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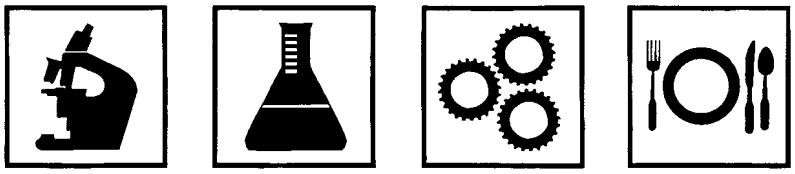

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ABSTRACTS

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IVAN PARKIN LECTURE ABSTRACT

HACCP: Seven Effective Principles for Every Reason, Season and 'Special Situation'

Rhona S. Applebaum
The Coca-Cola Company
Atlanta, GA, USA

First, a few factoids to set the stage —

HACCP: The acronym for Hazard Analysis Critical Control Points.

History: Developed by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF). Pioneered by the Pillsbury Company, with cooperation and participation from the National Aeronautic and Space Administration (NASA), Natick Laboratories of the U.S. Army, and U.S. Air Force Space Laboratory Project Group.

Definition and Purpose: A management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw material production, procurement and handling, to manufacturing, distribution and consumption of the finished product (USFDA Nov. 26, 2014).

Principles: Seven (7), beginning with the performance of a hazard analysis…

Application: Every segment of the food industry — from field to fork, seed to seat, grove to glass, farm to table, etc., etc.

This concludes the factoids on HACCP. However, before outlining the brief of what this presentation intends to offer and why the focus on HACCP, it is important to underscore what this presentation will not address and why:

- This presentation will not be a lecture on HACCP, detailing the classical preventative approach to food safety — the approach and system we have been trained in, understand, practice and implement on a daily basis.
- Nor should this be interpreted as minimalizing or trivializing HACCP — far from it. It remains essential, if not imperative, to maintain a lead on continued improvements (operational excellence) and shared learnings with regard to HACCP, its supporting, aligned, required conditions, practices, and any and all related programs and activities necessary to work alongside a HACCP system, including but not limited to the critical role of prerequisite programs, operational prerequsite programs and other analytical and/or controlling activities identified and now codified in the Food Safety Modernization Act (FSMA).
- HACCP has been addressed in some way, shape or form for at least five of the last 12 Ivan Parkin Lectures, and it will be addressed in a number of sessions, panels, talks and posters during the Annual Meeting. Consequently, for me to attempt to bring something new and insightful with regard to the classical use and deployment of HACCP would be missing a bigger opportunity.

During this presentation, I will offer different perspectives on the use of HACCP with focus on its application, utility and value to disciplines outside the food safety realm. I intend to demonstrate, using two non-food safety examples, how I have applied the HACCP approach and its seven principles to problem mitigation and resolution. As Stephen Covey, in his bestseller Seven Habits of Highly Effective People demonstrates how his seven principles are not limited exclusively to workplace, management, or leadership, I will discuss how HACCP is a methodology, a tool, a thought and action process whose benefits go beyond food safety. Consequently, these benefits are currently unknown, underused and/or underappreciated by many sectors — public and private — as an approach that may be effective for problem and risk mitigation, resolution and yes, even prevention.

No disrespect to Robert Fulghum, but maybe everything I need to know I didn't learn in kindergarten. In fact, my supplemental HACCP training was the icing on the cake. Many thanks to the food safety professionals who enabled this to happen — for me and many others.

JOHN H. SILLIKER LECTURE ABSTRACT

Challenging the Conventional

Francis (Frank) F. Busta
Professor Emeritus, Food Microbiology
Director Emeritus, National Center for Food Protection and Defense
University of Minnesota
St. Paul, MN, USA

Traditionally, food safety has been taught and professionals have been encouraged to analyze laboratory samples and solve problems using conventionally established and accepted methods. However, looking back over my and my colleagues' and students' careers, challenging of the conventional has been, in fact, the basis of much success, especially when intuition or data
suggested that the conventional was not telling the entire story.

Challenging the conventional has occurred by: Questioning, as part of a program of continuous improvement, the standard methods and approaches used; Never being quite satisfied with accepting the data if the outcomes seem too routine or being concerned if the variability is pushing the edge of statistical significance; Assessing extensive controls to verify the accuracy and precision of the method, especially with subtle but important differences from the predicted; When the outcome is not predicted, viewing the situation as an opportunity to discover why it occurred; Constantly searching for unintended consequences that invariably develop with new or improved processes or new approaches to solve existing problems; Anticipating a possible hidden and ongoing event even if it has never occurred before or a potential future problem that could develop.Questioning observations are the true basis for research and competence.

Research that reflects our challenging the conventional:

Heat injury of Staphylococcus aureus was discovered when established selective media results did not mesh with controls.

Freezing and dehydration damage of salmonellae and E. coli continued the theme of questioning the effects of selective media on cells exposed to processing conditions that damaged but did not inactivate the cells.

Apparent bacterial spore responses to heat varied depending on subculture conditions and ultimately revealed the roles of multiple spore germination systems.

Stressful conditions on C. perfringens demonstrated the extraordinary capability to grow rapidly over a broad range of constantly increasing temperatures.

Inhibition and growth of C. botulinum under various conditions in several food systems also verified the complexity of predicting effectiveness of accepted control measures and ingredients.

Response of B. cereus spores to changing environments highlighted the broad range of responses to germination, growth and inactivation conditions.

This background was ideal preparation for my transition to a food defense perspective addressing intentional contamination, where challenging the conventional is especially pertinent. Over my past 12 years, the application of these thought processes has been particularly useful in personal progression from food quality management and food safety through to food defense. Since many safety and defense principals are parallel, some felt their food safety programs were satisfactory deterrents in food defense. However, the thought patterns addressing potential threats demand different assessments considering that defense is dealing with intelligent adversaries. The success of food defense programs depends extensively on assessing and planning for events that have rarely if ever occurred to date. Ingenuity and inquiry play a great roll here as well as in food safety activities. The areas of concern range from considering whether threat agents would be biological, chemical or radiological; to vulnerability of entry points along the entire food supply chain; to effects of food processing and handling on various select agents; to relevant approaches in preparing to prevent, respond and recover should a threat or an event occur; to effectual risk and crisis communication on these rare but catastrophic events; to responses of consumers and the probable recovery of a commodity after an event; to the anticipated national and international economic impact and subsequent loss of faith in the government after an event where a trusted food supply had been violated and consumer protection had failed. Relevant concerns over intentional contamination of food have never been more important than today.

Symposium Abstracts

S1 Beyond the Standard Plate Count: Entering the Era of Food Microbiomics

ANDREW BENSON: *University of Nebraska-Lincoln, Lincoln, NE, USA*
MATT RAINIERI: *Acme Smoked Fish, Brooklyn, NY, USA*
BENJAMIN WOLFE: *Harvard, Cambridge, MA, USA*

Are we moving beyond the era of the standard plate count? Cultural microbiology remains the focus of the food microbiologist's tool box. Its utility cannot be overemphasized. However culture and the plate count technique are known to have two major drawbacks. First, estimates are that only approximately one percent of microorganisms have been cultured and secondly, of those that form colonies on an agar dish, their identity is largely unknown once a quantitative colony count is made. The practical use of the plate count does not include what is perhaps its most important potential offering, the identity of what has grown. The era of conducting food microbiome analysis is here and we are beginning to learn answers to the question of not only how many but also who they are, i.e. identity and relative proportion of the total population.

This symposium will examine this powerful tool by presenting case studies as well as the basic technique itself. Potential uses and discoveries for the food microbiologist will be a focus of the session using examples of refrigerated meats, smoked fish and cheese microbiomes.

S2 Metagenomic Analysis Pipelines: Really Learn about Your Food from Your NGS Data!

JAMES WHITE: *Resphera Biosciences, Baltimore, MD, USA*
STEFAN EDLUND: *IBM Almaden Research Center, San Jose, CA, USA*
DAVID CHAMBLISS: *IBM Almaden Research Center, San Jose, CA, USA*
NUR HASAN: *CosmosID, College Park, MD, USA*
YANYAN HUANG: *ConAgra Foods, Omaha, NE, USA*

The audience will hear from bioinformatic experts from academia, food industry, and government that are working to develop and validate different sequencing approaches to characterize the microbial composition of fresh produce, probiotics and other foods and food environments. Whole genome, 16S rRNA, and metagenomic sequencing are powerful tools to identify foodborne pathogens and improve understanding of how to prevent contamination. However, the large NGS data sets can present seemingly overwhelming bioinformatic challenges. As the scientific community, particularly those involved in food safety embrace the many options for data generation and analysis – some degree of standardization needs to occur. Methods for interpreting data will be presented and the road to validation for certain approaches will be discussed. This symposium will highlight some of the analysis platforms currently used to identify pathogens and characterize ecologies that play a role in contamination.

S3 Microbial Indicators: They are What You Make of Them

JEFF KORNACKI: *Kornacki Microbiology Solutions, Inc., Madison, WI, USA*
PETER TAORMINA: *John Morrell & Co., Cincinnati, OH, USA*
KAREN KILLINGER: *Washington State University, Pullman, WA, USA*
KALI KNIEL: *University of Delaware, Newark, DE, USA*
MIEKE UYTTENDAELE: *Ghent University, Ghent, Belgium*
MANAN SHARMA: *U.S. Department of Agriculture-ARS-EMFSL, Beltsville, MD, USA*

Microbial indicators for the presence of pathogens, fecal contamination, or unsanitary conditions are widely used in almost every facet of food microbiology. Many of our choices for microbial indicators have come from food testing and processing environments, where it has been appropriate to use traditional indicators like total aerobic plate counts, total coliforms, and *Escherichia coli* to indicate sanitary conditions, processing controls, and fecal contamination, respectively. However, as processors and handlers face new challenges with regard to foodborne viruses (norovirus, hepatitis A) and new testing environs (fruit and vegetable growing environments), it may be time consider if traditional indicators are sufficient to indicate the risk of contamination. This symposium will address how we have come to our current understanding of using microbial indicators, and then delve into the use of specific microbial indicators and what their presence means, and sometimes how these data can be misinterpreted. This will include the use of *Listeria innocua* and non-pathogenic *E. coli* to indicate the presence of *L. monocytogenes* and Shiga-toxigenic *E. coli* (STEC) in processed meats, respectively; the practicality and utility of using total or fecal coliforms to indicate the presence of STEC or fecal contamination in fruit and vegetable growing environments; and finally, the use of novel viral and bacteriophage-indicators to signify the presence of foodborne viruses in various foods. The symposium will conclude with the emerging use of specific culture-independent techniques to detect fecal contamination in various foods and processing environments, and the European perspective of using *Enterobacteriaceae* to indicate the presence of *Salmonella* spp. in foods, which may not have broad consensus among food microbiologists.

SS3 Listeria Special Session

ROBERT TAUXE: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
KATHLEEN GLASS: *University of Wisconsin-Madison, Madison, WI, USA*
YI CHEN: *U.S. Food and Drug Administration, College Park, MD, USA*
EDITH WILKINS: *Leprino Foods, Denver, CO, USA*

Abstract will be printed in the Program Addendum.

S4 Achieving Actionable Information from Food Safety Inspections

MIRIAM EISENBERG: *EcoSure, a Division of Ecolab, Lincolnshire, IL, USA*
JEFFREY LINDHOLM: *ICertainty, Inc., Omaha, NE, USA*
CHIRAG BHATT: *Bloomin Brands, Inc., Tampa, FL, USA*

The era of electronic data capture in the foodservice industry is with us. Numerous foodservice companies and regulators employ simple computerized inspection systems that capture a wealth of food safety data. As that data accumulates some have called it "big data." But data is just data unless something can be done with it. This session looks to address the technology of data capture from the perspective of getting the right data and then looking at several case studies of what some in the industry are doing with that information. How is inspectional data turned into classical metrics? How

might these inspectional lagging indicators be turned into food safety leading indicators? Can we predict the probability that a foodborne illness event might be on the horizon? Each presenter will be asked to summarize at least three actionable items from their talk that any food safety professional can immediately put to use.

This session is significantly different than one on "big data" presented in 2014, Indianapolis. In that session big data was presented more as a paradigm than a tool. This session evolved from many in attendance that said, "great, now what do you do with that data." In addition, this session will focus on the foodservice industry where many operators are chain units with hundreds to thousands of units scattered across the country. Any food company with multiple operations will benefit from this session by gaining insight into the means of capturing leading indicators on their own food safety system.

S5 Advanced Food Packaging Systems for Enhancing Product Safety

PAUL BUTLER: *IDTechEx Ltd, Cambridge, UK*
ALLEN ELY: *Packaging Progressions, Collegeville, PA, USA*
BRIAN THANE: *Tetra Pak Inc., Denton, TX, USA*
KATHY GOMBAS: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*

Most attention in the area of driving food safety had been placed on food ingredients, their transport, storage, processing, labeing and use by consumers. Yet, there have been a number of foodborne outbreaks where the packaging of the food was the or one of the causes of the outbreak. Food packaging systems and the packaging itself play a significant rolls in maintaining the safety of the food. This symposium will include an overview of current food packaging systems for wet and dry foods, then move into the relm of new and advanced packaging systems and packaging matierals that not only maintain the safety of the food but actually increase the confidence factor related to a given food's safety.

S6 Who's Going to Fill Your Shoes? Capacity Building for the Future

JULIA BRADSHER: *International Food Protection Training Institute, Battle Creek, MI, USA*
KATHERINE SATCHWELL: *University of Alberta, Edmonton, AB, Canada*
MARK OVERLAND: *Cargill, Bloomington, MN, USA*
BRIAN BEDARD: *Grocery Manufacturers Association, Washington, D.C., USA*
LONE JESPERSEN: *Maple Leaf Foods, Mississauga, ON, Canada*
LEE-ANN JAYKUS: *North Carolina State University, Raleigh, NC, USA*

As food safety professionals, we often spend our days overseeing food safety, guarding brand trust, and ensuring there's no interruption to product supply. This session forces audiences to go beyond assuring product supply and addresses the questions, "Is enough being done towards capacity building for the future? Are we adequately laying the groundwork to protect our legacy for those that come after us? Am I ever going to be able to retire in peace!?"

It's estimated that each day, 10,000 Americans (largely consisting of Baby Boomers) retire; many other countries are experiencing a similar phenomenon. This vacuum is creating a global skills gap, and the food safety industry isn't immune. We must proactively address this future talent shortage by implementing education and training strategies to ensure there is a sustainable succession of high quality practitioners.

This session will directly address many facets of this problem:

- Understanding the global skills gap in our industry through a case study involving auditor competency.
- Addressing the need for curriculum changes to ensure that this skills gap is addressed, and adequately equipping future professionals.
- The decline in incoming Food Science majors. How to recruit/train the next generation, directly from a Millenial's perspective.
- Ensure that current quality professionals receive the proper continuing development and education needed to combat emerging issues and future challenges.
- Capacity building for the future: a discussion of the link between competency, capacity, and protecting the health of our consumers.

This session will hear from food safety experts who have already begun to tackle these tough questions. They will present mitigation strategies for recruitment/engagement of present and future professionals and initiatives being developed to fill our global skills gap. Attendees will garner tips, share best practices for several innovative solutions, and discover collaboration opportunities that they can bring back to their own organizations.

S7 Preventing Foodborne Illnesses from Donated or Recovered Foods

MITZI BAUM: *Feeding America, Chicago, IL, USA*
FRANK FERKO: *US Foodservice, Rosemont, IL, USA*
BRIAN TURNER: *Sodexo, Downers Grove, IL, USA*
TODD ROSSOW: *Publix Super Markets, Inc., Lakeland, FL, USA*

Decreasing food waste through donations and recovery is a sustainability initiative that could have unintended consequences on public health and the company that donates the food. The purpose of this symposium is to have leaders responsible for developing and managing food safety systems throughout various segments of the supply chain share current practices taking place in the industry to assure that donated or recovered foods are safe to eat. The legal aspect for making food safety decisions on what foods can be donated and what foods should be destroyed will also be presented.

S8 Designing Microbiologically Safe Microwaveable Foods: Electromagnetic and Microbial Modeling Approaches

SANJAY GUMMALLA: *American Frozen Food Institute, McLean, VA, USA*
WILLIAM SHAW: *U.S. Department of Agriculture-FSIS, Washington, D.C., USA*
STEVE VLOCK: *ConAgra Foods, Omaha, NE, USA*
JEYAMKONDAN SUBBIAH: *University of Nebraska-Lincoln, Lincoln, NE, USA*
DAVID JONES: *University of Nebraska-Lincoln, Lincoln, NE, USA*
HARSHAVARDHAN THIPPAREDDI: *University of Nebraska-Lincoln, Lincoln, NE, USA*

Foodborne illness outbreaks resulting from consumption of microwaveable foods has increased the concern among the food processors, regulators and consumers on the microbiological safety of these products. While the knowledge of uneven heating of foods during microwave heating is common knowledge, methods to predict and evaluate the extent of heating non-uniformity have not been available. The variability in cavity design, wattage/power, turn table design, and magnetron feed port location affect the cooking performance of microwave ovens. Further, the differences in absorption of microwave energy by food components in a frozen and/or refrigerated food contribute to the non-uniform heating of those products. Properly designing frozen and/or refrigerated foods requires the product developers, food safety professionals and food engineers to collaborate to make these products microbiologically safe. Advances in research on development of predictive models for electromagnetic/microwave radiation and heat transfer, coupled with microbial death kinetics can provide insight on temperature distribution and assist in designing microbiologically safe foods. This symposium will address the safety of the microwaveable foods from industry and regulatory perspectives and new modeling techniques to assist food industry in properly designing and developing these products with improved microbiological safety and quality.

S9 Southeast Asia: Issues and Initiatives for Food Safety

CHEOW KEAT CHIN: *Ministry of Health Malaysia, Selangor, Malaysia*
MATHEW LAU: *Nanyang Polytechnic, Yio Chu Kang, Singapore*
M. AMAN WIRAKARTAKUSUMAH: *Institut Pertanian Bogor, Bogor, Indonesia*

Welcome to captivating Southeast Asia, one of the world's most popular tourist destinations, whose draws aretropical climates, gorgeous beaches, rich culture, unique food and reasonable prices.

Diverse Southeast Asia, population 618,000,000, consists of two geographic regions: Maritime Southeast Asia, comprising Philippines, East Malaysia, East Timor, Indonesia, Brunei, and Singapore, and mainland Southeast Asia, comprising Cambodia, Laos, Myanmar, Thailand, West Malaysia and Vietnam.

Like many countries, national food safety programs in Southeast Asia may lack detailed knowledge of both the nature and extent of food safety problems, as well as the consequences of contaminated food on the nation's health and economic development. Compounding these issues, there is a shortage of sound, cost-effective methods for identifying specific food safety problems in the region. The responsibility for ensuring food safety has been based on a multi-agency approach, and there has been lack of coordination among agencies. In addition, specific food safety policies are insufficient to ensure food safety in most countries. This situation has been further compounded by the presence of other areas of concern which compete for limited resources.

As the member countries of the Association of Southeast Asian Nations (ASEAN) strive towards greater economic integration to achieve the ASEAN Economic Community by 2015, risk-based food safety control measures are becoming increasingly important to protecting public health and ensuring fair trade practices in food and agricultural products. To that end, many countries in the region are working towards harmonizing their national food safety standards with international standards such as Codex Alimentarius, and are looking to adopt science-based risk analysis as the basis for developing their food safety systems.

This symposium presents an overview of food safety issues, infrastructures and initiatives in Southeast Asia, specifically showcasing three nations, Malaysia, Singapore and Indonesia.

S10 The Vegetarian Lifestyle: Molecular Responses of Enteric Pathogens to Fresh Produce

SHIRLEY MICALLEF: *University of Maryland-College Park, College Park, MD, USA*
FRANCISCO DIEZ-GONZALEZ: *University of Minnesota, Saint Paul, MN, USA*
MARIA BRANDL: *USDA-ARS-WRRC, Produce Safety and Microbiology Research Unit, Albany, CA, USA*
XU LI: *University of Nebraska-Lincoln, Lincoln, NE, USA*

Research on enteric pathogen colonization of fresh produce crops and their production areas has slowly changed the earlier notion that enteric pathogens are poorly adapted to survive outside animal hosts. Mounting evidence supports the view that enteric pathogens have developed physiological means to persist in the environment and to use plants as alternative hosts. Several pathogens, including Salmonella, enteropathogenic Escherichia coli and Listeria monocytogenes have been shown to effectively colonize and be able to multiply on a variety of fresh produce crops including leafy greens, vine-stalk vegetables, melons and sprouts. Recent research that makes use of molecular, microarray, next generation sequencing and mass spectrometry technologies have begun to elucidate the molecular mechanisms by which enteric pathogens can successfully achieve this. Genes involved in virulence, motility, stress and surface structures could be involved, and some of the same mechanisms used in animal infection could be at play. Published and ongoing work is also showing that different enteric pathogens appear to use different sets of genes for colonization, and differences also exist among serotypes of the same species, or pathogens attaching to a variety of crops. There is a pressing need to better understand the strategies used by pathogens to colonize fresh produce, and the genetic mechanisms by which conditions facilitate establishment and persistence, to help devise ways to mitigate contamination risks. This symposium will bring together perspectives and latest findings on the molecular responses induced in human pathogens successfully colonizing food plants.

