – 4.35, SD - 0.53). For these measures, a higher score is associated with stronger agreement. Scores were highest for the employee commitment measure; food workers agreed that food workers in their restaurant engaged in actions that ensured food safety. Management commitment had the lowest overall score; food workers were less likely to agree that their management prioritized food safety in their restaurant.

Significance: This study provides an assessment tool that can be used within the restaurant industry to assess FSC and changes in FSC over time. Our data indicate food workers have a positive view of FSC within their restaurants.

P3-182 Menu Selection on Food Safety Among Young Adult Consumers: Situational Factors and Beyond

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Introduction: Consumers rely on many cues when forming risk perceptions and in making decisions. Previous studies reported that situational factors affect consumers' sensory perception when selecting menu items in restaurants. However, there is a lack of knowledge on how situational factors affect consumers' perception of food safety and menu selection of high-risk food items.

Purpose: Evaluate the effect of situational factors on consumers' menu selection of high-risk food items in restaurants.

Methods: University students of food-related majors (n = 470) from three Northern California locations completed a web-based survey assessing menu selection. Using an evocation protocol, participants were shown pictures of dining areas (upscale and low-profile ambiances), descriptions of restroom cleanliness (clean or unclean), followed by multiple-choice questions on ordering food items. Survey responses were analyzed using logistic regression and ANOVA.

Results: The restaurant's ambiance, an individual's risk perception, prior food safety training and gender affect their menu selections. Decisions in the upscale restaurant were significantly more likely to be high-risk than in the low-profile restaurant when ordering a Caesar salad and beef burger (Odds Ratio = 1.61; 95% CI: [1.16, 2.23] and Odds ratio= 1.46; 95% CI: [1.09, 1.94], respectively). Participants were significantly more likely to think the high-risk salad was safe in the upscale restaurant (P < 0.05). Males were significantly more likely to make riskier decisions than females, regardless of restaurant ambiance or restroom cleanliness. Having food safety training improved food safety knowledge but was not a strong determinant of reducing self-reported risky menu selection. Amongst six possible factors, 'taste' was scored the most important when ordering food, followed by 'food safety.'

Significance: The findings provide insights on situational factors influencing self-reported menu selections of high-risk food items in restaurant settings. Consumer communication programs should be developed to increase public awareness of safe food choice, especially among populations with increased risk of foodborne illness.

P3-183 Isolation and Characterization of the Foodborne Pathogen *Vibrio parahaemolyticus* Variants Protected from Laboratory and Cooking Protocols from Retail Seafood Sources

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✦ ***Undergraduate Student Award Entrant***

Introduction: *Vibrio parahaemolyticus* is transmitted through the ingestion of raw, contaminated shellfish as well as ready-to-eat (RTE) foods. The viable but non-culturable (VBNC) state of this foodborne pathogen can endow a false-negative validation by the standard examination protocol, causing a high risk to food safety. Our recently recovered strains from environmental samples by heat-dependent enrichment method could more likely contribute to foodborne infections.

Purpose: We aimed to investigate the prevalence and characteristic of raw, retail-food-associated *V. parahaemolyticus* isolated by using the modified enrichment method.

Methods: Thawed raw seafood samples from various retail sources were enriched using the modified enrichment method and cultured on thiosulfate-citrate-bile salts-sucrose (TCBS) agar for selecting the *V. parahaemolyticus* species. Pure isolates of presumptive *V. parahaemolyticus* recovered by agar streak isolation were confirmed by 16S rDNA gene sequencing with ABI 3730XL sequencer. Subsequent virulence analyses investigated the availability of the Kanagawa phenomenon, urease, hemolysis, tdh, and tlh of the isolates. The significant antibiotic differential sensitivity was determined for strain isolates using Student's t-test (P < 0.05).