S11 The Rise of the Genomes II: Practical Integration of Whole Genome Sequencing into Food Safety

DAVID ENGELTHALER: *Translational Genomics Research Institute, Flagstaff, AZ, USA*
PETER GERNER-SMIDT: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
KATHIE GRANT: *Public Health England, London, England*
PETER EVANS: *U.S. Food and Drug Administration, College Park, MD, USA*
LEEN BAERT: *Nestlé Research Center, Vers chez le Blanc, Switzerland*
DAVID ENGELTHALER: *Translational Genomics Research Institute, Flagstaff, AZ, USA*
KATHIE GRANT: *Public Health England, London, England*

Whole genome sequencing is an emerging technology that has moved from research to practical food safety application at the speed of lightning. The technology has now moved into real-time surveillance in public health and food regulation, and the food industry is picking up. This symposium follows up where last year's "The Rise of the Genomes" symposium ended: Can whole genome sequencing really replace practical microbiology? Do we need epidemiology in the investigation of food safety emergencies any longer? What have we learned in the past year? Have all expectations been met? What are the gaps? Public health is overly excited with whole genome sequencing but is this revolution good or bad news for the food industry?

S12 Evolving Methods for Foodborne Illness Source Attribution

ANTONIO VIEIRA: *U.S. Centers for Disease Control and Prevention, Atlanta, GA, USA*
R. MICHAEL HOEKSTRA: *Centers for Disease Control and Prevention, Richmond, VA, USA*
MICHAEL BAZACO: *U.S. Food and Drug Administration, College Park, MD, USA*
CRAIG HEDBERG: *University of Minnesota, Minneapolis, MN, USA*
FRANK POLLARI: *Public Health Agency of Canada, Guelph, ON, Canada*

A major challenge for a risk-based food safety system is attributing to food sources the number of illnesses due to major foodborne pathogens like *Salmonella*, *E. coli* O157:H7, *Campylobacter*, and *Listeria monocytogenes*. Such attribution estimates are necessary to understand the burden of foodborne disease and provide critical information for risk-informed priority setting, program evaluation, and targeted risk management. Foodborne illness source attribution remains an area of active research, as scientists throughout the world continue to develop novel approaches, advance existing methods, and bring new data to bear on these questions.

This symposium will present six new studies underway or nearing completion. In a major effort, the Foodborne Disease Burden Epidemiology Reference Group (FERG) of the World Health Organization (WHO) has developed an expert elicitation for food source attribution to estimate the global burden of foodborne disease. Modeling efforts built on microbial fingerprinting continue to evolve rapidly; this symposium will include presentations on two novel microbial subtyping studies, one based on whole genome sequencing and another by FoodNet Canada using comparative genomics. The Interagency Food Safety Analytics Collaboration (IFSAC) – comprising joint efforts of the US Centers for Disease Control and Prevention (CDC), Food and Drug Administration (FDA), and USDA Food Safety and Inspection Service (FSIS) – has developed a new approach for estimating outbreak-based attribution based on a two-stage statistical model, and has critically assessed uncertainties associated with outbreak-based attribution estimates. IFSAC scientists have also advanced the U.S. version of the Hald model, a Bayesian frequency-matching model for attributing *Salmonella* serotypes across FSIS and FDA product categories.

S13 Pathogen Control Processes for Pet Food Manufacturing

MICHELE EVANS: *Hills Pet Nutrition, Topeka, KS, USA*
SIOBHAN REILLY: *Log10 Probiotics, Ponca City, OK, USA*
ADAM WATKINS: *Mars Global Petcare, Nashville, TN, USA*

Sales of pet foods in the U.S. have increased 68% in the past 13 years and now exceed $20 billion annually. In the same period, there have been several outbreaks linked to Salmonella in dry pet foods and pet treats. In the past year alone, there were seven recalls of pet food products due to potential Salmonella contamination. In that context, the recent FSMA Proposed Rule for Preventive Controls for Animal Food will soon put increasing pressure on the industry to demonstrate that systems and processes are in place for prevention and control of potential microbial hazards. Although the pet food industry already is implementing protocols and practices for ensuring product safety, the vast majority of food safety research being published and presented at conferences (including IAFP Annual Meetings) addresses processing of human foods, with little attention to special challenges associated with pet and animal foods. These include delivering a complete nutritional profile in a single product, managing animal-derived ingredients in a low-moisture manufacturing environment, preventing cross-contamination, and selecting and validating optimal critical controls. Consequently, this symposium will focus on the scientific, technical, and practical aspects of implementing such systems. The speakers will review current knowledge and the state-of-practice related to cGMPs and pathogen reduction, present new data on a novel application of competitive microflora in pet food manufacturing facilities, and end with a case study on pathogen reduction in pet food products. Attendees will gain a practical understanding of the fundamentals and challenges associated with implementing pathogen controls in pet food manufacturing systems.

S14 Safe Food for the Entire Family: The Global Pet Food Safety Frontier

ROBERT BUCHANAN: *University of Maryland, College Park, MD, USA*
LEE ANNE PALMER: *U.S. Food and Drug Administration, Rockville, MD, USA*
KARL NOBERT: *The Nobert Group, LLC, Sterling, VA, USA*

A whopping 68% of U.S. households own a pet, which equates to 82.5 million homes, according to the 2013-2014 American Pet Products Association National Pet Owners Survey. Some 56.7 million U.S. households own at least one dog, while 45.3 million households own at least one cat. About 83.3 million dogs and about 95.6 million cats are owned in the United States.

For most of us whose lives are enhanced and enriched by pets, our animals are beloved members of the family and we are devoted to their well-being. For 2014, it is estimated that $58.51 billion will be was spent on our pets in the U.S. and $23.62 billion of that is estimated to be spent on food.

Pet food and its many ingredients are susceptible to all of the same biological, chemical and physical contaminants that can threaten human consumables. The death of a pet due to contaminated food is devastating to the people that love them. Contaminated pet food also poses a health risk to people that handle it.

Since 2010, there have been more than 50 product recalls associated with pet foods potentially contaminated with *Salmonella*. As of May 1, 2014, FDA has received approximately 4,800 reports of pet illnesses which may be related to consumption of the jerky treats.The 2007 melamine contamination resulted in the biggest pet food recall in history and a $24 million class-action court settlement.

The importance of pet food safety cannot be over emphasized. Our pets depend on us to provide them safe food and treats free of deadly hazards.

This symposium addresses major pet food safety issues and developments, and provides information and tools to address them, including preventative controls, ingredient scrutiny and FSMA regulations.

S15 20 Years of Risk-based International Trade — Successes and Failures of the WTO

GRETCHEN STANTON: *World Trade Organisation, Geneva, Switzerland*
SARAH CAHILL: *Food and Agriculture Organization of the United Nations, Rome, Italy*
JENS KIRK ANDERSEN: *Technical University of Denmark, Copenhagen, Denmark*
ROBERT BUCHANAN: *University of Maryland, College Park, MD, USA*
ANDREW STEPHENS: *U.S. Food and Drug Administration, Washington, D.C., USA*
IAN JENSON: *Meat and Livestock Australia, North Sydney, Australia*

It is now 20 years since the World Trade Organisation's Sanitary and Phytosanitary (SPS) Agreement was signed. This agreement heralded an era in which international trade would be based on foods providing an acceptable level of protection to consumers in the importing country, that would be judged objectively, based on risk assessment.

The intervening 20 years has seen the development of microbiological risk assessment. These risk assessments have often been applied to international trade – sometimes they have reduced technical trade barriers – and sometimes they have created them.

There is a lot of controversy around the application of the SPS Agreement. This symposium aims to explain the agreement, the developing science, and examine how it is used, and how it benefits (or otherwise) international trade and public health.

S16 Is There a Role for "Modern Toxicology" in Regulatory Science?

SUZANNE FITZPATRICK: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
DAVID DIX: *EPA, Washington, D.C., USA*
ROBERT CHAPIN: *Pfizer Pharmaceuticals, Inc., New London/Norwich, CT, USA*
ROBERT SPRANDO: *U.S. Food and Drug Administration, Laurel, MD, USA*
HARVEY CLEWELL: *The Hamner Institutes for Health Sciences, Research Triangle Park, NC, USA*
HEIDI BIALK: *PepsiCo, Valhalla, NY, USA*

Many frequently used food chemical substances may affect different organs and their potential toxicity needs to be tested for determining their safety; several food chemical substances have not been tested for safety. Regulatory agencies rely on the best available science to obtain the information necessary to develop guidelines and regulations to ensure the safety of food chemical substances (food additives, colors, contact materials and dietary supplements). Current toxicological methodologies rely primarily on animal testing which is costly, laborious and time consuming. Additionally, traditional toxicological tests may not identify new and evolving areas of toxicological concern such as endocrine disruption, cardiotoxicity and neurobehavioral effects.

Advances in regulatory science are critical to effectively translate cutting-edge developments in science and technology. Agencies such as EPA and FDA are moving towards a systematic approach for developing research to fill data gaps identified by risk assessments and regulatory needs. More rapid higher throughput methods such as *in vitro*, *ex vivo*, and *in silico* models, are evolving and have been successfully used as screening tools. Their potential role for regulatory purposes, however, has yet to be established. There is also the need to determine how to translate these *in vitro* results into *in vivo* practical information and relate these results to the industry and the consumer. The objectives of this symposium are two-fold: 1) identify the regulatory needs for determining whether a substance is unsafe; 2) describe new and emerging methodologies and discuss how these new methodologies can be used to address our regulatory needs.

S17 Updating Our Knowledge in Assessing Food Safety Risk: Meta-analysis, Bayesian Statistics and Beyond

URSULA GONZALES-BARRON: *School of Agriculture, Polytechnic Institute of Braganza, Braganza, Portugal*
MOEZ SANAA: *ANSES, Maisons Alfort, France*
YUHUAN CHEN: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
MICHAEL WILLIAMS: *Risk Assessment Division, Food Safety and Inspection Service, USDA, Fort Collins, CO, USA*
VASCO CADAVEZ: *School of Agriculture, Polytechnic Institute of Braganza, Braganza, Portugal*
FRIEDRICH WESTERHOLT: *University College Dublin, Dublin, Ireland*

In order to address food safety problems, abundant research is conducted every year by academic institutions, governmental agencies and food industries. Although investigations aiming to provide answers to the same research question have in some instances comparable outcomes, and in others, contradictory outcomes, it is highly unlikely that they lead to the same results. A series of regular factors, such as the variability in composition according to production regions, or even differences in laboratorial procedures, bring about such divergences. Nevertheless, the outcomes from different experimental set-ups can still be combined, allowing our current knowledge on a food safety matter to be updated to facilitate microbial modelling and assessing food safety risk that requires the integration of data from multiple studies and sources. Although the interest in integrating existing knowledge on food microbial modelling is relatively recent, so far many applications have been done by using Bayesian statistics and meta-analysis. Bayesian inference mathematically describes the learning process: we may start off with a vague opinion, and then modify our opinion when presented with new evidence, and this can be done repeatedly. On the other hand, meta-analysis refers to the statistical analysis of a large collection of results from primary studies investigating the same research question. Apart from the capability to produce a more precise estimate of the effect of a particular intervention or treatment, meta-analysis can also pinpoint some of the study characteristics responsible for the heterogeneity in the outcomes from the different primary studies. Thus, the objective of this symposium is to review some applications of Bayesian statistics and meta-analysis in food microbial safety, as two powerful tools capable of updating, integrating and summarising research outcomes from different independent studies.

S18 Recent Developments in Food Mycology: From Safety to Spoilage

ROB SAMSON: *CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands*
JOS HOUBRAKEN: *CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands*
ULF THRANE: *Technical University of Denmark, Lyngby, Denmark*
JENS FRISVAD: *Technical University of Denmark, Lyngby, Denmark*
NARESH MAGAN: *Cranfield University, Bedfordshire, United Kingdom*
LUDWIG NIESSEN: *Lehrstuhl für Technische Mikrobiologie, Freising, Germany*
EMILIA RICO-MUNOZ: *BCN Research Laboratories, Inc., Rockford, TN, USA*

Filamentous fungi, also called molds, are one of the major causes of deterioration of foods such as pasteurized juices and beverages as well as of acid and acidified foods including fermented dairy products. This results in large economic losses. In addition, mold growing in these foods have the potential of negatively affecting human health due to the production of mycotoxins and other secondary metabolites. However, there has been very little research on the production of these metabolites under low oxygen conditions and at low pH. In this symposium, different aspects of the role of molds in both food safety and food spoilage will be discussed. The symposium will start with a general overview of the association of fungi with foods and their role in food safety and food spoilage. Other topics will include the most recent developments in detection and identification of fungi and filamentous fungi nomenclature (One fungus = One Name); the effect of pH and oxygen in mycotoxin production and the climate change factors and its effect on mycotoxin production in commodities. The latest model for the mechanisms involved in the induction of beer gushing due to particular mold metabolites will be presented. The role of heat-resistant mold ascospores found in the processing environment in the spoilage of juices and beverages as well as the best way for their elimination will also be discussed. This symposium is going to be presented by members of the International Commission in Food Mycology (ICFM; www.foodmycology.org) of the International Union of Microbiological Societies (IUMS).

S19 The Never-ending Quest for Discriminatory Power: Updates on Molecular Analytics of Foodborne Pathogens

JOHN BESSER: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
EDWARD DUDLEY: *The Pennsylvania State University, University Park, PA, USA*
ERIC BROWN: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
DAVID WHITE: *U.S. Food and Drug Administration, Silver Spring, MD, USA*

New genomic technologies present the potential for more rapid, reliable, and useful typing information for common foodborne pathogens. Microbial subtyping is one of the major methodologies to attribute foodborne infectious diseases to their putative sources (e.g., food, environment, animals). Currently, there are numerous molecular techniques being used globally to characterize microbial pathogens that IAFP members may not be familiar with. The proposed symposium will review the capabilities of several cutting-edge technologies that are being used in the food safety arena to discriminate genetic relatedness of foodborne pathogens. Speakers from academia and government will present their perspectives on the advantages and disadvantages of these technologies and how the most promising methods can be incorporated into industry and regulatory food safety strategies.

S20 I Want It All and I Want It Now: Metagenomics and Food Safety

JOHN BESSER: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
ERIC BROWN: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
STEVEN MASSEY: *University of Puerto Rico, San Juan, PR, PR*

Metagenomics will likely change the way we do business in food safety microbiology in just a few years. This emerging technology has the potential for revolutionizing our ability to understand and monitor almost every micro-environment which contributes to food safety...such as those of food plants and animals, processing facilities, finished products, and even food consumers. Metagenomics should make possible a wide range of applications for identifying foods and food ingredients, and understanding microbial community dynamics that lead to or prevent disease. However, the quantity and complexity of data needed for many metagenomic analyses currently make it impractical for routine use. What will it take for metagenomics to be accessible as a routine diagnostic tool for food safety testing? This session will describe the current bottlenecks and breakthroughs needed, and will review the state-of-the-art and future prospects for such diverse applications as determining the diets of ancient peoples and modern outbreak victims, determining the composition of foods, examination of microbial communities that make foods more-or-less susceptible to pathogen contamination, and in situ characterization of known, unknown, and un-culturable pathogens. Metagenomics is coming, and it may be sooner than you think.

S21 The Worlds of Shiga Toxin-producing E. coli and Beef Continue to Collide: So What's Happening Lately?

RANDALL PHEBUS: *Kansas State University, Manhattan, KS, USA*
MICK BOSILEVAC: *U.S. Meat Animal Research Center, Meat Safety and Quality Research Unit, Clay Center, NE, USA*
GARY ACUFF: *Texas A&M University, College Station, TX, USA*

The importance of safety of beef and beef products has been a focus of regulatory agencies, industry, as well as researchers due to the vast number of human illnesses caused by the consumption of beef contaminated with Escherichia coli O157:H7 and, more recently, even the non-O157:H7 shiga-toxin producing E. coli (STEC).

Despite the fact that several other foods, such as produce, dairy products, and also drinking water can act as vehicles for the transmission of STECs, reports have been well documented regarding the high prevalence of STECs in ruminants. According to recent reports, STECs are estimated to cause more than 265,000 illnesses each year in the United States, with more than 3,600 hospitalizations and 30 deaths. Typically STEC infections cause diarrhea, but some patients may develop hemolytic uremic syndrome (HUS), a severe complication characterized by renal failure, hemolytic anemia, and thrombocytopenia that can be fatal.

Most STEC infection and cases of HUS in the U.S. have been caused by STEC O157, however, non-O157 STEC have also caused U.S. outbreaks. In light of new developments in detection methods to rapidly and accurately detect STECs, it is now time to get an update on the current efforts on isolation, detection, and control of STECs in beef.

This symposium will focus on any recent changes over the past five years in the prevalence of STECs in beef cattle, development of novel, state-of-the-art methods for detection of STECs in beef cattle and current efforts to reduce the prevalence of STECs at the pre- and post-harvest stages, along with regulatory updates on reducing the risk of STECs in beef.

S22 Changing the Dogma on Meat Shelf Life

XIANQIN YANG: *Agriculture and Agri-Food Canada, Lacombe, AB, Canada*
MARK TAMPLIN: *University of Tasmania, Hobart, Australia*
IAN JENSON: *Meat and Livestock Australia, North Sydney, Australia*

Chilled vacuum-packed meat was introduced in the 1970s as a means of extending the shelf life of meat and has since successfully dominated international trade as well as significant domestic markets. Since the introduction of vacuum packing the shelf life of these products has become even more extended, but not documented in the scientific literature until recently. The shelf life of vacuum packed beef can exceed 6 months under controlled laboratory conditions.

Investigations have revealed that the microbial communities developing in these products seem to have changed from those described in the early literature- whether due to technological changes in the meat packing sector or advances in microbial systematics. Carnobacterium now seems to be the dominant genus rather than *Lactobacillus*.

Microbiologists like to concentrate on bacterial numbers in these products, whereas consumers are

influenced by appearance, odor, taste and tenderness. This discrepancy leads to debate about how shelf life should be determined. Additionally, the control of temperature in supply chains is frequently not the ideal conditions used in the laboratory.

This symposium will present the latest research on shelf life, microbial communities, and implications for supply chains, consumers and regulators.

This symposium is dedicated to the memory of Dr. Colin Gill, who made many scientific contributions to meat microbiology.

S23 Using Foodborne Disease Surveillance Performance Measures to Improve the Timeliness and Effectiveness of Foodborne Disease Outbreak Investigations

CRAIG HEDBERG: *University of Minnesota, Minneapolis, MN, USA*
GWEN BIGGERSTAFF: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
RACHEL JERVIS: *Colorado Department of Public Health and Environment, Denver, CO, USA*
JOSH ROUNDS: *Minnesota Department of Health, St. Paul, MN, USA*

The Council to Improve Foodborne Outbreak Response (CIFOR) developed its Guidelines for Foodborne Disease Outbreak Response to serve as a comprehensive source of information on foodborne disease investigation and control. The Guidelines included measurable performance indicators of effective surveillance for enteric diseases and for response to outbreaks by state and local public health officials.

Since publication of the first edition of the *Guidelines* in 2009, there has been more emphasis placed on performance, accountability and transparency by public health agencies. Therefore, a need was identified to develop target values to help state and local public health agencies document their performance and effectiveness for foodborne disease surveillance and outbreak control activities. Given the distributed public health system with multiple independent jurisdictions, the performance targets were intended to provide a framework for communicating best practices for surveillance activities and create clear expectations for performance to increase the likelihood of compliance.

The target ranges for selected CIFOR *Guidelines for Foodborne Disease Outbreak Response* performance measures were developed to comprise a set of core measures feasible for all states to collect, with explanations of how to do so and why. These were based on the performance measures in the CIFOR *Guidelines* and on those developed and used by the Centers for Disease Control and Prevention (CDC) Foodborne Diseases Centers for Outbreak Response Enhancement (FoodCORE). Improvements in many surveillance functions have been documented within FoodCORE sites. The Integrated Food Safety Centers of Excellence are using the CIFOR performance measures with target ranges to evaluate the timeliness and effectiveness of foodborne disease outbreak investigations, with a focus on assisting states outside of the Centers.

The session will summarize and evaluate the use of the performance measures to improve foodborne disease surveillance and outbreak control activities.

S24 The Puzzle of Microbial Traceability: Unraveling through Industrial and Regulatory Know-how

PETER GERNER-SMIDT: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
SHERRI MCGARRY: *U.S. Food and Drug Administration, College Park, MD, USA*
MICHAEL ROBACH: *Cargill, Minneapolis, MN, USA*

Microbial traceability is an important process to determine the source of contamination in the event of outbreak and industrial product development, and supply chain integrity failure. The merging of molecular typing methods with improved surveillance networks and databases has proven invaluable in enhancing recognition, investigation, and control of food borne disease outbreaks, particularly those that are separated in space and time. However, these molecular systems and the process of microbial traceability are not always that straightforward and on occasion either fail to lead to a source or mislead to a wrong source (e.g., tomato and pepper are often victims of outbreak investigations) that not only lead to resource mismanagement and delaying the process of microbial risk management and communication but sometimes also cause huge economical adversity to the misidentified food commodities in the marketplace. This speakers in this session will address the process of microbial traceability in the industrial (product contamination) and public health (outbreak) settings, the science used in the microbial traceability (relevant molecular techniques and databases), and the root causes of success and failure of microbial traceability and how we can prepare better from the lessons learned from previous stories of microbial traceability. This knowledge can be applied for future food safety (all IAFP members) and quality (industry concern) improvement programs.

S25 Importance of Codex, Regulations, Standardization, Validation, Certification, Verification and Accreditation in Global Trade

EMILIO ESTEBAN: *U.S. Department of Agriculture-FSIS-OPHS-EALS, Athens, GA, USA*
PATRICE ARBAULT: *BioAdvantage Consulting, Orlienas, France*
DANIELE SOHIER: *ADRIA Développement, Quimper, France*
ERIN CROWLEY: *Q Laboratories, Inc., Cincinnati, OH, USA*
ROY BETTS: *Campden BRI, Gloucestershire, United Kingdom*
TIM JACKSON: *Nestlé USA, Inc., Glendale, CA, USA*

World trade is important. No country can provide all things to all people. But how can countries have assurances that what is being imported is safe? How can an exporting economy meet requirements of the importing country? Regulation, Standardization, Validation, Certification, Verification, Accreditation are important processes that help provide these assurances.

In this symposium, presenters will use the framework of routine analysis in food microbiology to provide clear examples of where each of these processes fit and how they align to provide meaningful results that can be recognized and accepted for global trade.

S26 Molecular Methods to Characterize Microbial Pathogens: Outside the Whole Genome Sequence World

MARTIN WIEDMANN: *Cornell University, Ithaca, NY, USA*
STEPHANE EVOY: *University of Alberta, Alberta, Canada*
ARUN BHUNIA: *Purdue University, West Lafayette, IN, USA*

With the so much attention given to whole genome sequencing, other 'omics' disciplines, such as transcriptomics and proteomics, seem to be below the radar screen. In regard to food safety, these omics can provide much meaningful descriptive data on foodborne pathogens, such as monitoring the response of these microbes in several different environmental conditions. Some speakers in this session will address the application of these omic approaches beyond the development of detection assays, others will discuss molecular- and phage-based methods as alternate methods. Knowledge of how foodborne pathogens respond physiologically to their environment, as captured by transciptomics and proteomics can provide critical insight to the underlying genetic capability of these microbes to adjust and sustain themselves in foods. The impact on food quality (industry concern) and food safety (all IAFP members) can be significant, specifically if applied to improvements in HACCP and FSMA related programs to reduce foodborne illnesses.

S27 Novel Frontiers in Microbiology – Recent Advances in Non-DNA based Rapid Microbial Detection and Identification Methods

ARUN BHUNIA: *Purdue University, West Lafayette, IN, USA*
DAVID HAAVIG: *Micro Imaging Technology, San Clemente, CA, USA*
CURTIS STUMPF: *Crystal Diagnostics Ltd, Rootstown, OH, USA*
BRUCE APPLEGATE: *Purdue University, West Lafayette, IN, USA*

Rapid methods for the detection and identification of foodborne pathogens have seen significant developments since the discovery of PCR. Several robust methods have been developed by exploring different regions of the DNA and modifying existing PCR protocols.

However, the science of rapid detection and identification of foodborne pathogens should not be restricted to DNA. Several other molecules as well as physical and chemical properties of microorganisms can be used in developing these rapid methods. Researchers in the industry and academia have discovered these novel frontiers in microbiology. They have not only developed but also proven the effectiveness of rapid methods using certain unknown properties of microorganisms or developing a completely novel technique.

The symposium aims at showcasing the recent advances in the field of non-DNA based rapid methods for foodborne pathogen detection and identification. It also intends to open up discussions about non-DNA based methods, encourage microbiologists to discover novel methods and provide industry with an insight into the future of rapid detection and identification.

This symposium comprises of presentations from scientists representing industry and academia. Speakers from the industry will specifically talk on the science behind their technologies and will not be allowed to advertise their products.

S28 Identifying, Prioritizing, and Managing Chemical Hazards: The United States National Residue Program for Meat, Poultry, and Egg Products

KERRY DEARFIELD: *U.S. Department of Agriculture-FSIS, Washington, D.C., USA*
LOUIS BLUHM: *U.S. Department of Agriculture-FSIS, Athens, GA, USA*
SCOTT GOLTRY: *North American Meat Institute, Washington, D.C., USA*
DAVID HRDY: *EPA, Crystal City, VA, USA*
AMBER MCCOIG: *U.S. Food and Drug Administration, Rockville, MD, USA*
CHARLES SANTERRE: *Purdue University, West Lafayette, IN, USA*

The Food Safety and Inspection Service (FSIS) oversees the United States National Residue Program (NRP) for meat, poultry and egg products in partnership with the Food and Drug Administration and the Environmental Protection Agency as well as with the support of other federal agencies. In 2012, FSIS announced significant enhancements to the NRP including the implementation of multi-analytic methods to analyze veterinary drugs and pesticides and a new sampling scheme to better evaluate chemical hazard exposure. These changes are the foundation for the additional improvements FSIS is making to the current framework to identify, prioritize, and manage chemical hazards identified in FSIS-regulated products. This symposium will outline what has occurred to date within the NRP, including laboratory developments which enable FSIS to gain more information about a single sample as well as the chemical exposure of a herd or flock represented by that sample. Going forward, FSIS will rely even more on FDA and EPA to communicate information on emerging and remerging chemical hazards as well as on academic research exploring the possibilities of known or unexpected exposure. This symposium is an important step towards introducing an area not traditionally addressed at the IAFP and continuing the dialogue and relationship FSIS has with experts possessing knowledge of chemical hazards and food safety.

S29 Controlling Bacterial Pathogens in Low-water Activity Foods and Spices

SOFIA SANTILLANA FARAKOS: *U.S. Food and Drug Administration, Silver Spring, MD, USA*
SUSANNE KELLER: *U.S. Food and Drug Administration-NCFST, Bedford Park, IL, USA*
DAVID BEAN: *Federation University, Ballarat, Australia*
JANE VAN DOREN: *U.S. Food and Drug Administration-CFSAN-OFDCER, College Park, MD, USA*
WAYNE BENNETT: *McCormick and Company, Sparks, MD, USA*
JEFFREY KORNACKI: *Kornacki Microbiology Solutions, Inc., McFarland, WI, USA*

Low water activity (aw) and dried foods such as dried dairy and meat products, grain-based and dried ready-to-eat cereal products, powdered infant formula, peanut and nut pastes, as well as flours and meals have increasingly been associated with product recalls and foodborne outbreaks due to contamination by pathogens such as Salmonella spp. and enterohemorrhagic *E. coli*. In particular, recent foodborne outbreaks and product recalls related to Salmonella-contaminated spices have raised the level of public health concern for spices as agents of foodborne illnesses. Presently, most spices are grown outside the U.S., mainly in 8 countries: India, Indonesia, China, Brazil, Peru, Madagascar, Mexico and Vietnam. Many of these countries are underdeveloped and spices are harvested and stored with little heed to sanitation. The FDA has regulatory oversight of spices in the United States; however, the agency's control is largely limited to enforcing regulatory compliance through sampling and testing only after imported foodstuffs have crossed the U.S. border. Unfortunately, statistical sampling plans are inefficient tools for ensuring total food safety. As a result, the development and use of decontamination treatments is key. This symposium will provide an understanding of the microbial challenges to the safety of low aw foods and provide a historic backdrop to the paradigm shift now highlighting low aw foods as vehicles for foodborne pathogens. Up-to-date facts and figures of foodborne illness outbreaks and product recalls will be provided. Special attention will be given to the uncanny ability of Salmonella to persist under dry conditions in food processing plants and foods. A lecture is specifically dedicated to processing plant investigations, providing practical approaches to determining sources of persistent bacterial strains in the industrial food processing environment. A lecture will also address the range of decontamination processes for spices

S30 Chasing "Zero": How Likely to Reach Success?

ROBERT BUCHANAN: *University of Maryland, College Park, MD, USA*
PAMELA WILGER: *Cargill, Wayzata, MN, USA*
DAVID GOMBAS: *United Fresh Produce Association, Washington, D.C., USA*
CHRISTOPHER WALDROP: *Consumer Federation of America, Washington, D.C., USA*

Providing foods that are free from chemicals, microbial pathogens and any other material that could be hazardous or deleterious to public health is an underlying basic for all parties involved with food safety. As we do not live in a perfect world, what are the issues and what will it take to achieve that "perfect world" of foods free from any risk of contamination. The food industry has a major stake in taking "reasonable" efforts to ensure a safe food supply. But they do not act alone as governments tend to act as an oversight function, in cooperation with food producers, and the public also plays

an active role beyond just being consumers. In this symposium, we provide a forum in which an overview of risk is discusses then followed by industry, public and government perspectives on the current state of affairs and with that magic wand, what the future could bring.

S31 I Found a Positive, Now What Do I Do?

DOUGLAS MARSHALL: *Eurofins Scientific, Inc., Fort Collins, CO, USA*
RICHARD BROUILLETTE: *Commercial Food Sanitation, Harahan, LA, USA*
EDITH WILKIN: *Leprino Foods, Denver, CO, USA*

Product testing and environmental pathogen monitoring programs can be used to verify the implementation and effectiveness of preventative controls as appropriate to the facility processing steps and the nature of the food. Each monitoring program is essential to reduce the contamination of human pathogens in ready to eat (RTE) food products. Although both testing programs were not included in the current proposed preventive control rule of the US food safety modernization act (FSMA), a facility is expected to have written procedures for product testing, environmental monitoring, and corrective actions to address the presence of a pathogen in RTE products or in the processing environment. How to react to positive test results found in product testing and environmental monitoring has not been well illustrated and communicated. The objective of this symposium is to share the experience in finished product testing and environmental monitoring by food safety experts with hands on experiences. The symposium will be focused on the corrective actions when positive results are found to help improve product testing and environmental monitoring programs for food manufacturing plants.

S32 Today's Food Safety and Risk Communication Environment: Solutions-based Strategies to Improve Public Understanding

ROBERT GRAVANI: *Cornell University, Ithaca, NY, USA*
WILLIAM HALLMAN: *Rutgers University, New Brunswick, NJ, USA*
CHAD WEIDA: *Abbott Nutrition, Columbus, OH, USA*
DEBORAH BLUM: *University of Wisconsin-Madison, Madison, WI, USA*
MATT RAYMOND: *International Food Information Council and Foundation, Washington, D.C., USA*
AURORA SAULO: *University of Hawaii at Manoa, Honolulu, HI, USA*

According to the 2014 IFIC Foundation Food & Health Survey, consumer confidence in the safety of the U.S. food supply is on the decline from 78% at its peak in 2012 to now only 66%. Despite advances in food safety technologies and a seemingly more transparent food system, it is therefore intriguing to observe such a decline in consumer confidence. While we can't isolate any one factor to account for this decline, a few observations are worth discussing. Over the past five years, an uptick in food misinformation disseminated by "food activists" such as Food Babe, Dr. Mercola, and Dr. Oz have not only been influential in rousing consumer outrage and advocacy, but have also impacted and influenced the food industry. Such influential so-called "experts" have adapted well by employing diverse methods of communication and media. Their language, tone, and delivery resonates with consumer audiences through personal salience and reaches concerned parents and foodies much more effectively than the multi-syllabic and technical jargon that most scientists use. This disconnect inevitably creates a broad chasm between scientific communication and public knowledge. The discourse has now spread into the diverse American culture in which scientists are finding similar difficulties communicating science and food safety to varying socio-ethnic-economic communities. Preconceived notions, prejudices, and lack of transparency leading to mistrust of both consumers and scientists continue to plague science communication. This proposed session is outlined to begin or continue the dialogue among food safety experts and professionals and identify a practical approach to reach consumers with science communications. The ultimate goal is to enhance attendee knowledge about public perceptions from producer to consumer; advance knowledge about today's traditional and social media environments; and provide best practices for effectively reaching target audiences.

S33 Filling the Food Safety Void in Small and Very Small Food Companies

MANPREET SINGH: *Purdue University, West Lafayette, IN, USA*
DINA SCOTT: *Darden Restaurant, Kennesaw, GA, USA*
BENJAMIN CHAPMAN: *North Carolina State University, Raleigh, NC, USA*
JUDY HARRISON: *University of Georgia, Athens, GA, USA*

Local foods being sold in grocery stores and farmers markets have gained significant popularity among consumers. Several entrepreneurs have successfully established small and very small food businesses selling unique products including unpasteurized goat cheeses to locally harvested produce. However, their potential to grow is restricted by little or lack of food safety knowledge. Consequently, these businesses cannot comply with the regulations and face potential shutdown or sell products without appropriate food safety measures.

The food safety issues faced by smaller businesses are slightly different than the well-organized ones. Most importantly, small businesses do not have a trained food safety professional on staff and their knowledge on food safety rules is minimal. Although they are capable of implementing food safety programs, they need guidance from food safety professionals.

The proposed symposium will focus on understanding the food safety needs of small and very small food businesses, discuss the hurdles they face in implementing food safety programs, and provide some suggestions that can help them to ensure food safety.

S34 Validation of Multi-hurdle Lethality Treatments for Specialty/Niche Meat and Poultry Products Produced by Small Establishments

MERYL SILVERMAN: *U.S. Department of Agriculture-FSIS, Washington, D.C., USA*
MOHAMMAD KOOHMARAIE: *IEH Laboratories & Consulting Group, Lake Forest Park, WA, USA*
BARBARA INGHAM: *University of Wisconsin-Madison, Madison, WI, USA*

With increasing interest in specialty fermented and dried meat products such as jerky, biltong, basturma, and soudjouk there is a need for validated processes that utilize multi-hurdle approaches to achieve lethality of pathogens and that can be implemented by small and very small processors. Multi-hurdle approaches (lethality achieved in multiple stages) are used for many ready-to-eat (RTE) products to achieve lethality of pathogens since these processes do not often include sufficient heat treatment. Outbreaks associated with these products have been attributed to inadequate scientific support for the lethality treatment. Under Hazard Analysis and Critical Control Point (HACCP) regulations, FSIS regulated processors are required to control the food safety hazards in their products and validate that their systems work as intended. This symposium will bring together speakers from academia, industry, and government to present tools that small and very small processors can use to support the safety of RTE meat and poultry processes to comply with FSIS regulations and reduce the risk of foodborne illness from their products.

S35 Benefits of an International Standardization for Challenge Tests for Fair Food Trade

DANIELE SOHIER: *ADRIA Développement, Quimper, France*
JENNY SCOTT: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
RHONDA FRASER: *Fonterra Research and Development Centre, Palmerston North, New Zealand*
ALEJANDRO AMEZQUITA: *Unilever, Sharnbrook, United Kingdom*

To foster fair and open food trade, regulatory harmonization between exporting and importing countries remains a key issue. Facing the expansion and diversification of the food trade, the Codex Alimentarius provides internationally recognized standards, guidelines and codes of practice related to food manufacture. Those guidelines have served the worldwide development of national and international guidelines/regulations integrating challenge tests as a requirement for various food items. For ready-to-eat and/or perishable food which supports microbial growth, various guidelines have been developed to facilitate the validation of preventive controls and product shelflife that is required by regulations, HACCP plans and food safety management systems, to ensure microbiological safety and quality for specified processing, storage and handling conditions. Thus food business operators and establishments responsible for the manufacture of a product need to demonstrate compliance with the food safety criteria throughout shelflife, which may include conducting challenge test studies combined or not with the use of predictive models to estimate the effectiveness of preservation conditions and the effects of modifying product composition or storage conditions on microbial growth behavior. In 2010, the U.S. National Advisory Committee on Microbiological Criteria for Foods published guidelines for conducting challenge studies on pathogen inhibition and inactivation studies in a variety of Foods. Recently, an ISO TC34/SC9 working group was created to establish international standard on guidelines for conducting challenge tests and assess food shelflife with the recommendation to address both i) bacteria growth and ii) bacteria survival and inactivation.

During this session, lectures will address these issues and will cover questions such as what are the current guidelines and requirements worldwide? Why an international standard? How to comply with microbial quality indicators in the food industry when regulation mainly concerns pathogen? What are the needs and requirements for food industries and for regulatory stakeholders?

S36 What's on Your Plate or What's That in Your Suitcase: What Exactly is Coming in from Our Imports?

MICHAEL ROBACH: *Cargill, Minneapolis, MN, USA*
JOAN PINCUS: *McCormick and Company, Sparks, MD, USA*
GILLIAN KELLEHER: *Wegmans Food Markets, Inc., Rochester, NY, USA*

Focus of this symposium will be on what microbial hazards that may be present in imported foods including the importation of bushmeat, whether legally or smuggled. Many foods are single ingredient commodities yet many would be surprised on the number of foods that have up to 100 ingredients which in this day of global commerce truly provides an international flavor. Speakers will detail how foods are made with ingredients from around the world, discuss the grave concern about fraud in foods, e.g., is that really horsemeat in my meatballs or where did that fish originate from; and an overview of how the world has changed and the effect this has had on the global food supply. Bushmeat is defined as game meat made from wild animals that are hunted and slaughtered, particularly from the tropics of Africa, Asia and the Americas. Of note is the amount that is smuggled illegally into European countries and the U.S. are staggering. Although bacterial pathogens are always a concern, more attention is given to viruses and the potential for rapid spread to both human and animal populations. The importance of these measures can easily be demonstrated by the rapid spread of viruses through local populations and onto larger scales, from within a country and beyond international borders, as we have witnessed with the latest Ebola outbreak as well as other emerging infectious disease agents, such as MERs, Avian flu viruses. Another example of the rapid spread of a microbial pathogen has been documented with porcine virus that had ravaged the pig population in China as well as the pig population in the United States with staggering number of deaths. This symposium will bring to light the potential impact of microbial pathogens in imported and smuggled foods on public and animal health.

S37 Sustainable Sanitation and the Use of "Green" Technologies to Protect the Public Health

NANCY LINDE: *NSF International, Ann Arbor, MI, USA*
FRANK YIANNIS: *Walmart, Bentonville, AR, USA*
DANIEL DAGGETT: *Sealed Air, Sturtevant, WI, USA*

As more and more food companies strive to develop sustainability strategies, food safety professionals may face a challenge to not compromise best practices for sanitation and hygiene. While sustainability initiatives to reduce energy, conserve water, and use 'green chemistry' may have good intentions, what happens if they are at odds with sanitation requirements. The session will examine how sustainability can be used as a lens for innovation and an asset to food sanitation professionals. Presenters will share "pitfall" experiences and lessons learned regarding how to evaluate and implement new 'green' technologies to achieve the right balance, where instead of being compromised, food safety is an integral part of an organization's sustainability platform.

S38 Microbiological Air Quality Considerations in the Processing Environment

JEFF KORNACKI: *Kornacki Microbiology Solutions, Inc., Madison, WI, USA*
DAVID BLOMQUIST: *Ecolab, Inc., St. Paul, MN, USA*
DAVID SWINEHART: *Controlled Environment Equipment, Waukesha, WI, USA*

The role of airborne contamination of processed foods is controversial and fraught with contradictory opinions. Some feel that airborne contamination of foods is of great public health significance, whereas others feel it is of little importance. The proposed symposium will address insights on aerosols as a contamination mechanism with respect to exposed food products and food processing equipment. Both food processing plant and air handling equipment design and maintenance will be address with regard to traffic routes, turbulent air movement, filtration and air handling unit condensation. Case studies will be presented along with best practice techniques to monitor air quality. Finally, applicable air quality regulations will be discussed. The symposium will provide food safety, quality, research and engineering professionals an understanding of plant air as potential contamination source during food processing and present applied solutions.

S39 Application of Predictive Risk, Threat, and Vulnerability Tools for Food Safety and Defense

ALAN ERERA: *Georgia Tech, Atlanta, GA, USA*
ANTHONY PAVIC: *Birling Avian Laboratories, Bringelly, Australia*
JOSEPH SCIMECA: *Cargill, Wayzata, MN, USA*
JESSICA COX: *Department of Homeland Security, Aberdeen, MD, USA*
CLINT FAIROW: *ADM, Decatur, IL, USA*
ASHLEY KUBATKO: *Battelle Memorial Institute, Columbus, OH, USA*

The theme of the proposed session is examples or case studies of applications of predictive risk, threat, or vulnerability tools for the purposes of food safety and defense. Though there are a plethora of predictive models that address a wide array of topics from risk to microbial growth and decay to product vulnerability to potential human health consequences, the application of those tools to real world scenarios is relatively atypical. This session aims to illustrate how such tools can be applied to help answer the tough questions facing food industry today.

S40 Steps toward the Practical Use of Microbial Models for Food Safety Assessments by the Food Industry

DENNIS SEMAN: *Kraft Foods, Madison, WI, USA*
PAW DALGAARD: *Technical University of Denmark, Kongens Lyngby, Denmark*
JEFFREY FARBER: *University of Guelph, Guelph, ON, Canada*

Microbial models are becoming an important tool for food safety research for assessing microbial risks that may rise from food manufacturing. Many of these models have become common place and are used by the food industry to determine effects of formulations, processes and handling on the safety of food products. Examples are models for fate of L. monocytogenes in deli meat as affected by many factors including lactate/diacetate content in formulation, thermal processing time and temperature, cross-contamination during slicing, and outgrowth of Clostridium perfringens during cooling. However, many in industry, especially the small food producers, may not be able to use these models effectively. This is largely due to their lack of food safety expertise and/or resources to fully understand, interpret, and validate the models and results. In addition, when using models, the industry professionals may misapply a microbial model or have no confidence on models that are not specific and overly conservative. All these are believed to lead to the limited adaption of microbial models by the food industry. The goals of this symposium are to 1) Illustrate examples and the usefulness of microbial models in food safety assessments for products and processes, 2) illustrate some of the limitations of using microbial models "off the shelf" and provide improvement strategies, 3) provide guidelines for appropriately using microbial models, and 4) examine areas of microbial modeling that need further research.