Results: Using the modified 2-step technique, a total of 17 presumptive *V. parahaemolyticus* were recovered from 17 retail kinds of seafood, suggesting that these isolates be utilized for subsequent investigations. 16S rDNA bacterial analyses for genomic DNA of these isolates confirmed the prevalence of *V. parahaemolyticus* (~12%) in retail samples tested. Subsequent analyses for virulence phenotypes and genotypes of these isolates determined that these isolates were clinically linked strains and possessed hemolytic, urease, and/or Kanagawa phenomenon activities. Antibiotic differential inhibition analyses for strain determination did not significantly distinguish these isolates.

Significance: The VBNC state of *V. parahaemolyticus* can lead to false-negative examinations and foodborne outbreaks. Our findings suggest for the first time, the prevalence of the novel group of *V. parahaemolyticus* in retail kinds of seafood which could bypass current testing and cooking protocols and thereby become an emerging source of infections.

P3-184 Food Safety Best Practices at Food Service and Retail Sector in the Face of COVID-19

Muhammad Shahbaz[1], Abdul Moiz[2], Shugufta Mohammad Zubair[3] and Mohammad Ahmad Amjad[4]

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Introduction: COVID-19 has adverse effects on all fields of life, but the condition of food sector is more alarming. It's challenging to maintain food safety and security during this pandemic. To ensure the safe food supply is some preventive and curative actions must be taken. Food itself is not a cause of coronavirus spread but during handling it can be transmitted from one person to another. Proper hygiene should be maintained from Farm to Plate. All food handlers should maintain social distancing, SOPs should be followed, checking of temperature, use of hand sanitizers. Food business chains should not come in direct contact with customers. Food audits should be done online. Use CCTVs to check on the workers and SOPs keep record and if someone shows symptoms related to COVID-19 contact the health professionals.

Purpose: The purpose of this study was to develop the practical guideline for handlers to assure Food Safety Best Practices at Food Service and Retail Sector during the COVID-19 pandemic.

Methods: The instructions and the guidelines published during this pandemic specifically from the food regulatory authorities was demonstrated in pictorial and self-explanatory the driveway to the Food handlers should follow the instructions.

Results: Being a critical in implementation of these practices particularly in the quick service restaurants the team have discovered in the selected restaurants the staff with the appropriate training and following of Strict protocols accompanied by PPE has minimum infection rate as compared to the others. The food handlers have a major challenge towards managing and maintaining the protocols on the customer side.

Significance: The outcome of this investigation is highly valuable to understand and implement the Food Safety Best Practices at Food Service and Retail Sector in the Face of COVID-19 for organizations in quick service restaurants and retail sector. As area of food sector is highly essential and having most busy interference with public

P3-185 SARS-CoV-2 on High Touch Surfaces at Food Retailers

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Introduction: The COVID-19 pandemic has generated increased interest in potential transmission routes of SARS-CoV-2. Lockdown and quarantine protocols have significantly restricted activities and channeled concerns towards public accessible spaces and essential services such as food retailers. Indirect transmission through high-touch surfaces has been proposed as a potential route. However, limited information exists on the presence, survival and infectivity of SARS-CoV-2 on surfaces, particularly outside laboratory settings.

Purpose: The purpose of this project was to fill this gap by assessing the potential presence of the virus on commonly found surfaces at food retail stores and the role that these spaces may play in virus transmission.

Methods: A total of 957 samples were collected in four food-retail stores located within the province of Ontario, Canada. Sampling was performed on each store twice a week, before opening and after closing operations, for a month (Oct to Nov 2020). Trained personnel sampled the surfaces using environmental swabs. High-touch surfaces were identified and surveyed in 4 zones within the store: payment station, deli counter, refrigerated food section and shopping carts and baskets. The samples were immediately refrigerated and analyzed using a molecular method, i.e., reverse transcriptase quantitative Polymerase Chain Reaction (RT-qPCR). Positive and negative controls were performed at each run.

Results: Regardless of store's location (urban vs. suburban), sampling day or time, location of the surface within the store or surface material, all samples tested negative for SARS-CoV-2 (i.e., values < the detection limit of the method). These results suggest that the risk of exposure from potentially contaminated high-touch surfaces within a food retailer store is low if social distancing guidelines and recommended cleaning protocol are followed (e.g., enforced mask use, sanitation protocols).