S41 Viruses in Shellfish: Filtering Expertise toward a New Foundation for Risk Reduction Policies and Practices

WILLIAM BURKHARDT: *U.S. Food and Drug Administration, Dauphin Island, AL, USA*
ENRICO BUENAVENTURA: *Health Canada, Bureau of Microbial Hazards, Ottawa, ON, Canada*
DAVID LEES: *Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth, United Kingdom*
SOIZICK LE GUYADER: *IFREMER, Nantes, France*
MARION CASTLE: *Ministry for Primary Industries, Wellington, New Zealand*
DAVID KINGSLEY: *U.S. Department of Agriculture-ARS, Dover, DE, USA*
LEE-ANN JAYKUS: *North Carolina State University, Raleigh, NC, USA*

Norovirus is a leading cause of foodborne illnesses worldwide. Contamination of food, particularly bivalve molluscan shellfish, with this pathogen most often occurs during production, and is only infrequently associated with improper storage, handling, or preparation. The risk of norovirus infection associated with consumption of bivalve molluscs is elevated relative to other commodities because these animals are often eaten raw or lightly cooked. In order to lead successful efforts at reducing consumer exposure to norovirus through this food commodity, it is imperative that appropriate safeguards be implemented to minimize the potential impacts of viruses that enter shellfish harvest areas from municipal and other wastewater sources. Systems level quantitative risk assessments for norovirus in bivalve molluscs can provide critical insight to inform the development of updated food safety risk management practices and policies.

This symposium will focus on new approaches and recent advances in the characterization of potential hazards, exposure, and risk posed by norovirus in bivalve molluscs. Six experts from a variety of disciplines will present evidence of shellfish contamination and human illness from consumption of shellfish, development of risk assessments that quantify exposure and risk of illness, and development of risk management options to reduce human health risk from exposure to contaminated shellfish. Significantly, this symposium brings together subject matter experts, risk assessors, and risk managers to discuss an important worldwide food safety issue.

S42 Everything But Salmonella – Other Microbiological Hazards in Low-water Activity Foods

EDITH WILKIN: *Leprino Foods, Denver, CO, USA*
LISA LUCORE: *Kellogg Company, Battle Creek, MI, USA*
SUSANNE KELLER: *U.S. Food and Drug Administration, Bedford Park, IL, USA*

The burden of foodborne illness and the number of food recalls associated with microbial hazard contamination of low water activity foods (LAwF) has been on the rise in recent years. These foods are naturally low in moisture or are produced from higher moisture foods through dehydration and other processes. LAwF are generally perceived as safe by consumers, and many LAwF are consumed as ready-to-eat products with no consumer-level pathogen reduction step such as cooking. While LAwF are susceptible to contamination from a wide range of microbial hazards, Salmonella has emerged as the pathogen of concern in many of these products, and most control efforts have been directed at this pathogen. Other relevant microbiological hazards of concern may include: Cronobacter sakazaki; Listeria monocytogenes; shiga toxin producing Escherichia coli; Pseudomonas spp.; the spore formers Clostridium perfringens; Staphylococcus aureus; and Bacillus cereus; and toxigenic molds. Although pathogens cannot grow in LAwF due to the low Aw, many pathogens can survive and remain viable for extended periods. Pathogen populations may increases after reconstitution and/or during preparation of food products prior to consumption. Traditionally effective processing interventions achieving >5 log reduction used for high moisture level foods, are generally not as efficacious when applied to LAwF. The combination of low Aw with the high solute and/or fat content may contribute to enhanced survival and thermal resistance of pathogens in these foods. The most important control measures for LAwF involve preventing cross-contamination, good manufacturing practices, and hazard analysis critical control point (HACCP) programs that vary depending on the microbiological

hazard and LAwF. Audits and microbial monitoring of LAwF products and food processing environments are also important strategies for industry to monitor food safety and will vary based on microbiological hazards.

S43 In-process High Moisture Foods: *Staphylococcus aureus* and *Bacillus cereus* Food Safety Concerns

ERIN HEADLEY: *Schreiber Foods, Inc., Green Bay, WI, USA*
LORALYN LEDENBACH: *Kraft Foods, Glenview, IL, USA*
BALASUBRAHMANYAM KOTTAPALLI: *ConAgra Foods, Omaha, NE, USA*

New food safety regulations for HACCP and Food Safety plans include rigorous hazard analysis processes, as well as increased transparency requirements for the scientific basis of hazard analysis decisions. During the hazard analysis of food processing steps, considerations must be given to in-process high moisture food systems that may support the growth of *Staphylococcus aureus* ((materials at 4.5 < pH< 9.6; aw >0.85) and/or *Bacillus cereus* (materials at 4.5 < pH< 9.6; aw > 0.91). Given favorable conditions, these in-process materials may produce heat stable toxins during routine manufacturing, in the absence of proper controls. This symposium will discuss various in-process risk scenarios and possible mitigation strategies during routine manufacturing conditions. This will also help manufacturing facilities generate scientific basis for the hazard analyses which are necessary for their food safety plans.

S44 How Do I Validate That? Assuring Credibility of Process Controls for Pathogen Reduction

NANCY BONTEMPO: *Mondelez International, East Hanover, NJ, USA*
MARTIN WIEDMANN: *Cornell University, Ithaca, NY, USA*
BALASUBRAHMANYAM KOTTAPALLI: *ConAgra Foods, Omaha, NE, USA*
RICHARD WHITING: *Exponent, Inc., Bowie, MD, USA*
DARINKA DJORDJEVIC: *ILSI North America, Washington, D.C., USA*
LINDA J. HARRIS: *University of California, Davis, CA, USA*
MICHELLE DANYLUK: *University of Florida, Lake Alfred, FL, USA*
STEVE CALHOUN: *American Peanut Council, Cleveland, TN, USA*
ROBERT BUCHANAN: *University of Maryland, College Park, MD, USA*
DONALD W. SCHAFFNER:

The validation of control measures has historically been a requirement of food safety management systems and is increasingly required by certification bodies, regulatory agencies and customers. Criteria for the design, execution, and interpretation of validation studies are often unavailable or unclear for control measures beyond traditional canning, acidification, or pasteurization. A wide range of products are subjected to processing steps that provide varying levels of pathogen reduction. The approach used for almond process validation has provided an example of the establishment by an industry segment of standardized criteria for the design and evaluation of a challenging process. This session discusses approaches to validation of control measures and control measure combinations as well as the qualification and role of an expert Process Authority in determining the credibility of process validation.

S45 Foodborne Pathogens in Apples, Stone Fruits, Avocados, Mangos, Papaya…A New Trend or Sporadic Incidence?

DUMITRU MACARISIN: *U.S. Food and Drug Administration, College Park, MD, USA*
YI CHEN: *U.S. Food and Drug Administration, College Park, MD, USA*
MICHELLE DANYLUK: *University of Florida, Lake Alfred, FL, USA*
ANDERSON DE SOUZA SANT'ANA: *University of São Poalo, São Paolo, Brazil*
ANA LUCIA PENTEADO: *Embrapa Meio Ambiente, Campinas, Brazil*

Fresh produce-associated outbreaks of foodborne illnesses frequently involved leafy greens, berries, vegetables, and fruit vegetables (e.g. cantaloupes, tomatoes, bell pepper, etc.). However, in the past few years, other fresh produce commodities have emerged as new concerns for human pathogen contamination. The 2015 multistate listeriosis outbreak associated with contaminated caramel apples, the 2014 recall of stone fruits due to *L. monocytogenes* contamination, the 2014 surveillance findings of *L. monocytogenes* on avocados, and previous *Salmonella* outbreak associated with contaminated mangos have brought tree fruits to our attention as an emerging food safety concern. Limited research has been performed on tree fruits because these fruits are not in direct contact with soil, irrigation water, or organic fertilizers and thus were considered relatively safe. Therefore, an improved understanding of the origin and spread of human pathogens on tree fruits is needed. The purpose of this symposium is to educate the scientific community on the prevalence, contamination level, risks, routes of contamination, behavior, persistence and control of human pathogens on select tree fruits.

S46 Approaches to the Management of Viruses in the Food Industry

NIGEL COOK: *The Food and Environment Research Agency, York, United Kingdom*
RUTH PETRAN: *Ecolab, Inc., Eagan, MN, USA*
FABIENNE LOISY-HAMON: *Ceeram, La Chapelle Sur Erdre, France*

Although human norovirus is a major cause of foodborne illness in many parts of the world, the risks and methods for control along the food system are still not well understood.

In this symposium, the audience will be provided with information about the challenges faced in the management of foodborne viruses, along with practical approaches to their control.

Attendees will learn about the risk of viruses in the retail environment, and will gain insights into the best practices for their control. The control of viruses in primary production will also be addressed, incorporating perspectives from recent European and international initiatives. In addition, the role of monitoring viruses in food will be discussed, highlighting the challenges faced when embarking on a testing regime, while regulatory considerations and successes from implementing virus testing in the food industry will also be covered.

It is anticipated that attendees will be better equipped to address virus risks in their products after attending the symposium. They will have a greater understanding of how susceptible their raw materials are to viral contamination, how reliable methods of control are in preventing contamination from reaching consumers, and of the pros and cons of virus testing along the food production chain.

S47 Infodemiology: Let's Turn Big Data into Knowledge for Decision Making

JEANNE JONES: *ConAgra Foods, Omaha, NE, USA*
TARA CLARK: *ConAgra Foods, Omaha, NE, USA*
AMY KIRCHER: *University of Minnesota, St. Paul, MN, USA*
HUBERT DELUYKER: *European Food Safety Authority, Parma, Italy*
MARTIN WIEDMANN: *Cornell University, Ithaca, NY, USA*

Each day the world's data footprint increases exponentially amassing large and complex collections of data sets. There is great power in the plethora of this data to inform our decisions. Analyzing the diverse and unstructured data in a meaningful way is the key to informing operational decision and policy.

Harnessing the power of big data will aid us in finding food safety and defense issues which is a number one priority for all persons working in the food and agriculture sector. Early identification and warning of contamination minimizes human and animal suffering, reduces economic consequences, and provides trust in our food supply. Better yet are predictions of disruption that allow decision makers to adjust their behavior.

Based on the success of last year's session, "Big Data: Food Safety's Holy Grail or Pandora's Box?", that introduced the topic, this symposium will build a cohort of professionals that can apply the principles of infodemiology through the collection and analysis of big data, to inform operations, decision-making, and public policy. The symposium will feature three segments: overview of big data and infodemiology, methodologies for using big data, and applications of infodemiology in practice with examples from the industry, academy and government. Attendees will leave with knowledge to find food system disruption, anticipate unknown problems, and rapidly identify emerging issues they have never seen before.

S48 Nibbles, Bits, and Bytes: Leveraging Big Data and Analytics to Inform Food Safety Risk Analysis

BARBARA KOWALCYK: *RTI International, Research Triangle Park, NC, USA*
VASUDHA REDDY: *New York City Department of Health and Mental Hygiene, New York, NY, USA*
ARON HALL: *Centers for Disease Control and Prevention, Atlanta, GA, USA*

Vast amounts of data are being collected throughout the food system: on the farm, in processing, during distribution, at retail, in the home, and in the public health system. There is increasing interest in how to leverage these diverse sources of "big data" to identify and manage food safety risks in both the public and private sectors. The siloed nature of data sources and the persistent lack of data sharing within and between sectors are barriers that need to be overcome, but they are not the only challenges. Although the potential power of big data is widely recognized, many struggle with how to realize that power.

Big data analytics, including statistical methods and data visualization tools, are needed to transform data into actionable information that can support risk-based decision making. A plethora of analytic tools—including synthetic populations, geospatial agent-based models, time-series analysis, and forecasting—have been used to analyze trends, as well as to detect, predict, mitigate, and rank risks.

This symposium will discuss the potential of big data analytic tools for improving food safety and provide attendees with practical examples of how these tools have been applied in the food safety arena. The symposium will conclude with an interactive discussion between presenters and attendees.

S49 Metals Exposures in Foods

JORGE G. MUÑIZ ORTIZ: *U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, D.C., USA*
LEILA BARRAJ: *Exponent, Washington, D.C., USA*
JI-EUN LEE: *Kellogg Company, Battle Creek, MI, USA*

As numerous metals are a normal part of the environment, they conceivably have always been a constituent of foods. The presence of metals in foods and food ingredients has long been recognized as an important factor in assuring a safe and secure food supply. However, the effect of "chasing zero" from an analytical chemistry perspective has led to the ability to detect metals including Arsenic, Cadmium, Lead, and Mercury in foods at very low (ppb) levels. A number of stakeholders have communicated that there can be no safe level of certain metals in the food supply. This is an unrealistic view, but one that must be addressed from a scientific perspective. Placing the proper context on analytical data for metals in foods or food ingredients by critically evaluating the exposure in such situations is important in the development of sound risk assessments and risk management decisions. A tool, based on NHANES intake data, was developed that provides individuals highly trained in fields such as toxicology or risk assessment with information on exposure to metals from various foods that can be incorporated into risk management decisions associated with findings of certain metals in those foods. This symposium will provide: a) An overview on occurrence of metals in food b) Use of the heavy metal screening tool for risk assessment and c) Examples to demonstrate the effective use of the heavy metal screening tool in evaluating the low, medium and high risk of the occurrence of heavy metals in foods.

S50 Bacteriophages for Food Safety: Advances in Prevention and Detection

JASON GILL: *Texas A&M University, College Station, TX, USA*
AMANDA KINCHLA: *University of Massachusetts, Amherst, Amherst, MA, USA*
SAM NUGEN: *University of Massachusetts, Amherst, Amherst, MA, USA*
MICHAEL KOERIS: *Sample6, Boston, MA, USA*

Bacteriophages are proving to be versatile and effective tools in food safety. Their unique ability to recognize and infect potential human pathogens has allowed them to be utilized for several aspects including decontamination of foods, and detection of foodborne pathogens. The use of phages on foods has even been granted GRAS status by the USDA and FDA for meat and poultry as processing aids to defend against *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes*. As a medical tool, interest in phage therapy has reemerged due to a high prevalence of antibiotic resistance. This symposium will update the audience on advances in these areas. The first half of the symposium will address the use of phages in a decontamination rinse for meat and produce. Speakers will discuss the use of individual phages as well as cocktails to reduce the bacterial load in food application. The effect of the sample matrix on the decontamination effectiveness will also be addressed. The second half of the symposium will discuss the use of phages for the rapid detection of bacteria. These discussions include advances in the genetic engineering of phages and detection schemes. Included will be the advantages and limitations of phage-based detection for food safety in applications relevant to produce. The increased need for food safety requires us to investigate new technologies. This symposium will update the audience on research in this emerging area.

S51 Global Lab Capacity Building – Training for Ensuring Food Safety

PALMER ORLANDI: *U.S. Food and Drug Administration, Washington, D.C., USA*
LESLIE BOURQUIN: *Michigan State University, East Lansing, MI, USA*
JANIE DUBOIS: *University of Maryland, College Park, MD, USA*
PAMELA WILGER: *Cargill, Wayzata, MN, USA*

Although lab capacity building has taken on a life on its own over the past few years, there are other components of the food safety umbrella that remain under-appreciated. The entire spectrum of what is necessary to produce a safe food supply has drawn the attention of countries worldwide as well as all the other entities, e.g., international organizations dedicated to food safety, food industry, NGOs, and regulatory agencies to address the need to improve the food supply in the 21st century. The objective of this symposium is to demonstrate the utility and integration of lab training within individual countries food safety program. This is to ensure that their food production conforms to international standards for food safety, not only their own domestic consumption but also as a trusting partner in the global economy. Each speaker will share their experiences that addressed how countries or their respective organizations used analytical and microbiological, hands-on laboratory training to improve food safety programs. Specifically, they will provide insightful models that were successful and furthermore, identify the challenges and obstacles that lie ahead. These will reflect the findings of a number of international organizations, including government, food industry and the private sector, that have identified critical gaps in lab capacity building that includes training and retention of lab personnel. Training is not a stand-alone caveat to fix the challenges faced by many countries. In addition to lab training and a suitable physical laboratory facility, any improvement to an individual country's ability to respond to food safety issues needs to focus on a number of diverse but integrated aspects; appropriate management and leadership, laws, regulations and policies, an understanding of QA policies and practices, procurement, information management system, and maintaining and improving the physical facility infrastructure. Lastly, the commitment of resources and efforts to train lab analysts for proficiency and to learn the necessary skills to adapt to and adopt new technology.

S52 Environmental Monitoring: A Preventative Control Requiring the Closed Loop Method of Food Safety Education

JOHN BUTTS: *Land O Frost, Lansing, IL, USA*
HOLLY MOCKUS: *Alchemy Systems, Austin, TX, USA*
MIRIAM EISENBERG: *EcoSure, a Division of Ecolab, Lincolnshire, IL, USA*

By August of 2015, the FDA will issue the final rules for preventive controls under the Food Safety Modernization Act. One of the preventive controls that will be expecially impactful and fully scrutinized by regulators is the comprehensive environmental monitoring program. The success of an environmental monitoring program is dependent on plant employees and their knowledge the process and how each of them plays an important role. A workforce that is empowered to recognize critical environmental conditions that can contribute to pathogen growth is the first line of defense against contamination issues. The purpose of this session is to communicate the three legs of a successful environmental monitoring program using the closed loop method of employee engagement – designing and developing a monitoring program and understanding how the pieces fit together, behavior based food safety education, and establishing clear communication for an employee driven food safety culture.

First, the session goes back to basics on building a comprehensive environmental monitoring program through sanitation and sampling, benchmarking through the use of the key metrics, and identifying desirable employee behaviors that impact preventative controls. Secondly, the session will address the reality that food safety is dependent on human behavior. Understanding how to leverage behavioral science to fuel a successful education program is imperative to prevent the proliferation of pathogens in the manufacturing environment. Studies in the industry are proving that post-training observations mixed with corrective actions, and positive reinforcement coaching is an effective process for allowing employees to recognize their role in the prevention of pathogen growth. Finally, a culture of effective communication brings the process full circle by empowering employees to take action when they detect a potential contamination issue. The session will close with insights into the best ways to facilitate both top-down and bottom-up communication to drive continuous improvement.

S53 Nanotechnology from Farm to Table: Implications to Food Safety and Human Health

SANGEETA KHARE: *U.S. Food and Drug Administration, Jefferson, AR, USA*
GEORGIOS PYRGIOTAKIS: *T.H. Chan Harvard School of Public Health, Boston, MA, USA*
MARIA KHODAKOVSKA: *University of Arkansas at Little Rock, Little Rock, AR, USA*
TERESA CROCE: *U.S. Food and Drug Administration, Laurel, MD, USA*

Application of nanotechnology in foods covers a broad range of uses, from detection methods for the presence of pathogens and chemicals in foods to the incorporation of nanoparticles in food packaging materials. Furthermore, nanomaterial is used as taste enhancers, antimicrobials, immune regulators, and also in cosmetic applications. Nanoparticles have been used to address major issues pertaining to food safety; food preservation to extend shelf life of foods and antimicrobial activities against bacterial, fungal and viral pathogens. As a relatively new field of science, concerns can be raised as to the safety of such particles either added directly to foods (pathogen and spoilage microbes reduction), as part of food packaging material and the possible migration into foods, and its use in agriculture. Uncertainty remains as to the exposure of nanoparticles and any potential human health complications, including accumulation of metal nanoparticles in various organs, effects on the blood-brain barrier and perturbation of gut associated immune responses as well as the effect on the human gut microbiome. This symposium will present different aspects of the application of nanotechnology in foods and crops and a view from the regulatory perspective.

This symposium will focus on the innovative applications of nanotechnology in the food protection and public health and will bring together the experts in the field of nanomaterials engineering, Nanomedicine, nano-agriculture and risk analysis and risk assessment. Attending this symposium will provide the latest updates in the field of nanotechnology related to the food arena. Furthermore, it will allow the participant to establish and enhance collaborations and identify new alliances for future research.

S54 Delivery Systems for Introduction of Natural Antimicrobials into Foods: Need, Formulation, Applications and Current Trends

P. MICHAEL DAVIDSON: *University of Tennessee-Knoxville, Knoxville, TN, USA*
KANIKA BHARGAVA: *University of Central Oklahoma, Edmond, OK, USA*
JASDEEP SAINI: *WTI, Inc., Jefferson, GA, USA*

Application of lipophilic natural antimicrobials such as essential oils and polyphenols in foods are of great interest for the food industry to meet consumer's needs in terms of food safety, defense and quality. Although, the antimicrobial activities of these compounds have already been proven, they face challenges with their incorporation in complex food systems due to insolubility in water, physical instability or integrity of the food chemistry, and degradation of the antimicrobial activity of these compounds. Moreover, there is a need to optimize concentrations which are high enough to inhibit microbial growth within the limits imposed by food regulations, but at the same time minimally alter the qualitative and aesthetic properties of the product. Nano- or microencapsulation of these antimicrobials represents an efficient approach to overcome these challenges. This strategy can decrease the minimum inhibitory concentration of these natural antimicrobials and increase the concentration of the bioactive compounds in food areas where microorganisms are preferably located, for example water-rich phases or liquid–solid interfaces. This session will discuss the formulation, characterization, and application of these antimicrobial emulsions. Experts in the field of food engineering, processing, and microbiology will present their perspectives on this emerging technology.

S55 Recent Developments in Food Fraud Prevention

SHAUN KENNEDY: *University of Minnesota, The Food System Institute, St. Paul, MN, USA*
ANUP SHARMA: *Alabama A&M University, Huntsville, AL, USA*
KAREN EVERSTINE: *University of Minnesota, Minneapolis, MN, USA*
JEFFREY MOORE: *United States Pharmacopeia, Rockville, MD, USA*

Prevention of food fraud and economically motivated adulteration present a significant challenge to industry and regulatory agencies. As illustrated by melamine adulteration of dairy products in China and horse meat adulteration of ground beef in the EU, large-scale food fraud incidents can result in catastrophic health consequences, widespread recalls, and brand damage. The Food Safety Modernization Act will require additional control efforts by industry aimed at food fraud. However, since food fraud is an intentional act, traditional food safety and quality approaches to assessment and mitigation are not sufficient. This session will present recent developments in the area of food fraud prevention and mitigation. USP has developed a framework to help industry and regulators develop their own systems to assess and mitigate food fraud vulnerabilities. This session will outline USP's proposed food fraud vulnerability Guidance chapter. Conventional Raman spectroscopy is widely used as a research tool in food-science laboratories, but is not typically used for routine applications within the food industry. This session will describe a standoff Raman technique for detecting food fraud-related adulterants in edible oils, milk, and flour, and its potential for use by the food industry. While multiple reports have cited the sale of mislabeled fish species at retail, little information has been provided about the supply chain for these seafood products and the location of the substitution. This session will address the results of a study to determine the prevalence of substitution in three fish species at retail in Minnesota and to describe the supply chains for these products. Finally, this session will describe a tool-box of methods under development aimed at discriminating authentic skim milk powders from those that have been adulterated with both known and unknown adulterants. Some of the tools being explored represent a new paradigm for verifying food ingredient integrity.

S56 The True Prevalence of Food Fraud in Our Global Supply Chain

MITCHELL WEINBERG: *INSCATECH Corporation, New York, NY, USA*
GEORGE HUGHES: *U.S. Food and Drug Administration, Rockville, MD, USA*
ANDREW CLARKE: *Maple Leaf Foods, Etobicoke, ON, Canada*

There has been recent, renewed attention to the practice of intentional adulteration which has been triggered by several scandals and resulted in new expectations in regulations and global standards. Past food defense initiatives mainly revolved around facility security and protective measures to prevent sabotage, tampering, or resulting acts of agroterrorism. It has become apparent that the greater risk might come from economically motivated adulteration, or food fraud, which occurs through intentional substitution, willful blindness, gross negligence, mislabeling, etc. Increased global trade has expanded our access to an international food supply and with it, an increased prevalence of fraud.

The potential impact of food fraud is two-fold: the risk to public health (milk melamine incidents) and the risk to brand trust (Whole Foods Chinese organic produce scandal). Much of this sabotage is motivated by profit and not intended to cause harm. Unfortunately, much of this fraud has caused a huge risk to public health through lack of knowledge on how the adulteration will affect certain populations.

Today's consumer is demanding increased transparency and intention adulteration scandals can be devastating to a company's brand and to the reputation of that country's regulatory body that is charged with protecting our food supply. This is truly an emerging issue that is only going to increase unless companies and governmental bodies put forth mitigation strategies to prevent these acts.

This session will hear from professionals that have dealt with food fraud, first-hand and those that specialize in preventing intentional adulteration. Attendees will get a better understanding of the risks involved in this practice, the true prevalence in our global supply chain, how this has affected food legislation and global standards. They will also learn ways to assess the risk of their raw material supply through vulnerability studies and use mitigation strategies to protect their company or organization.

S57 *Clostridium botulinum*: A Recurrent Emerging Foodborne Pathogen

MIKE PECK: *Institute of Food Research, Norwich, United Kingdom*
SIMBARASHE SAMAPUNDO: *University of Ghent, Ghent, Belgium*
FRANK DEVLIGHERE: *University of Ghent, Ghent, Belgium*
JEANNE-MARIE MEMBRÉ: *INRA, Nantes, France*

Foodborne botulism is a serious disease resulting from ingestion of preformed Clostridium botulinum neurotoxin in foodstuff. Since the 19th century, the heat resistance of this spore forming bacteria has been studied in order to guarantee the public health associated with low acidic, ambient stable products. However, still nowadays, Clostridium botulinumremains an emerging issue. Indeed, there is a consumer demand for high-quality, preservative-free foods that require little preparation time. In particular, to increase the quality (taste, nutrition value, texture), the food manufacturers tend to reduce the thermal treatment of their products.

The symposium will begin with a short introduction on *C. botulinum*, including both proteolytic and non-proteolytic *C. botulinum*, and on the approaches used to control this pathogen in a large variety of foods. The next presentation will consider the safety of minimally heated chilled foods with respect to non-proteolytic *C. botulinum*, and the advancements that have been made using quantitative risk assessment. Then, an example of nitrite level reduction in processed meat products will be presented and more generally, the potential application of fermentates to inhibit the growth of *C. botulinum* will be discussed. Finally, a quantitative risk assessment of proteolytic *C. botulinum* in canned foie gras in France will be introduced. The model outputs have been interpreted into a risk-based food safety management perspective. This work illustrates that despite receiving a mild heat treatment, this canned product does not present any public health risk.

S58 Influence of Climatic Conditions and Climate Change on the Microbial Safety of Food

ISABEL WALLS: *National Institute of Food and Agriculture, USDA, Washington, D.C., USA*
CHENG LIU: *Wageningen University, Wageningen, Netherlands*
RENATA IVANEK: *Texas A&M University, College Station, TX, USA*

Climate is commonly defined as the weather averaged over a long time with a standard averaging period of thirty years while climate change refers to any change in climate over time, due to natural variability or as a result of human activity. Climate change has been described as 'the single most important issue that we face as a global community,' with compromised food safety being an important concomitant. It is reported that, between 1900 and 2005, there has been a 0.45°C rise in the average world temperature and models forecast that the ambient temperature will continue to increase gradually over time, resulting in a 2 to 5°C increase in the extreme daily maximum temperature by the late 21st century. It is thus anticipated that there will be a steady rise in the incidence of gastrointestinal illnesses in the coming years. Indeed, the incidence of salmonellosis and vibriosis has been shown to follow a seasonal pattern, which is statistically correlated with the mean monthly temperature. Moreover, climate change may also have an impact on the routes of transmission of important zoonotic and geonotic pathogens to food crops, such as leafy green vegetables, during the pre-harvest phase. It is worth mentioning that the survivability of these pathogens in the environment is intrinsic to the microorganism in question and may be affected by several climatic factors including temperature, precipitation and sunlight exposure. Thus, controlling microbial contamination of crops in the face of climatic changes relies on a sound consideration of putative contamination routes and meteorological factors affecting pathogen viability. This symposium seeks to highlight the current state of knowledge on the impact of climate change on the microbial safety of food and offer future perspectives with the view to mitigating the risks of food contamination and foodborne illnesses.

S59 Survival of the Fittest: Controlling Listeria at Retail

KRISTINA BARLOW: *U.S. Department of Agriculture-FSIS, Washington, D.C., USA*
KEVIN SMITH: *U.S. Food and Drug Administration, Washington, D.C., USA*
KRISTIN DELEA: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
HILARY THESMAR: *Food Marketing Institute, Arlington, VA, USA*
DAVENE SARROCCO-SMITH: *Lake Couny General Health District, Painesville, OH, USA*

Despite great strides that have been made to reduce product contamination with Listeria monocytogenes (Lm), it continues to cause foodborne illness, particularly from products purchased at retail stores. Control of Lm continues to be a struggle because its growth characteristics (growth at refrigeration temperatures, survival in low pH conditions, and endurance during drying) allow it to survive where other pathogens cannot. Retail facilities are subject to Lm contamination from the environment, incoming raw products, employees, and consumers. Research has shown that Lm forms harborages in retail environments, sometimes for weeks or months, and can contaminate ready-to-eat products, including fresh cut produce, deli meats, salads, and other items. Several retail associations have developed action plans that retailers can take to keep Lm from contaminating products. In addition, government agencies have developed best practice guidance and model regulations that retailers can adopt to help ensure product safety. This symposium will bring together speakers from government, retail associations, and a local health department to discuss best practices and tools that retailers can use to control Lm in their facilities, and ultimately decrease the risk of foodborne illness from their products.

S60 Biofilm Update 2015: Where We Can Find Them, and How We Control Them

HALEY OLIVER: *Purdue University, West Lafayette, IN, USA*
PETER BODNARUK: *Tyson Foods, Chicago, IL, USA*
DIANE WALKER: *Montana State University - Bozeman, Bozeman, MT, USA*
STEPHEN TOMASINO: *EPA, Fort Meade, MD, USA*

Almost all microorganisms outside of a microbiology lab live in microbial communities called biofilms. These communities greatly impact the nature of the organisms that live in them and can affect characteristics such as resistance to antimicrobial agents, tolerance of adverse conditions, nutritional requirements, and even gene expression. Microbial communities are closely linked to the food that we eat and the safety of our food supply. Biofilms are found in the environments where we grow, process, serve, and sell food. They are naturally occurring on foods that we consume and are even present in and on our own bodies. Biofilms are dynamic systems with changing populations and conditions within them. Our understanding of biofilms and how and when we should try to control them is equally dynamic. This symposium will provide an update on biofilms and their relevance to the food industry. Speakers will address the latest information on topics such as laboratory methods to study biofilms, the claims that can be made for systems to mitigate them, the relevance of biofilms in retail and food processing industries, how to control them, and how to detect them.

S61 Challenges to Allergen Detection and Method Selection

TONG-JEN FU: *U.S. Food and Drug Administration, Bedford Park, IL, USA*
TRACIE SHEEHAN: *ARYZTA, LLC, West Columbia, SC, USA*
BRENT KOBIELUSH: *General Mills, Inc., Minneapolis, MN, USA*
SAMUEL GODEFROY: *World Bank Group, Washington, D.C., USA*

Reliable detection methods play a key role in an effective allergen control program. An increasing number of allergen test kits based on different formats for either quantitative or qualitative analyses are commercially available. Food manufacturers use allergen test kits in many areas of food production, including ingredient analysis, validation of cleaning and sanitation procedures, and routine finished product testing. These test kits are also used by public health authorities to investigate consumer complaints and to monitor industry compliance with food allergen labeling regulations. In many cases, the perceived risks are linked to levels of allergen measured. As the number of commercial test kits with different formats and detection targets continues to increase, food manufacturers are faced with the constant challenge in choosing the right tests for their applications. Questions such as what allergen molecules should be targeted, how food processing conditions may affect allergen quantitation and thus the perceived risk, should a quantitative or a qualitative test be used, and what are the best methods and locations for sampling are often raised. This symposium addresses the challenges associated with the use of commercial test kits for analysis of allergen residues in processed foods and for allergen cleaning validation. Speakers will also highlight key consecrations in the selection and application of tests for allergen control and discuss how follow up actions are decided based on test results, from both food manufacturer and government agency's perspectives.

S62 A Bridge between Research and Regulatory Science: Research Prioritization and Outcome Measures

CHRISTOPHER BRADEN: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
MARY TORRENCE: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
DAVID WHITE: *U.S. Food and Drug Administration, Silver Spring, MD, USA*
DAVID GOLDMAN: *U.S. Department of Agriculture-FSIS, Washington, D.C., USA*

As food safety knowledge and activities have evolved, the objectives of the Federal agencies responsible for the safety and security of the food supply have as well. The research goals across these agencies provide both broad and focused strategic approaches depending on their respective regulatory responsibilities. However, there is a continued need to foster a culture of research collaboration between Federal and State government agencies, academia, and private industry to expand scientific capacity and ensure public health metrics are developed and achieved. With the establishment of transparent, collaborative processes for prioritizing science and research needs, Federal agencies can achieve a clear and consistent focus on food safety goals and leverage the regulatory and research capabilities of partners to help meet the highest priorities of protecting the U.S. food supply This symposium will include government speakers that will provide perspectives on how strategic directions and prioritization for research are conducted and discuss a current effort toward measuring outcomes. A common theme will be the discussion of strategic planning efforts by Federal agencies and how they can leverage and strengthen research capabilities of academic and industry partners to meet the highest priorities of protecting the U.S. food supply. The outcome of this symposium is important in light of the 2011 Food Safety Modernization Act which calls for a science-based, public health prevention-oriented food safety system, and the establishment of an infrastructure needed to support such a system. The intended outcome of this symposium is to inform academia and industry of potential research directions and opportunities as well as provide a forum for Federal agencies to highlight new efforts in prioritization and outcome measurement. The expectation is to create new collaborative and strategic efforts in food safety research.

S63 Mobile Food Establishments; Beyond the Brick and Mortar

JEFF MARTIN: *Multnomah County Health Department, Portland, OR, USA*
BRENDA FAW: *California EHS-Net Food Safety Program, Sacramento, CA, USA*
MICHELLE WOLLENZIEN: *Quality Concepts, San Antonio, TX, USA*
JESSICA JONES: *Chick-fil-A, Inc., Atlanta, GA, USA*

The purpose of this symposium is to discuss the unique challenges faced by mobile food establishments (MFE) and how we can work together to identify and overcome these obstacles. The food truck phenomenon has gained national media attention and with an estimated 20,000 plus trucks nationwide has rapidly grown to become the newest food craze. In a 2009 industry survey annual revenue from food trucks was around $1.2 billion and growth rates from 2007-2012 was approximately 8.4% annually. With the growth of food trucks comes a variety of food safety obstacles such as operating in a small space, reliance upon generators to keep food at correct temperatures, licensing concerns and limited water supply. Since the trucks are on the move another challenge is the opportunity for regulators to see the truck in service at an event. This symposium will address specific MFE inspection challenges and risk factors and provide participants ideas on how to overcome these challenges. According to the FDA over 2,000 different state and local agencies are responsible for inspecting food trucks, which results in food truck safety standards that vary widely across the country. The symposium will strive to provide insight into specific state regulations regarding food truck standards. Mobile food operation concepts widely vary in size and complexity and include self-sufficient trucks, trucks that must report back to a commissary, canteen style trucks where all serving or cooking of food is done from outside of the truck and trucks that only sell pre-packaged non-TCS. Speakers will include information on food truck design, commissary and mobile support unit requirements.

S64 Challenges in Dairy Spoilage – From Sporeformers to Yeast and Molds

REID IVY: *Kraft Foods, Glenview, IL, USA*
NICOLE MARTIN: *Cornell University, Ithaca, NY, USA*
MANSOUR SAMADPOUR: *IEH Laboratories & Consulting Group, Lake Forest Park, WA, USA*
MICHELE GORMAN: *Chobani, LLC, Norwich, NY, USA*

Spoilage issues in dairy products have made a number of headlines in recent years and cause consumer dissatisfaction and concerns. Similar to PulseNet allowing for linkage of common source foodborne disease cases that occur over wide spatial and temporal scales, social media (e.g., Twitter) facilitate linkage between rare food spoilage issues that would have gone unrecognized even 5 years ago. Consequently, there is a need to share industry experiences on dairy spoilage issues and to develop new approaches to control the wide range of organisms that can cause spoilage in different dairy products. This symposium will cover spoilage issues ranging from Yeast and Molds, to sporeforming bacteria and lactic acid bacteria that can cause dairy product spoilage and off flavors. In addition, this symposium will provide information on the development and implementation of environmental sampling plans that are intended to prevent spoilage issues, including how to balance use of indicator and spoilage organism tests. Presenters will also discuss use of molecular tools for source tracking of spoilage organisms, including trace-back for sporeformers to on-farm sources.

S65 Foreign Supplier Verification Programs: Challenges and Opportunities

ANDREW STEPHENS: *U.S. Food and Drug Administration, Silver Spring, MD, USA*
JOSEPH SCIMECA: *Cargill, Wayzata, MN, USA*
YABIN SUN: *Liaoning Entry–Exit Inspection and Quarantine Bureau, Dalian, China*
POJJANEE PANIANGVAIT: *Thai President Foods, PLC., Bangkok, Thailand*

The amount of food imported into the U.S. has increased rapidly in the past two decades, by more than 200% during the period of 2003–2013. Recent data indicated that 15% of the U.S. food supply is imported, including about 50% of fresh fruits, 20% of vegetables, and 80 % of seafood. Accompanying this increase, concerns about the safety of imported food have also increased. The Food Safety Modernization Act (FSMA) provides FDA new tools and authorities to make certain imported foods meet the same safety standards as foods produced in the U.S. In 2013, FDA issued a proposed regulation on Foreign Supplier Verification Programs (FSVP) that would establish requirements for importers to verify that their foreign suppliers are implementing the modern, prevention-oriented food safety practices called for by FSMA. This symposium will provide an overview of the proposed FSVP regulation, showcase the approaches taken by U.S. food companies in establishing effective and robust foreign supplier verification programs, and highlight how companies work in partnership with foreign suppliers to implement modern food safety management systems and to ensure that international standards are met. In addition, this symposium will discuss the potential impact of FVSP on foreign suppliers, with a specific focus on food exporters in China and Southeast Asia. The speakers will highlight the approaches taken to establish prevention-based food safety systems and will comment on the

challenges and opportunities in meeting the new requirements. This symposium is unique in that it provides a diverse view from different stakeholders in addressing the issues associated with foreign supplier verification and in meeting the requirements set forth in the proposed FVSP regulation.

S66 Challenges to Modernization for Safe and Secure Food in the Middle East

EWEN TODD: *American University of Beirut, Lebanon and Ewen Todd Consulting, Okemos, MI, USA*
ATEF IDRISS: *MEFOSA, Beirut, Lebanon*
BOBBY KRISHNA: *Dubai Municipality, Dubai, United Arab Emirates*
EWEN TODD: *American University of Beirut, Lebanon and Ewen Todd Consulting, Okemos, MI, USA*
ZEINA KASSAIFY: *Mars Chocolate - Middle East, Turkey and Africa, Dubai, United Arab Emirates*
WALID ALALI: *Qatar National Research Fund of Qatar Foundation, Doha, Qatar*
JENNY SCOTT: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*

Most Middle East (ME) countries depend on much imported food, partly because of lack of infrastructure and investment and partly because most land is not rehabilitated or suitable for agriculture. However, traditional food industries need to modernize to be competitive to reach export markets, and multi-national companies need to find the niche where they can develop markets within the region. Safe, secure and sustainable food supplies are especially critical where there is regime uncertainty and conflict, as we see in many ME countries today. On top of this, there is the risk of new diseases that can affect both agriculture and tourism, such as Middle East Respiratory Syndrome (MERS-CoV) now linked to camels, milk, and possibly meat. Climate change is forcing farmers to use more untreated water for irrigation increasing the risks of pathogen transmission. This symposium will address both the local production challenges and those for developing or expanding food export markets, especially as now FSMA stipulates preventive actions in food safety. This is forcing some ME companies to address FSMA requirements, with a particular need for new technologies and certified food safety labs, especially where there are long supply chains from Sub-Saharan Africa. Foodborne disease surveillance is unable to identify and characterize most food-pathogen combinations. The challenges for the Greater Arab Free Trade Area (GAFTA), include the diverging needs for specific Knowledge Based Bio-Economy (KBBE) values. The increasing demand for a sustainable supply of food, raw materials and fuel is the major economic driving force behind growth of the KBBE in Europe over the last few decades and is now reaching the ME Region. Defining KBBE values regionally will be required for objectives, priorities, and actions, amongst which are harmonizing benchmarks by bioeconomy sector across GAFTA, and outlining preventive food safety regional drivers from farm to fork.

Roundtable Abstracts

RT1 Debate: Current Perspectives in Food Safety

MARTIN WIEDMANN: Cornell University, Ithaca, NY, USA
ERIC BROWN: U.S. Food and Drug Administration-CFSAN, College Park, MD, USA
KATHY GOMBAS: U.S. Food and Drug Administration-CFSAN, College Park, MD, USA
KATHLEEN GLASS: University of Wisconsin-Madison, Madison, WI, USA
PETER TAORMINA: John Morrell & Co., Cincinnati, OH, USA

This interactive roundtable is intended to engender lively discussion of important food safety topics. It is assumed audience participants will have a basic understanding of the unresolved issues surrounding the topics to be discussed in the symposium. The session will cover three topics: "Is shoe leather epidemiology dead in the age of Whole Genome Sequencing?"; "Is sustainability treading on food safety?"; and "Is sodium reduction in processed foods a risk to food safety?" Each topic will include a 7 minute presentation in support of (YES) followed by a 7 minute presentation in opposition of (NO) the proposed topic question. Each speaker will have 3 minutes for extemporaneous rebuttals. A 6 minute question/answer session will then follow to allow for audience participation. We will have electronic polling of the audience to allow for a Yes/No vote on each topic question prior to and following the discussion to evaluate whether people's views have been changed by the presentations.

1. Is Shoe Leather Epidemiology Dead in the Age of Whole Genome Sequencing?
2. Is Sustainability Treading on Food Safety?
3. Is Sodium Reduction in Processed Foods a Risk to Food Safety?

Panelists:

Eric Brown, FDA/CFSAN

Martin Wiedmann, Cornell University

Kathy Gombas, FDA/CFSAN

Peter Taormina, John Morrell & Co.

Kathleen Glass, University of Wisconsin-Madison

RT2 Poultry Slaughter Modernization and Evaluation of Process Control

DANIEL ENGELJOHN: U.S. Department of Agriculture-FSIS, Washington, D.C., USA
SCOTT EILERT: Cargill Turkey and Cooked Meats, Witchita, KS, USA
DAN ZELENKA: Tyson Foods, Springdale, AR, USA
ASHLEY PETERSON: National Chicken Council, Washington, D.C., USA
SHELLY MCKEE: USA Poultry & Egg Export Council, Stone Mountain, GA, USA
ROY BIGGS: Tegel Foods Ltd, Auckland, New Zealand

Salmonella Performance Standards have been in place in the poultry industry since the implementation of the Pathogen Reduction; Hazard Analysis Critical Control Point (HACCP) System in 1998. Prior to that implementation, there was also a regulatory declaration of a "Zero Tolerance" standard for visible fecal contamination in all species. Also the PR/HACCP rule required testing of biotype 1 E. coli as a quantitative measure of process control of sanitary dressing.

During the first years of the PR/HACCP rule, the per cent of broiler carcasses positive for Salmonella fell sharply from the industry baseline of 20% positive. However, that level began to creep back up causing FSIS to refocus attention on the Performance Standard by creating Categories within that Performance Standard Framework.