Significance: These results emphasize the importance of preventive measures to reduce the probability of encountering SAR-CoV-2 on common and frequently touched surfaces in retail stores.

P3-186 Focus Group Studies on *Listeria* Control at Retail – Outcomes and Next Steps

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Introduction: *Listeria monocytogenes* (*Lm*) has been found to contaminate ready-to-eat meat and poultry products at retail and has been a significant cause of human listeriosis cases and outbreaks.

Purpose: The Food Safety and Inspection Service (FSIS) conducted focus groups to assess the clarity and utility of guidance materials to address *Lm* contamination at retail.

Methods: FSIS conducted the focus groups in collaboration with the Food and Drug Administration (FDA) and the Centers for Disease Control and Prevention (CDC). Overall, 13 focus group sessions were held, with a total of 71 participants from State/local public health and agriculture departments, retail, and academia (Cooperative Extension Specialists). Focus group sessions were conducted by webinar and each session was recorded. Questions were grouped in the following topic areas: distribution/availability of communication materials; *Lm*-specific communication content, and FSIS-specific *Lm* communication tools.

Results: The results showed that stake holders receive information from federal agencies, state and local agencies, industry associations, and academia. Participants stated that they prefer email distribution of food safety communications rather than paper documents. They also recommended that FSIS simplify materials and add more visuals to make the materials easy to understand. In addition, the focus groups recommended that FSIS coordinate with CDC and FDA to harmonize its outreach materials and to work with industry groups and academic partners to review and distribute its outreach materials.

Significance: In response to the focus group findings, FSIS plans to update its Retail *Lm* Guideline and to work with its public health partners and industry groups to review its outreach materials. By making changes in outreach materials, FSIS can help protect public health by driving adoption of food safety practices to control *Lm* at retail.

P3-187 Antimicrobial-resistant Non-Typhoidal *Salmonella* in Various Foods at Retail in the United States: A Rapid Systematic Review and Meta-Analysis

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❖ ***Developing Scientist Entrant***

Introduction: Antimicrobial resistance (AMR) is a major public health threat worldwide, against which ambitious actions have been taken in the United States. Resistant bacteria in foods at the retail stage have been frequently reported, indicating the potential of food consumption as an important exposure pathway. Among foodborne pathogenic bacteria, *Salmonella* is the leading cause of foodborne diseases in the U.S.

Purpose: The study was aimed to comprehensively collect and critically review quantitative and qualitative information about the contamination of antimicrobial-resistant non-typhoidal *Salmonella* in various foods at retail in the U.S. using systematic review (SR) and meta-analysis (MA) approaches.

Methods: The CoCoPop framework (condition, context and population) was followed to determine eligible literature from electronic databases including: Web of Science Core Collection, Biological Abstracts, MEDLINE, CABI, BIOSIS Citation Index, Scopus and NARMS annual reports. Stratified Meta-analyses of resistant *Salmonella* prevalence were performed by major commodities (beef, chicken, turkey, pork) and classes of antibiotics.

Results: From 11,839 identified citations, 44 were considered relevant. In addition, NARMS data from 2002 to 2017 were included. In general, results showed a higher prevalence of resistant *Salmonella* in chicken and turkey, compared with pork and beef, and lowest in vegetables and imported foods (data mainly available for spices). As for resistance to various antibiotic classes, tetracycline resistance was observed highest among major commodities (27.99%-54.64%). Albeit a moderate level of resistance to beta-lactam antibiotics, the threat to public health can be profound due to their critical roles in clinical use. Surprisingly, resistance to macrolides, an important antibiotic class used in veterinary settings, was considerably lower for all major commodities, which however was estimated based on less data currently available.

Significance: Results of the present study will facilitate the application of quantitative microbial risk assessment methods in identifying and evaluating potential mitigation strategies for controlling human exposure to foodborne AMR.

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