The USDA-FSIS has introduced the Poultry Inspection Modernization Rule which aims at improving safety at poultry slaughter facilities. The rule rescinds the biotype 1 E. coli testing because the Agency concluded that it may not be as useful as originally believed for indication of control of sanitary dressing procedures for broiler processing. "The Agency will allow establishments to use other more relevant indicators of process control" (USDA, FSIS 2014. FR 79(162): Pages 49565-49637). One of the provisions in the rule states that poultry slaughter facilities can choose an indicator organism(s) to monitor process control as well as testing for Salmonella and Campylobacter per the new regulations. It also may be possible to look at other indices of process control for Sanitary Dressing Procedures.

This Roundtable will be organized for USDA and the poultry industry to share experience with HIMP, the precursor to NPIS, as well as discuss with the audience possibilities for the use of the indicator organism option from FSIS. The roundtable will be comprised of regulatory and industry representatives, both domestic and international.

RT3 The Black Box of Qualitative Performance Standards for Meat and Poultry: The Drive to Quantitate *Salmonella*

EMILIO ESTEBAN: U.S. Department of Agriculture-FSIS-OPHS-EALS, Athens, GA, USA
SHARON WAGENER: Ministry for Primary Industries, Wellington, New Zealand
JEFFREY FARBER: University of Guelph, Guelph, ON, Canada
IAN JENSON: Meat and Livestock Australia, North Sydney, Australia
ANGELA SIEMENS: Cargill, Wichita, KS, USA
JOHN MARCY: University of Arkansas, Fayetteville, AR, USA

At present, performance standards involving qualitative microbiological criteria (presence/absence) are used in the food industry for reducing the risk associated with *Salmonella* contamination in raw meat and poultry. However, the incidence of *Salmonella* remains on the top of all foodborne illnesses and well above the national *Healthy People* target (FoodNet 2013). There is need to revisit the existing regulatory performance standards for meat and poultry and facilitate an open systematic discussion among the key stakeholders from academia, industry and regulatory agencies to contemplate alternate science driven risk-based approaches to mitigate the risk of *Salmonella* in meat and poultry. Making decisions about product acceptability based on quantitative contamination levels of *Salmonella* is a potential alternate model but developing an effective mitigation strategy based on such model will entail a depth of baseline data and knowledge of risk factors associated with *Salmonella* infectivity including but not limited to baseline data, serotypes, infectious dose, strain pathogenicity etc. In addition, the development of validated methods to support such an approach need to be established.

Although *Salmonella* is often the subject of discussion at IAFP meetings, previous sessions have not yet addressed the challenges of existing *Salmonella* performance standards with a specific focus on the meat and poultry industry. The proposed roundtable will bring together key industry stakeholders, representatives from global regulatory agencies and engage the audience in an interactive discussion to deliberate the issues with existing performance standards and outline the 'what-if scenario' for the alternate performance standards based on *Salmonella* enumeration.

RT4 Aquaponics: How Do We Safely Produce Fish and Vegetables in the Same Water?

KATHLEEN RAJKOWSKI: U.S. Department of Agriculture-ARS-ERRC, Wyndmoor, PA, USA
TREVOR SUSLOW: University of California, Davis, CA, USA
MICHELLE SMITH: U.S. Food and Drug Administration, College Park, MD, USA
SARAH TABER: The Aquaponics Association, Gainesville, FL, USA
PAUL HARDEJ: FarmedHere, LLC, Bedford Park, IL, USA
STEVEN HUGHES: Aquaculture Research Education Center, Cheyney University, Cheyney, PA, USA

The technique of growing seafood and plants together in a single system, using the same water, may have originated as early as the Aztec civilization. It is currently reemerging as a novel idea for modern time with many potential benefits. However, the existing scientific research on which to base regulations and recommended best practices for aquaponics to ensure food safety is limited. Aquaponics combines hydroponics (growing plants in water) and aquaculture (raising aquatic species) into one system. As pumps recirculate water, nitrifying bacteria convert ammonia created by the fish to nitrates which are taken up as nutrients by plant roots. Aquaponic production reduces dependence on land for soil-based agriculture and irrigation water. It is also efficient, sustainable, and increasingly popular. Backyard gardeners and schools are setting up small-scale systems, while commercial producers have larger systems to satisfy demand for locally produced foods, even in urban areas. Because plant and animal-based food production systems are usually separate, even in diversified operations, current food safety programs may not have fully considered to what extent these programs are relevant or sufficient to ensure safety of aquaponically grown foods. This roundtable session will bring together researchers, regulators, and aquaponic growers to exchange information on aquaponic production and food safety, including discussion of the current state of knowledge with respect to aquaculture and agricultural-water treatment microbiology, potential produce safety hazards, and future directions for research to better characterize potential hazards and support best practices that mitigate risk. Since this is the first time aquaponics has been discussed at an IAFP meeting and attendees may not be familiar with it, a brief introduction to this topic will be given by Sarah Taber, Food Safety Director of The Aquaponics Association, before the discussion begins.

RT5 Preharvest Control of Zoonoses: Opportunities and Hurdles

TREVOR SUSLOW: University of California, Davis, CA, USA
MOHAMMAD KOOHMARAIE: IEH Laboratories & Consulting Group, Lake Forest Park, WA, USA
DANIEL ENGELJOHN: U.S. Department of Agriculture-FSIS, Washington, D.C., USA
MARK BERRANG: U.S. Department of Agriculture-ARS, Athens, GA, USA
BOB O'CONNER: Foster Farms Poultry, Livingston, CA, USA

Preharvest control of zoonoses, food-animal-borne human pathogens, is critical to the safety of meat, poultry, fruits, and vegetables. Epidemiological and research results in the past decades have demonstrated zoonotic contamination of crops and waterways by food animal facilities, including dairies. Additional research has shown that despite improvements in slaughter processes over the past two decades, pathogens going into an establishment will emerge on the final product. There are successes in reducing and even eliminating on-farm zoonotic carriage but most of the successes have been for animal pathogens, not human pathogens. While "farm to fork", and "gate to plate" has been touted for over a decade, little progress has been accomplished on the farm end. There are economic, practical and political hurdles to overcome for reducing or eliminating zoonoses on the farm. This round table will include speakers from industry, academia, a regulatory agency and USDA Agriculture Research Service. The round table will offer a lively discussion of the science, opportunities, need, and hurdles to implementing more effective preharvest control of zoonoses. IAFP 2015 will be an excellent venue for discussing these issues.

RT6 Food Safety Training for Non-native English Speakers

PHILIP CRANDALL: University of Arkansas, Fayetteville, AR, USA
LONE JESPERSEN: Maple Leaf Foods, Mississauga, ON, Canada
DANIEL OKENU: HEB, San Antonio, TX, USA
KATEY KENNEDY: U.S. Food and Drug Administration, Beaverton, OR, USA
GINA KRAMER: Savour Food Safety International, Columbus, OH, USA

Teaching and training Adult learners can bring many challenges including understanding how and what motivates them to learn. The challenge increases significantly when students speak English as a second language. Nearly one in ten adult workers is considered Limited English Proficient (LEP), and many are working in the food industry. How do we effectively teach Food Safety to non- native English speakers? As trainers, we can employ many different strategies including creating programs that address both native and non-native English speakers in one classroom, conducting a separate training for the LEP population in their own language, or even abandoning traditional classroom training for a more hands on approach. Which strategy produces the best results? This round table will feature panelists with a deep understanding of food safety training from a number of perspectives including food retail, food processing, food service, academia, and regulatory. At this round table we will discuss ways that instructors have found to successfully teach LEP food workers to utilize safe food practices they learned in training with the goal of creating strategies for LEP populations that can affect real change in the workplace.

RT7 The Evolving Patterns for Publishing Science

DONALD W. SCHAFFNER: Rutgers University, New Brunswick, NJ, USA
MARCEL ZWIETERING: Wageningen University, Wageningen, Netherlands
ANDERSON SANT'ANA: University of Campinas, Campinas, São Paolo, Brazil
DANE JENSEN: Rutgers University, New Brunswick, NJ, USA
AMANDA FERGUSON: Institute for Food Technologists, Chicago, IL, USA
LAUREN JACKSON: U.S. Food and Drug Administration-CFSAN, Bedford Park, IL, USA

At the 2014 Annual Meeting a Round Table panel discussed some of the issues surrounding publishing science in today's world, touching on open access vs. traditional publishing formats, plagiarism, and the increasing number of "rogue" journals to avoid. This RT will explore in more depth the very likely scenario that all journals and authors will have to adapt to open access publishing and this will cost more all round. Is this an advantage or deterrent to younger scientists and how will traditional journals adapt to the pressure to change? Will Source Normalized Impact per Paper (SNIP) replace the impact factor (IF) as a best measure of journal quality or is it a gimmick by a major publishing house? There is no standard for being able to review, usually being an author, but this does not mean they are effective reviewers. Many experienced authors refuse to do reviews leaving it to others. Another issue is the low quality of submissions, either poor or repeated research, not well-thought out methodologies, too long a text relative to the merit of the research, and unacceptable English. Journals also want reviews to reject perhaps 50% of submissions as there are more submissions than journal space for monthly or quarterly publications. With the likelihood that all journals will become electronic, space will no longer be an issue for publication only quality. Scientific societies such as IAFP will have to appoint forward-thinking editorial boards to be ready to consider several publication alternatives well in advance of what publishing companies start to promote before it may be forced upon them by dwindling submissions. This roundtable will have panelists representing four scientific journals, and a young scientist to review the issues and suggest ways out of the publication maze. There will opportunities for audience input as well.

RT8 Taking a Detour: Innovative Careers in Food Safety

BARBARA MASTERS: Olsson Frank and Weeda, Shenandoah Junction, WV, USA
JOHN ALLAN: International Dairy Food Association, Washington, DC, D.C., USA
MICHELLE DANYLUK: University of Florida, Lake Alfred, FL, USA
SERGIO NIETO-MONTENEGRO: Alimentos y Nutricion, Chihuahua, Mexico
JUSTIN DERINGTON: Food Safety Net Services, San Antonio, TX, USA

Food safety is a very diverse field and, as such, career opportunities are ample and interesting. Our industry is constantly changing and this has led to several emerging opportunities for expanding traditional career paths and hidden facets inside the traditional options: industry, academia, government. Industry typically encompasses manufacturing, but what about the supporting companies, such as third-party training? Research and teaching are associated with academia, but extension work is also a key component. Food Safety careers in government are normally associated with the USDA, FDA, or CDC, but foreign organizations, such as the World Health Organizations are also vital to our field. There are also emerging fields such as Food Law and Public Relations, which are growing rapidly. Panelists in this session will include representative from each of these fields. In this session, the Student PDG has teamed up with the Developing Food Safety Professionals PDG. This roundtable will provide the audience with broader perspective of alternative career choices and new opportunities for collaboration and support. Our diverse panel of speakers will share their thoughts on opportunities and challenges for these innovative food safety career paths, the skill set required, the contribution they are making to public health, and emerging new careers they see on the horizon. Attendees will gain exposure to these new fields and will have the opportunity to inquire about recommended and necessary technical background, where to look, and how to reach out to collaborate with these different facets of the food industry.

Technical Abstracts

T1-01 An Integrated Approach to a Process-Based Quality and Food Safety Management System at Dr. Pepper/Snapple Group Multi Sites

FREDERIC CHERNE, George Russel, Andrew Smith
Dr. Pepper/Snapple Group, Williamson, NY, USA

Introduction: The Dr. Pepper Snapple Group (DPSG) serves its customers and consumers via 21 owned plants and a network of licensed bottling facilities/warehouses. Over 15 years ago, DPSG designed and implemented a unique process-based Management System for Quality Management, called VMS (Value Management System). There is no need for a Quality Manual; the DPSG Process Framework identifies business processes that are part of the Management System scope. Process Designs define how activities are to be performed and measured. Processes are electronically accessible and common to any site at which they are executed. Good Manufacturing Practices (GMPs) are defined and applicable during execution of any process. Process Owners are responsible and accountable for all of process design, execution, performance and compliance.

Purpose: The purpose of this case study is to outline the advantages and benefits of a centralized process-based Management System. It also explores how to take advantage of such a system through the use of a multi-site approach to Management System certification.

Methods: This presentation explains the critical role that Process Ownership plays during implementation and how it is measured. It explains how to realize the potential for significant audit efficiencies, by assessing centralized processes once, and then verifying their implementation at each subsequent site audit.

Results: DPSG has successfully applied a Process-Based Management System to their organization resulting in a streamlined control of processes which help minimize food safety risks whilst at the same time maximizing the efficiency of verification processes

Significance: Effective and efficient management systems and robust verification processes are two key drivers of a successful food safety culture. This case study demonstrates how these principles have driven DPSG's process design and the rationale for selection of FSSC 22000 as its GFSI approved certification scheme.

T1-02 Import Alert Action Taken by the US Food and Drug Administration as a Result of Regulatory Food Testing by the New York State Rapid Response Team During an International *Salmonella* Outbreak Investigation

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Introduction: From May-August 2014, an outbreak of *Salmonella* infections with 31 cases from 16 states in the US and 63 cases from 4 provinces in Canada was identified and linked to the consumption of organic sprouted chia seed powder through epidemiologic and traceback investigations by the Centers for Disease Control and Prevention (CDC), the Food and Drug Administration (FDA), the New York State Rapid Response Team (NY-RRT), the Public Health Agency of Canada (PHAC), the Canadian Food Inspection Authority (CFIA), and several state, provincial, and local public health and regulatory food safety agencies. Under the Food Safety Modernization Act (FSMA), FDA is charged with both improving state and local food safety capacities as well as reliance on inspections by federal, state, and local agencies and data from International Organization for Standardization (ISO) 17025 accredited laboratories.

Purpose: This investigation was aimed at identifying the source of the *Salmonella* outbreak and taking necessary regulatory actions to protect public health.

Methods: Epidemiologic and traceback investigations implicated a single Canadian firm as a common supplier of the organic sprouted chia seed powder. The NY-RRT sampled sealed implicated product from a NY retail establishment and submitted to their ISO 17025 accredited laboratory for *Salmonella* testing (screening by polymerase chain reaction [PCR] and culture confirmation using the FDA Bacteriological Analytical Manual [BAM]). Pulsed-field gel electrophoresis (PFGE) was performed according to PulseNet protocols.

Results: *Salmonella* Newport was isolated from the implicated product, reported to PulseNet, and was found to be indistinguishable from the outbreak pattern. FDA used NY-RRT test result data to support issuance of an import alert for the implicated product.

Significance: Public health partnerships between public health and regulatory food safety agencies led to the rapid issuance of an FDA import alert on the basis of NY-RRT testing data to expedite control of this outbreak.

T1-03 A Novel Approach to FSIS Intensified Sampling in Response to a *Salmonella* Outbreak in Chicken Products

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Introduction: In a recent *Salmonella* outbreak associated with raw chicken products, traceback evidence implicated multiple brands of raw chicken products from multiple establishments under a single corporate umbrella and across a range of production dates. In order to evaluate this relationship, FSIS initiated intensified sampling at implicated establishments using a novel weighting scheme.

Purpose: FSIS developed an intensified sampling program to sample raw chicken products at six implicated establishments. Once three establishments were identified as the most likely producers of implicated products, FSIS monitored implementation of corrective actions and their effectiveness in reducing *Salmonella* at these establishments through continued intensified sampling.

Methods: FSIS applied a probability-based weighting scheme to choose establishments and product types most likely associated with this particular illness cluster for inclusion in the intensified sampling project. For over 12 months, FSIS analyzed samples for the presence of *Salmonella*, including routine subtyping analyses (e.g., serotyping, pulsed-field gel electrophoresis, and antimicrobial susceptibility testing). Product was collected in final retail packaging at these establishments and shipped to FSIS field service laboratories for subsequent analysis.

Results: During the initial phase of testing, the results showed approximately 25% *Salmonella* positive in chicken products at the implicated establishments. After corrective actions were implemented, FSIS testing in conjunction with establishment testing showed a reduction to at or below 5% *Salmonella* positive at the three implicated establishments.

Significance: This is the first time FSIS utilized a weighted approach for focusing intensified sampling resources as part of an outbreak investigation. Through this type of sampling, we were able to rule out establishments under the same corporate umbrella and focus on the three establishments where *Salmonella* appeared to be most elevated. As a result of corrective actions implemented in these establishments, the incidence of *Salmonella* was reduced, and over time the associated illnesses returned to background levels.

T1-04 A Regulatory Agency Model for Identifying and Triaging Emerging Food Safety Issues: Best Practices and Lessons Learned

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Introduction: As the public health agency of the U.S. Department of Agriculture, the Food Safety and Inspection Service (FSIS) ensures that the nation's commercial supply of meat, poultry, and egg products is safe, wholesome, and correctly labeled and packaged. This includes addressing imminent food safety incidents, as well as scanning the horizon for emerging food safety issues before they are implicated in a specific incident. FSIS has successfully developed and refined tools and processes that satisfy both of these aims.

Purpose: In early 2014, FSIS's Hazard Identification Team (HIT) implemented a systematic approach to identify, track, and triage emerging and re-emerging food safety issues.

Methods: Issues are initially submitted by any FSIS employee, including inspectors and veterinarians in slaughter and processing establishments, using a standardized issue initiation form. HIT prioritizes submitted issues for further agency action using several criteria, including the potential public health impact, the prevalence of the hazard and the severity of its effect, data availability and research needs, previous FSIS action or existing regulation related to the issue, and perceived levels of stakeholder concern. For high-priority issues, dedicated task forces consisting of relevant subject-matter experts are assembled to provide actionable recommendations.

Results: This presentation will highlight best practices developed, and challenges encountered, by FSIS while designing and implementing this program and will share lessons learned from reviews of the HIT process. Examples of emerging food safety issues identified and triaged by HIT will demonstrate the breadth of emerging issues facing regulatory food safety agencies.

Significance: The HIT process enables FSIS to focus resources on the issues of greatest concern, while creating a record of all emerging issues that can be accessed as conditions change. The approach is adaptable and may be of interest to anyone active in the food safety arena.

T1-05 Impact of Handling Practices on the Microbiota of Inshell California Walnuts

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Introduction: Steps during harvest and hulling potentially expose walnuts to diverse microbial communities that may serve as reservoirs for two-way transfer of microbes.

Purpose: To evaluate the impact of harvest and hulling practices on the microbiota of inshell walnuts using a non-culture-based classification methodology.

Methods: Water from the "float tank" (used to separate inshell walnuts from rocks) and inshell walnuts from several locations before and after the float tank were collected from a commercial walnut huller over multiple days during the walnut harvest. Duplicate or triplicate samples were agitated in 0.1% peptone and centrifuged to obtain a bacterial pellet. Total genomic DNA was extracted and the V4 domain of bacterial 16S rRNA genes was amplified by PCR and subsequently sequenced on an Illumina MiSeq instrument. The 16S rRNA gene sequences were assigned to operational taxonomic units (OTUs) at the 97% similarity level, and a representative sequence from each OTU was assigned a taxonomic identity. Alpha-diversity and beta-diversity analyses were performed in QIIME.

Results: Walnuts received at the huller had proportionately lower amounts of Enterobacteriaceae (31%) than the float tank water (66%) or the walnuts collected after passing through the float tank water (54%). Bacterial communities on individual pre-float tank walnut samples were highly variable but beta-diversity analysis showed significantly different microbiota associated with incoming walnuts than with walnuts exposed to the float tank. Several OTUs were recovered at proportionally higher levels in float tank water and on walnuts collected after passing through this water than on walnuts collected at receipt.

Significance: Beta-diversity analyses of inshell walnut microbiota provides evidence that passage through a float tank can alter the microbiota of walnuts.

T1-06 Evaluation of Vacuum Steam Pasteurization to Inactivate *Salmonella* PT30, *Escherichia coli* 0157:H7, and *Enterococcus faecium* on Flaxseed

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❖ ***Developing Scientist Competitor***

Introduction: With increasing demand for nutritious food, low moisture foods such as flaxseeds, sunflower seeds, and sesame seeds have been studied a great deal for their nutritious value. However, such foods are minimally processed and are usually consumed raw and several outbreaks due to *Salmonella*, and *E.coli* 0157:H7 have been attributed to them. Previous studies show that these pathogens are more resistant to dry heat in low moisture foods and processes such as chemical treatments and blanching may have negative effects on product quality and functionality.

Purpose: Vacuum steam pasteurization is a technology that injects saturated steam under vacuum to supply heat which can be controlled to achieve desired temperatures. We wanted to determine the efficacy of this technology in inactivation of pathogens on flaxseeds.

Methods: Flaxseeds were separately inoculated with bacterial lawns of *Salmonella* PT30, *E.coli* 0157:H7 and *E. faecium* to obtain a homogenous distribution. The inoculated flaxseed (25 g) were pasteurized at 75°C, 85°C, 95°C, and 105°C after 24 and 48 hours post inoculation. Three 25 g samples were run at each condition, and the experiment was repeated three times.

Results: Similar average log reductions of 5.5 ± 1.2, 5.7 ± 0.4, and 5.3 ± 0.5 was observed after 1 min at 75°C for *Salmonella, E.coli* 0157:H7 and *E. faecium,* respectively ($P > 0.05$). The optimum time and temperature for maximum log reduction was determined to be after pasteurization for 3 min at 85°C on flaxseeds.

Significance: Steam vacuum pasteurization is an effective method for inactivation of these pathogens on flaxseed achieving a 5-log reduction after just 1 min at 75°C. *Enterococcus faecium* may be used as a surrogate for *Salmonella*, and *E.coli* 0157:H7 when evaluating vacuum steam pasteurization.

T1-07 Influence of Sucrose, Fructose, and Sorbitol on the Survival of *Salmonella* in a Low-water Activity Whey Protein Model System at 70°C

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Introduction: In recent years, numerous *Salmonella* spp. outbreaks have been attributed to low a_w foods. Many of these food products recalled for *Salmonella* contain sugars. The impact of reducing sugar (fructose), non-reducing sugar (sucrose), and sugar alcohol (sorbitol) at low a_w levels (below a_w < 0.60) has yet to be determined.

Purpose: The purpose of this study was to determine if sugar in low a_w whey protein powder affects the survival of *Salmonella* independent of water activity at 70°C.

Methods: A four-strain cocktail, *Salmonella* Typhimurium, Tennessee, Agona, and Montevideo, was dried and added to either 0, 20, or 40% (w/w) sugar supplemented protein samples that were equilibrated to a_w of 0.20 ± 0.02, 0.42 ± 0.01, or 0.54 ± 0.01. Sugars tested included sucrose, fructose and sorbitol. After heat treatment (70°C for up to 48 h), survivors were enumerated using supplemented tryptic soy agar and data were analyzed using two-way ANOVA.

Results: After 48 h of heat treatment, log reduction at 40% sugar was significantly increased (P = 0.0042) by an average of 2.5 log units (a_w = 0.42) and 3 log units (a_w = 0.54) when fructose or sorbitol was added to the protein powder. Addition of fructose or sorbitol (20 or 40%) significantly (P < 0.0001) increased survival of *Salmonella* over 48 h of treatment. Overall, the water activity was a significant (P < 0.02) factor in survival when comparing both the types of sugar and the amount of sugar used per treatment.

Significance: Sugar has an effect on the survival of *Salmonella* spp. independent of water activity at 70°C for 48 h. This should be taken into account in the development of predictive models.

T1-08 The Influence of Fat Content on *Salmonella* Survival in a Low-water Activity Model Food System at 50, 60, 70 and 80°C

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Introduction: Low-water activity (a_w) foods and ingredients are naturally dry or have been dried through processing. Low a_w is a barrier to growth for pathogens, including *Salmonella* spp. However, *Salmonella* has demonstrated ability to survive in low-water activity foods for long periods of time. The role of fat composition of the food in the survival of *Salmonella* in low-water activity foods remains unclear.

Purpose: To evaluate the effect of fat on the survival of *Salmonella* in a whey protein isolate (WPI) model food system at 50, 60,70 and 80°C at water activities of 0.18, 0.26, 0.34, 0.42, and 0.57.

Methods: Peanut oil was homogenized with whey protein isolate to achieve 20 and 50% (w/w) fat concentration and was equilibrated to target water activity levels. A dried cocktail including *Salmonella* Typhimurium, Tennessee, Agona, and Montevideo was inoculated and treated at 50 and 60°C for up to 28 d and at 70 and 80°C for up to 48 h. Survivors were recovered at various times using supplemented Tryptic Soy Agar. Log CFU/g of surviving *Salmonella* in 0, 20 and 50% peanut oil at each time point were compared using a two-way ANOVA.

Results: Survival differed with water activity at 70°C and was affected by fat content at 50, 70 and 80°C (P = <0.0001, α = 0.05). At 60°C, survival was not affected by fat content or water activity (P = 0.8253). There was 4.0 log greater survival at 50% fat level compared to no added fat after 48 h at 80°C.

Significance: An understanding of the role of fat in the survival of *Salmonella* in low-water activity foods will improve the ability to predict and control the behavior of *Salmonella* in these foods.

T1-09 Mesophilic and Thermophilic Sporeformers are of Primary Concern in Dairy Powders: A Survey Across the United States

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Introduction: Aerobic sporeforming bacteria are important contaminants in dairy foods due to their ubiquitous nature and ability to survive heating processes. They can cause product specification compliance issues or product spoilage if present in sufficient numbers and/or where conditions favor growth for biofilm formation in processing plants.

Purpose: To evaluate mesophilic and thermophilic sporeforming bacteria commonly found in raw and milk powder samples collected from 11 dairy powder plants across the US.

Methods: Raw ingredient (R) and finished powder (FP) samples were collected from 11 dairy powder processing plants located across the US for over a period of 6 months. All samples were subjected to 3 spore treatments which included spore pasteurization (SP; 80°C for 12 min), highly heat resistant (HHR; 100°C for 30 min) and specially thermoresistant (STR; 106°C for 30 min) spore tests. Samples were enumerated for mesophilic (incubated at 32°C) and thermophilic (incubated at 55°C) sporeforming isolates, either obtained by direct plating or enrichment following the spore treatments.

Results: Overall, 52.3% (92/176) and 48.1% (127/264) of R and FP samples collected from 11 plants were positive (≥ 2 CFU/ml or g) for 412 mesophilic and thermophilic sporeforming isolates, either obtained by direct plating or enrichment from each spore test type. Out of 227 sporeformers isolated at 55°C, 34 represented groups of obligate thermophiles selected by STR method, also considered as primary organisms of interest in dairy powders. The given isolates will be characterized to species and/ subtype level using *rpo*B identification technique.

Significance: The results provide an understanding of the sporeformers commonly found in dairy samples obtained from different powder processing plants across the US. Characterization of groups of sporeformers of interest is necessary in order to develop strategies for their control with the goal of improving dairy quality.

T1-10 Prevalence of Pathogenic Shiga Toxin-producing *E. coli* (pSTEC) O157 and Non-O157 in Raw Milk Samples

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Introduction: Detection of pathogenic Shiga Toxin-producing *E. coli* (pSTEC) typically rely on two successive PCR screening steps as described in the ISO/TS 13136 (EU) and MLG 5B (US) reference methods. In order to reduce both the rate of presumptive PCR positive results and the time to result, we developed a one-step multiplex PCR based method using the GeneDisc® technology.

Purpose: With the aim to evaluate the prevalence of pSTEC in raw milk products, 4,064 raw milk samples (3,331 cow milk and 733 ewe milk samples), collected over one year in France, were analyzed according to the ISO/TS 13136 standard and the GeneDisc method.

Methods: Twenty-five ml of raw milk sample were enriched in 225 ml BPW supplemented with 10 mg/l acriflavine for 18 h ± 2 h at 37°C. DNA extracted from the enrichment broth was analyzed with the GeneDisc Cycler, according to both PCR approaches. Confirmation of presumptive positive samples was also done following own procedures for each method. Isolated strains were characterized using qPCR on chips allowing carrying out 48 different PCR reactions (Fluidigm).

Results: One thousand nine (28.3 %) presumptive positive results for pSTEC were obtained according to the ISO/TS 13136 standard while only 456 (11.2 %) were positive with the GeneDisc multiplex PCR. The number of atypical enteropathogenic *E. coli* (aEPEC) and pSTEC strains isolated was, respectively, 41 and 28 with a majority of O26 strains (62%), followed by O103 (34.4 %). Molecular characterization of the isolates showed that aEPEC O26 strains might be in fact sub-divided in *stx*-negative pSTEC derivatives and aEPEC O26 strains.

Significance: The accuracy of the GeneDisc screening method provides a lower rate of presumptive positive for pSTEC making it suitable for routine testing condition. It would allow the dairy industry to accurately sort their milk and confidently direct it to the right processing.

T1-11 Food Safety Concerns Regarding the Consumption and Sale of Unpasteurized Milk in Ireland

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Introduction: Many families who live on farms in rural Ireland still consume, and in some cases sell, unpasteurized (raw) milk for direct human consumption. The Food Safety Authority of Ireland (FSAI), a statutory, independent and science-based body with the responsibility for coordinating the enforcement of food safety legislation in Ireland, opposes the sale of unpasteurized milk from all farm animals for direct human consumption. In response the Irish Department of Agriculture is planning to introduce legislation which may prohibit or impose further restrictions on the sale of unpasteurized milk for direct human consumption in Ireland.

Purpose: To support this policy decision opposing the sale of unpasteurized milk from all farm animals for direct human consumption in Ireland.

Methods: A total of 600 samples of unpasteurized bulk tank milk and corresponding milk filters were collected nationally from 211 dairy farms between June 2012 and June 2013 and tested for a range of pathogens using internationally recognized ISO methods.

Results: Approximately 45% (86/190) of milk filters tested positive for one of four foodborne pathogens (*Salmonella* spp., verocytotoxin producing *Escherichia coli* (VTEC)*, Listeria monocytogenes* and *Campylobacter* spp.). In the unpasteurized milk samples 7% (15/208), 1.5% (3/200) and 0.5% (1/206) of samples tested positive for *Listeria monocytogenes*, *Campylobacter* spp. and *Salmonella* spp., respectively. There was no correlation between herd size, herd species, or season and the detection of pathogens in samples tested.

Significance: Based on these findings, the FSAI continues to recommend that the sale of unpasteurized milk for direct human consumption should be prohibited in Ireland and that farm families that drink milk produced on their own farm should pasteurize it before drinking.

T1-12 Validating Environmental Pathogen Monitoring Programs in Small Dairy Processing Facilities

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❖ ***Developing Scientist Competitor***

Introduction: Environmental pathogen monitoring programs are uniquely designed for individual plants. While these programs may be in place, few programs have been validated to ensure representative sampling.

Purpose: Validation sampling was performed to ensure the *Listeria* spp. frequency detected during routine sampling did not underestimate the *Listeria* spp. frequency in a given facility.

Methods: Using six "artisan" US dairy plants' environmental pathogen monitoring programs, sample size calculations were performed to determine how many environmental samples needed to be collected to have an 80% power to determine whether the *Listeria* spp. frequency observed during validation sampling was no more than twice the frequency as estimated by the routine sampling. Sample size calculations were performed using the routine sampling results to estimate the minimum detectable difference in the validation sampling using a normal approximation to the binomial distribution. A minimum of 50 samples were collected and tested for *Listeria* spp. according to the FDA BAM from each plant to ensure a confidence interval within the validation goal.

Results: Prevalence of *Listeria* spp. varied by plant during both routine sampling (< 0.3% - 22.64%) and validation sampling (1.33% - 14%). Five of the six plants in the study met their validation prevalence goal, suggesting effective environmental pathogen monitoring plans.

Significance: This study provides a model for a science- and statistics-based method of validating environmental pathogen monitoring programs.

T2-01 Neural Network Model for Behavior of *Salmonella* in Chicken Meat during Cold Storage

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Introduction: Mathematical models that predict behavior of human bacterial pathogens in food are valuable tools for assessing and managing this risk to public health.

Purpose: A study was undertaken to develop a model for predicting behavior of *Salmonella* 8,20:-:z6 in chicken meat during cold storage and to determine how well the model predicts behavior of other serotypes of *Salmonella* stored under the same conditions.

Methods: Ground chicken thigh meat (0.75 cm^3) was inoculated with 1.7 log of *Salmonella* followed by storage for 0 to 8 days at -8 to 16°C. An automated mini-most probable number method was used for enumeration of *Salmonella*. Commercial software (NeuralTools) was used to develop

a multi-layer feedforward neural network model with one hidden layer of two nodes. Model performance was evaluated using the acceptable prediction zone method.

Results: Number of *Salmonella* in ground chicken thigh meat stayed the same ($P > 0.05$) during 8 days of storage at -8 to 8°C but increased ($P < 0.05$) over time of storage at 9 to 16°C. The proportion of residuals in an acceptable prediction zone (pAPZ) from -1 log (fail-safe) to 0.5 log (fail-dangerous) was 0.939 for data (n = 426) used in model development. The model had pAPZ of 0.944 or 0.954 when it was extrapolated to test data (n = 108/serotype) for other serotypes (Typhimurium var 5-, Kentucky, Typhimurium, Thompson) of *Salmonella* in ground chicken thigh meat stored for 0 to 8 days at -4, 4, 12, or 16°C.

Significance: A pAPZ ≥ 0.7 indicates that a model provides predictions with acceptable bias and accuracy. Thus, results indicated that the model provided valid predictions of survival and growth of *Salmonella* 8,20:-:z6 in ground chicken thigh meat stored for 0 to 8 days at -8 to 16°C and that the model was validated for extrapolation to four other serotypes of *Salmonella*.

T2-02 Development of a Graphical-user Interface to Optimize the Temperature for the Supply Chain of Leafy Greens Using Nonlinear Programming

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Introduction: Consumption of fresh and fresh-cut leafy green vegetables in the United States has increased by about 27% in the last 30 years. However, leafy green are highly susceptible to microbial contamination because they are minimally processed and consumed raw. Pathogenic bacteria of concern include *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes*. Leafy greens are a perishable commodity, with a postharvest shelf life limited to one week.

Purpose: This study aimed to optimize the maximum temperature of leafy greens during supply chain taking into account cost of refrigeration, sensory quality parameters (fresh appearance, wilting, browning, and off-odor), and microbial safety of leafy greens using nonlinear programming (NLP).

Methods: The coefficient of performance (COP) was used to determine the cooling costs of refrigeration. The loss of sensory quality parameters (for fresh-cut iceberg and Romaine lettuce and fresh-cut chicory) was expressed using Arrhenius equation, and pathogen growth was represented by a three-phase linear (primary) and square-root (secondary) models. The objective function representing cost was minimized. The nonlinear constraints were growth of pathogens (*E. coli* O157:H7, *Salmonella* and *L. monocytogenes*), and loss of sensory characteristics. An interactive graphical user interface, 'SHELF' was developed in the MATLAB (MathWorks, ver. 2013b) software.

Results: For a desired shelf life of 2-3 days, pathogen growth poses a higher concern than loss of sensory quality. However, for longer shelf times it is difficult to maintain sensory qualities. The results vary for different leafy greens. *E. coli* O157:H7 and *Salmonella* do not grow at lower temperature, whereas *L. monocytogenes* is capable of growing at refrigerated temperatures. Browning poses the worst concern for iceberg and Romaine lettuce, whereas off-odor is the biggest concern for fresh-cut chicory.

Significance: The results can inform decisions on the maximum refrigeration temperature for leafy greens along the supply chain.

T2-03 Dynamic Analysis, Prediction, and Monte Carlo Simulation of Growth of *Clostridium perfringens* in Cooked Beef

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Introduction: *Clostridium perfringens* is a spore-forming anaerobic pathogen that can cause acute abdominal pain, stomach cramps, and diarrhea in consumers. This pathogen is one of the most common causes of foodborne illness in the United States. Improper cooling and handling of meat products after cooking is a major cause of outbreaks of *C. perfringens.* Therefore, rapid cooling is critical to limit the germination of the spores and prevent the growth of this microorganism.

Purpose: This study was conducted to develop and validate a new dynamic method to directly construct a tertiary model for prediction of the growth of *C. perfringens* in cooked beef.

Methods: This method was based on a numerical algorithm to simultaneously solve the primary and secondary equations using multiple dynamic growth curves obtained under different conditions. A bootstrap method was used to calculate the 95% confidence intervals of kinetic parameters. Monte Carlo simulation was used to validate the models.

Results: The results demonstrated a close agreement between the predictions and experimental observations. The mean residual of predictions (RP) was -0.02 ± 0.23 log CFU/g. The RPs were < 0.4 log CFU/g for relative growths < 1 log CFU/g. Overall, 74% of the RPs were < 0.2 log CFU/g, 7.7% > 0.4 log CFU/g, while only 1.5% > 0.8 log CFU/g. In addition, the dynamic model also accurately predicted four isothermal growth curves arbitrarily chosen from the literature. Finally, the probability of > 1 and 2 log CFU/g relative growth at the end of cooling was provided by the Monte Carlo simulation.

Significance: This study provides a new probabilistic approach to estimate of the growth of *C. perfringens* in cooked meat during cooling. The results of this study can be used by the food industry and regulatory agencies to assess the safety of cooked beef in the event of cooling deviation.

T2-04 Modeling the Growth/No Growth Response of Non-O157 Shiga Toxin-producing *Escherichia coli* to Temperature, pH and Water Activity

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Introduction: The role of non-O157 Shiga Toxin-producing *E. coli* (STEC) in food safety is gaining public concern. Several STEC foodborne outbreaks have been associated with acidic and dry foods.

Purpose: The objective of this study was to model the effect of temperature, pH and water activity (a_w) on the growth/no-growth response of non-O157 STEC.

Methods: A six-serogroup STEC (*E. coli* O26, O45, O103, O111, O121 and O145; ca. 6 log CFU/ml) cocktail was inoculated into brain heart infusion broth (BHI) at 48 combinations of pH (4.5, 5.0, 5.5, 6.0, 6.5, and 7.0) and a_w (0.950, 0.956, 0.963, 0.971, 0.977, 0.984, 0.989, 0.993), and incubated at ten

temperatures (6, 9, 12, 18, 24, 30, 37, 41, 45, and 47°C). After 62 days of incubation, growth was determined by turbidity and plating on tryptic soy agar, and the binary data (0 [no growth] or 1 [growth]) were fitted using non-linear logistic regression. The model was validated with independently generated data of the six serogroups of non-O157 STEC, a cocktail of *E. coli* O157:H7, and two panels of six-serogroup STEC cocktail.

Results: A non-linear, logistic regression model was developed to predict growth/no-growth boundary for non-O157:H7 STEC in a microbiological medium and validated in a beef system. Non-O157 STEC did not grow at temperature ≤ 6°C and ≥ 47°C, and the most tolerance to low pH and a_w occurred at 25°C. Although slight difference on growth/no-growth response existed among serogroups of STEC, the developed model can be applied to all the STECs with an average concordance of 92.3%.

Significance: The developed model will be useful to the industry in designing food products with minimal risk of STEC growth.

T2-05 Development of Modeling and Validation Software Called FAME

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Introduction: Predictive modeling software such as Pathogen Modeling Program (PMP), Combase, and Growth Predictor were developed to predict growth or inactivation of foodborne pathogens in various conditions, but the software is equipped with only kinetic models.

Purpose: The objective of this study was to develop software program to predict bacterial growth, to calculate growth probability, and to validate developed models with experimental data.

Methods: Foodborne bacteria Animal product Modeling Equipment (FAME) was programmed with Java script and Html programming languages. Kinetic models developed with the modified Gompertz model with 5,400 samples of experimental data (packaging condition × temperature × NaCl × $NaNO_2$) on frankfurters for *Pseudomonas* spp., *Listeria monocytogenes,* and *Salmonella* were loaded in FAME, and probabilistic models developed with 345,600 samples of experimental data for *L. monocytogenes*, *Staphylococcus aureus*, and *Salmonella* for combinations of the fixed effects were also loaded in FAME. In addition, validation function was added in the software.

Results: Using FAME, cell counts of foodborne bacteria at various conditions (packaging condition, storage temperature, NaCl concentration, and $NaNO_2$ residual) can be calculated in part of kinetic model, and growth probability (P = 0.1, 0.5, and 0.9) of foodborne bacteria can also be estimated by probabilistic models. At the same time, automatic validation function by calculating bias factor, accuracy factor, and root mean square error can be processed with experimental data. In addition, there is a function which users can load and edit own equations.

Significance: FAME should be useful in predicting foodborne pathogen growth and growth probability, especially for non-specialists in predictive models.

T2-06 A Meta-analysis Model Based on the Bigelow Equation to Determine Thermal Inactivation Parameters of *Alicyclobacillus acidoterrestris* in Fruit Beverages

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Introduction: Since *Alicyclobacillus acidoterrestris* (AAT) is the major target for fruit juice pasteurization, numerous studies estimate their thermal inactivation parameters in different kinds of beverages. Yet, in many instances, the *D*- and *z*- values reported therein have been found to be divergent, even among studies investigating the same type of fruit beverage.

Purpose: Thus, the objective of this study was to summarize all published findings on the heat resistance of AAT, and to explain the differences among the *D*- and *z*-values by assessing study characteristics such as type of beverage (juice, concentrate), application of clarification, use of bacteriocins and fruit type.

Methods: The Bigelow secondary predictive model was used to interpret the combined results of the primary studies. As the meta-analytical data encompassed a number of coded study characteristics, the basic Bigelow model was transformed into a linear mixed-effects model in order to assess whether each of the moderating variables had any effect on *D* and *z*.

Results: The meta-analysis demonstrated that the heat sensitivity of AAT had on average log *D** (log *D* at 95°C and pH 3.5) values higher than juices by 0.115 units ($P < 0.01$), while the *z*-values were higher ($P < 0.01$) in concentrates than in juices. AAT possesses lower thermal resistance in clarified beverages than in non-clarified beverages ($P < 0.01$), with log *D** higher by 0.26 units, while clarification did not affect *z*-values. In addition, the effect of a bacteriocin on the thermal sensitivity of AAT became more evident at higher pH.

Significance: Because of its design, the meta-analysis model is able to provide *D* and *z* estimates for single-strength juices and concentrates of ten different fruits, with or without clarification and, with or without bacteriocins.

T2-07 Quantitative Risk Assessment of *Toxoplasma gondii* Infection through Consumption of Fresh Pork in the United States

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❖ ***Developing Scientist Competitor***

Introduction: *Toxoplasma gondii* is one of the leading foodborne pathogens in the U.S. The Centers for Disease Control and Prevention (CDC) reported that *T. gondii* is one of three pathogens, which together account for >70% of all deaths due to foodborne illness in the U.S. Consumption of undercooked pork products in which *T. gondii* has encysted is a common transmission route for human infection.

Purpose: The goal of this study was to develop a farm-to-table quantitative microbial risk assessment (QMRA) model to analyze the public health burden through consumption of fresh pork in the U.S.

Methods: *T. gondii* prevalence in pigs was described by a weighed prevalence and bradyzoites concentration was calculated in each pork cut (picnic, butt, ham, loin and belly) of an infected pig. A logistic regression and a log-linear regression were developed to model the reduction of *T. gondii* during moisture enhancement and home cooking, respectively. A Beta-Poisson dose response model was developed by using the experiment data of mice orally infected with Type II strain. The QMRA model was developed in a Monte Carlo probabilistic framework to account for uncertainty and variability in model parameters and input variables.

Results: The mean probability of infection per serving of fresh pork products ranges from 2.4×10^{-7} to 6.69×10^{-6}, corresponding to 136,470 new infections annually in general population. Approximately 1,059 new infections occurred each year in pregnant women, which associated with 346 congenital toxoplasmosis cases per annum. Sensitivity analysis suggested that cooking is the most important parameter impacting human health risk.

Significance: This study addressed the disease burden of *T. gondii* infection and quantified the effects of processing on the viability of cysts, which provides the scientific basis for risk management and also could serve as the baseline model for the QMRA of *T. gondii* in other meats.

T2-08 Quantitative Microbial Risk Assessment of Salmonellosis from Australian Pork Burgers

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Introduction: In 2014, ~16,000 cases of Salmonellosis were notified in Australia; a *per capita* incidence that is similar to other industrialized nations. Various foods have been shown to contribute to Salmonellosis in Australia, including pork products. The Australian pork industry is promoting new forms of pork products, including pork burgers. Due to their comminuted nature, pork burgers could pose an increased risk of Salmonellosis compared to intact cuts of pork due to pathogens being internalized and being inactivated more slowly during cooking.

Purpose: Estimate the risk of Salmonellosis from Australian pork burgers.

Methods: A two-dimensional stochastic model, separating variability and uncertainty was constructed, to describe changes in the prevalence and concentration of *Salmonella* on pork mince/burgers between the time of purchase at retail and consumption. The model includes the effects of time and temperature on i) potential for *Salmonella* growth during retail display, consumer transport and domestic storage and ii) inactivation of *Salmonella* during cooking, to iii) enable estimation of the probability of illness from consumption of a pork burger in Australia. A 'worst-case' scenario for the expected number of illnesses from pork burgers per year was estimated by assuming that all pork mince sold in Australia during 2012 (4.3 million kilograms) was consumed as pork burgers.

Results: The mean probability of illness for Salmonellosis from consumption of a 100 g Australian pork burger was estimated at 7.25×10^{-9} per burger (with a 95% uncertainty credible interval between 7.44×10^{-10} and 2.76×10^{-8}), or 0.73 illnesses per 100,000,000 burgers consumed on average. This would lead to an expected 0.32 cases of Salmonellosis in Australia caused from pork burgers during one year among a population of ~24 million people.

Significance: This model can be modified to estimate the risk in other countries by changing the country-specific inputs.

T2-09 Quantitative Analysis of the Public Health Impact of *E. coli* O157:H7 Cross-contamination in Beef Processing Plants

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Introduction: *E. coli* O157:H7 can persist and proliferate on cutting and conveyor equipment surface despite stringent cleaning and sanitation protocols and continue to cross-contaminate the meat contacting surface during the fabrication process, which could result in a higher health risk to the exposed population.

Purpose: The purpose of this study was to quantify the relative impact of cross-contamination from the cutting and conveyor equipment surface in slaughter house on *E. coli* O157:H7 contamination on end beef products and subsequent health risks associated with its consumption.

Methods: A QMRA model was developed to evaluate the public health risk associated with consumption of ground beef and other beef products. Three potential contamination sources were evaluated and compared, including hides, gut and cross-contamination. The concentration of *E. coli* O157: H7 were then incorporated into a dose-response model, to estimate and compare the public health risks associated with the consumption of different beef products. Attributable risks to the evaluated potential contamination sources were evaluated as well.

Results: When all the contamination sources were considered, the model predicted the mean probability of illness associated with consumption of ground beef to be 6.3×10^{-4} with a 95% C.I. (1.25×10^{-5} - 8.9×10^{-2}). When cross-contamination was not modelled, the mean probability of illness associated with consumption of ground beef was calculated as 3.9×10^{-4} with a 95% C.I. (1.9×10^{-6} - 1.6×10^{-2}), indicating 38.1% of the ground beef relevant *E. coli* O157: H7 infections can be attributable to the cross-contamination in processing plant.

Significance: The findings suggest that relative risks of *E. coli* O157:H7 infection associated with consumption of beef products could be significantly higher as a result of cross-contamination from cutting & conveyor equipment surface.

T2-10 Risk Assessment of *Vibrio parahaemolyticus* and Interventions for Raw Oysters in Taiwan

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Introduction: *Vibrio parahaemolyticus* has been recognized as a cause of gastroenteritis linked to the consumption of seafood, particularly oysters consumed raw or inadequately cooked or contaminated after cooking. In Taiwan, *V. parahaemolyticus* is the most prevalent foodborne pathogen in Taiwan. However, information is limited about the average risks per serving of raw oysters.

Purpose: This study aimed to develop a risk assessment of *V. parahaemolyticus* associated with raw oysters produced and consumed in Taiwan and suggest effective interventions steps to control the risks.

Methods: Interviews with producers and processors were carried out to collect data regarding the current production, processing, and consumption of oysters in Taiwan. An exposure assessment was performed to estimate the prevalence and density of *V. parahaemolyticus* in raw oysters from harvest to consumption with data from literatures and sample analysis. The data was combined with a Beta-Poisson dose-response model to estimate the probability of illness.

Results: Results indicated oyster production chain started with harvest, wholesaling and then retailing before consumption. After harvested, shucked oysters were washed by tap water. The oysters were then packed by styrofoam box with crushed ice and transported in non-refrigerated vehicles to wholesalers and then to retailers. The estimated average risks per serving of raw oysters was 2.2×10^{-4}. The influential interventions on the risk of illness were transport temperature and washing water temperature.

Significance: The findings could be used by the food regulatory agencies in Taiwan to establish seafood food safety policies and develop risk management strategies to reduce *V. parahaemolyticus* risk associated with the consumption of oysters.

T2-11 Norovirus Transmission during Produce Harvest and Packing: A Quantitative Microbial Risk Assessment Model Approach

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Introduction: Norovirus (NoV) is a major cause of acute gastrointestinal illness and responsible for 40% of all produce-associated foodborne disease outbreaks in the U.S. Risk modeling can be used to understand how key factors in the production chain impact pathogen contamination in fresh produce.

Purpose: Develop and apply a quantitative microbial risk assessment (QMRA) model that simulates the likelihood and magnitude of NoV contamination of produce from infected workers during harvest and packing.

Methods: A stochastic QMRA model was developed in R using data (derived from the literature or experts) on key parameters related to: NoV load on worker hands, worker behavior, and transfer, persistence, and dissemination of NoV. A stochastic sensitivity analysis identified the most relevant parameters. Scenario testing assessed the impact of hand washing, reduced produce contact, and worker illness severity on produce contamination.

Results: Produce contamination was greatest for the first several items harvested immediately after infected workers used the restroom, declining by over 2 log to approximately 20 virions/cm^2 by the 10th item. Complete compliance of workers with recommended handwashing reduced contamination by 26.3% (SD = 33.2%) (packers) and 20.2% (SD = 23.8%) (harvesters), compared to the baseline model. A doubling of handwashing efficacy reduced contamination by 29.9% (SD = 35.2%). Contamination of produce declined to a negligible level (10 virions/cm^2) when both packers and harvesters were shedding less than 10^7 NoV virions/g stool. This suggests that it might be relatively safe for workers to return after the period of peak viral shedding has passed, as long as they adhere to rigorous hand hygiene.

Significance: QMRA modeling can identify key factors that contribute to NoV transmission and allows for prioritization by magnitude of these effects. Model results support guidelines that reduce acutely ill workers' contact with produce and encourage brief furlough and handwashing compliance to reduce the risk of NoV contamination.

T2-12 A Food Processing Vulnerability Tool Exploring Public Health Risks

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Introduction: The Food Safety Modernization Act (FSMA) Section 106 requires that a vulnerability assessment of the food system be conducted, including biological, chemical, radiological or other risk assessments. The Department of Homeland Security (DHS) has defined vulnerability as "likelihood that an attack is successful, if it is attempted." The success of an attack on the food system depends on the ability of the hazard to persist in the product at concentrations sufficient to cause harm after distribution.

Purpose: We have developed a web-based food processing vulnerability assessment tool for agents of concern that may be used in attacks targeting food production systems. The aim is to inform decisions focusing upon protecting the production system from attacks.

Methods: The tool is based upon an underlying stochastic simulation model tracking the levels of a suite of agents of concern in a user-defined food production system. Results are presented incorporating assessments for a set of multiple agents of concern, both microbial and chemical (e.g., *Bacillus anthracis*, *Clostridium botulinum*, *Yersinia pestis*, and other agents that may be used in attacks on the food supply).

Results: The tool provides quantitative estimates that directly measure risk and vulnerability using public-health based metrics. Adopting a multi-hazard approach, results provides a holistic view of the vulnerability of the production system, as opposed to single hazard-food based assessment. Results of the vulnerability assessment of illustrative processing systems will be presented.

Significance: Our tool is generic in nature, and can be applied to a multitude of food production systems. It enables exploration of the impact of risk mitigation measures upon the vulnerability of a food production system. Use of the tool will provide stakeholders with science-based quantitative information that can be directly used to inform decisions enhancing the resiliency of the supply chain, and minimizing the risks to the consumer.

T3-01 Assessment of Pre-Enrichment Conditions for Molecular and Cultural Detection of *Listeria* Species and *L. monocytogenes* from Produce Cooling and Packing Environmental Samples

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Introduction: Environmental monitoring for harborage sites of *Listeria* spp. and *L. monocytogenes* and differentiation of resident and transient sources is increasingly expected of raw agricultural commodity (RAC) facilities. *Listeria* detection may be limited by the performance of the enrichment media in complex matrices.

Purpose: To evaluate *Listeria* enrichment broth (LEB), Half-Fraser Broth (HFB) and analytical sample volume for the detection/recovery of *Listeria* spp. at RAC handling facilities using automated Atlas molecular detection (Roka Bioscience) and culture-based methods.

Methods: Four hundred forty-five sterile sponges and swabs were collected from seven RAC facilities. One hundred eighteen samples were placed in 190 ml of HFB and incubated at 37°C for 24 hours. Three hundred twenty-seven samples were enriched in 90 ml of supplemented LEB and incubated at 37°C for 48 h. Following incubation, 12 µl (validated volume) were subjected to Atlas-Listeria protocols. In addition, 400 µl (sediments and highly oxidized organic matter) were evaluated in 161 paired samples. After incubation, CHROMagar Listeria incubated at 37°C for 24 - 48 h was used for confirmation of *Listeria* spp. with qPCR using *23S r*DNA and *hlyQ* gene targets for *Listeria* spp. and *L. monocytogenes*, respectively.

Results: A total of 172 (38.4%) samples were positive for *Listeria* spp. Forty-seven of the positive samples were enriched in HFB (39.8%) and 125 in LEB (38.2%). *Listeria* spp. was isolated from 97 of 172 positive samples (56.4%). Forty-two strains were confirmed as *L. monocytogenes* (43.2%). The molecular detection of *Listeria* could be affected by the volume used to inoculate the Roka Atlas transfer tube. When 400 µl were used 71 of 161 samples were positive, in contrast 59 paired samples were positive with a 12 µl analytical sample.

Significance: The results of this study contribute to improved *Listeria* detection and recovery. The use of supplemented LEB without primary enrichment was effective for environmental detection of *Listeria* in these complex matrices.

T3-02 Assessment on the Sensitivity and Specificity of Five Culture Media in the Detection of Environmental *Escherichia coli* O157

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❖ ***Developing Scientist Competitor***

Introduction: *Escherichia coli* is a large and diverse group of bacteria. Most of them are harmless and are naturally found in the human intestinal tract, but others can be deadly. *E. coli* O157:H7 has become an important problem in human health in the United States. Scientific studies have indicated that cattle herds are the primary reservoirs of *E. coli* O157:H7. The ability of *E. coli* O157:H7 to survive in soil and manure for extended

periods of time could explain its spread into the water supply and onto crops. Prior to confirm the presence of *E. coli* O157:H7 in a sample, proper isolation of *E. coli* O157 is necessary.

Purpose: The purpose of this study was to establish the sensitivity and specificity of five commercial media, and establish which of them is/are the best option for isolation for presumptive identification of environmental *E. coli* O157 from cattle farms.

Methods: One hundred thirty-eight samples of fresh cattle fecal matter on the ground, water from water troughs and ponds, and swabs from troughs, salt and hay bunks were tested for *E. coli* O157. For the isolation of the *E. coli* O157, samples were enriched in TSB, followed by an Immunoseparation and then plating onto SMAC, CT-SMAC, CHROMagar™, T CHROMagar™, and VCC CHROMagar™. RT-PCR was used to identify the strains by using the FDA's BAM methodology and the genes were stx_1, stx_2, and *eae*.

Results: SMAC tested positive to *E. coli* O157 in 109 samples, an 81% of these samples were false positive. Using logistic regression CT-SMAC and CHROMagar® was the best combination for the isolation of *E. coli* O157, showing a 79% of positive and only 0.05% of false negative results.

Significance: The findings of this study can be used by regulatory agencies for detection of environmental *E. coli* O157:H7 in cattle and produce farms.

T3-03 Development of an Improved Sampling Method for Concentrating Viruses from Bioaerosols

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Introduction: Bioaerosols are a potential source of food contamination with microorganisms, including viruses. Among the most effective platforms to capture viruses from bioaerosols is the SKC Biosampler, an all-glass impinger employing a high-volume sonic flow pump to trap aerosolized particles in liquid matrices approximately 20 ml in volume. However, this relatively large sample volume limits the sensitivity of molecular detection methods in which only small sample volumes are typically analyzed.

Purpose: Here, the performance of a novel modification to SKC Biosamplers compatible with molecular detection strategies was tested. IRA-900 anion exchange resin was added to the SKC BioSampler liquid matrix to adsorb and concentrate negatively charged MS2 and ϕ6 bacteriophages (also surrogates of human enteric viruses and influenza virus, respectively).

Methods: MS2 and ϕ6 (10^{-3} to 10^{6} PFU/ml inocula) in PBS were aerosolized to particle concentrations of 5 mg/m^3 in a HEPA-filtered bioaerosolization chamber. For each experiment (performed in triplicate), two SKC BioSamplers containing 20 ml of PBS with and without 0.5 g of IRA-900 were co-located in the chamber and each attached to a pump calibrated to sample 500 liters of air in 40 min. RNA was isolated from the PBS and IRA-900 for real time reverse transcriptase PCR (RT-PCR) analyses.

Results: The addition of IRA-900 to SKC BioSamplers markedly improved RT-PCR detection sensitivity for MS2 by an average of 8.15× ($P < 0.05$), and allowed for reliable detection of 1 log fewer MS2 (10^2 PFU/ml MS2 inoculum) compared to direct testing of unmodified SKC BioSamplers. For ϕ6, the improvement in detection sensitivity was not as pronounced as with MS2 (average of 2.08 × improvement) and allowed for 10^3 PFU/ml of the ϕ6 nebulized inoculum to be detected.

Significance: The addition of IRA-900 to SKC BioSamplers improved the sensitivity of RT-PCR detection with the tested aerosolized bacteriophages.

T3-04 Challenges to Develop a Detection Method for Hepatitis A Virus of Culture or Clinical Origin from Frosting Containing Berries

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Introduction: The number of reported outbreaks caused by foodborne viruses and more specifically human noroviruses and hepatitis a virus (HAV) has been on the rise. However, even though the means of transmission can be identified as foodborne, the virus cannot always be isolated from the suspected food vehicle.

Purpose: Berries have frequently been associated with HAV outbreaks worldwide. A study was undertaken to detect HAV from artificially contaminated strawberries in a cake frosting mix.

Methods: HAV was used as inoculum from either purified cell culture lysate or clinical samples to seed a 30 g of strawberries-frosting mix sample. HAV was eluted with a 0.1 M Tris-HCl, 0.05 M glycine, 1% beef extract, pH 9.2 (TGBE) containing 2% Polyvinyl Pyrrolidone (PVP). After a brief spin, the eluate was combined with chloroform to separate the fats from the frosting, and after a second re-extraction was precipitated with 10% polyethylene glycol overnight. The next day the pellet was washed with chloroform-butanol, the virus was eluted with TGBE buffer and concentrated with a second PEG precipitation. Finally, virus RNA was isolated from the pellet with a commercial kit and detected with a real-time RT-PCR.

Results: HAV could be detected at a level of seeding of at least 10^4 PFU of purified cell culture lysate per 30 g of sample. When the frosting-strawberries mix was inoculated with a clinical isolate of HAV, recovery was achieved at a low contamination level of less than 200 RNA copies per 30 g sample.

Significance: We developed a rigorous methodology to isolate HAV in a strawberries-frosting mix, that also provides insight into useful steps for reducing the inhibitory effect of polyphenolic and fat substances often present in produce and produce-related food items, with the aim of providing a tool for critical response during disease outbreaks.

T3-05 Characterization of Outer Membrane of *Salmonella* Responding to Different Inactivation Treatments Using Surface Enhanced Raman Spectroscopy

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❖ ***Developing Scientist Competitor***

Introduction: *Salmonella* is a one of the most common causes of food poisoning in the US and the inactivation of *Salmonella* cells can be achieved through heating, ethanol and sodium hypochlorite treatments. The outer membrane of *Salmonella* cells plays an important role in responses to these treatments. Surface enhanced Raman spectroscopy (SERS) is a combination of Raman spectroscopy and nanotechniques and it is a perfect tool to characterize the molecules specifically in bacterial outer membranes.

Purpose: The objective of this study is to characterize the changes of *Salmonella* cell outer membrane after heating, ethanol and sodium hypochlorite treatments using SERS.

Methods: In this study, *Salmonella* Enteritidis 710 and 1045 were cultured and inactivated by heating, ethanol and sodium hypochlorite. After inactivation, cells were washed in the saline water and then mixed with 50 nm gold nanoparticles for SERS characterization. Data were analyzed by principle component analysis.

Results: The results show the SERS spectra changed significantly after treatments and the variations in the SERS spectra between treatments demonstrated different mode of actions of these treatments. Among all these treatments, the SERS spectra after sodium hypochlorite treatment showed the largest discrimination from the control. Significant alternations of the lipid peaks were observed, demonstrating the destruction of the phospholipid and lipopolysaccharides in the outer membrane after the treatment. The heating and ethanol treatments altered the protein peaks.

Significance: These results demonstrated the capacity of SERS to characterize the outer membrane of *Salmonella* cells responding to different treatments, which can help us to further understand the behaviors and mechanisms of bacterial outer membrane responding to different treatments, from a biochemical perspective. The spectral discrimination can be also set as a base for prediction of the sensitivity and viability of cells responding to different treatments using SERS.

T3-06 Development of a Novel CRISPR-based Molecular Typing Method of *Salmonella*

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Introduction: *Salmonella* is one of the major foodborne pathogenic bacteria; moreover, *Salmonella* Enteritidis is the most important *Salmonella* serovar around the world. Molecular typing for *Salmonella* isolates can reveal their origin, variation and evolution, providing key support data for hazard tracing, risk assessment and hazard control.

Purpose: The purpose of this study was to establish 2 novel rapid, effective and simple typing methods for *Salmonella* including CSST (CRISPR initial three Spacers Sequence Typing) and TCSST (Target-CRISPR initial three Spacers Sequence Typing) based on the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) sequences.

Methods: CSST and TCSST are applied to subtyping different *Salmonella* serovars and *Salmonella* Enteritidis, analyzing CRISPR1 and CRISPR2 spacer sequence profiles, computing the discrimination power of CSST/TCSST typing method, discussing the relationship between the source and virulence with TCSSST type.

Results: A total of 86 *Salmonella* strains representing 30 serovars were differentiated by CRISPR, S69, CSST and TCSST with discrimination power 0.9692, 0.9199 and 0.9423, respectively. TCSST typing were used to discriminate 90 *Salmonella* Enteritidis isolates from the year of 2008 to 2012 from different sources in Shanghai with discrimination power 0.7506. TCSST types were correlated with the sources, isolation years and virulence genes of *Salmonella* Enteritidis isolates. In addition, when the number of strains was amplified to 198 *Salmonella* Enteritidis strains, 31 TCSST types were obtained, and the value of discrimination power was 0.6555.

Significance: Two novel molecular typing methods were developed in this study as CSST and TCSST; CSST typing could be used in the classification of different *Salmonella* serovars with advantages of simple operation and low cost; and when S69 was involved, TCSST typing could be used for the discrimination of different *Salmonella* isolates from one serovar such as *Salmonella* Enteritidis.

T3-07 Immunosensor-Based Simultaneous Detection of 7 Major STEC Serotypes

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❖ ***Developing Scientist Competitor***

Introduction: Shiga Toxin-producing *Escherichia coli* (STEC), including the most recognized serotype O157,and other major non-O157 STEC serotypes (O26, O45, O103, O111, O121, and O145), commonly known as "Big Six," have been associated with multiple outbreaks and they are considered as adulterants in certain meat products. Therefore it is highly desirable to develop assays that can simultaneously detect multiple STEC serotypes.

Purpose: The goal of this study was to develop a sensitive and rapid immunosensor-based multiplexing array system to simultaneously detect and identify 7 major STEC serotypes.

Methods: The mixture of 7 different types of immunosensors, which are pre-encoded magnetic beads functionalized with antibodies to each of 7 STEC serotype, was loaded into a 96-well microplate and used as an array platform. Presence of target STEC was determined and identified by reading fluorescent signals from binding of fluorescently labeled detection antibodies to target STEC cells. The developed array system was tested for its sensitivity and specificity.

Results: Results from this study showed the developed immunosensor array was an easy-to-operate, high-throughput detection system for multiple STEC serotypes. The array could be completed in less than 5 h. The optimized multiplex array was able to detect STEC as low as 10^4 CFU/ml without any enrichment and 10-100 CFU/ml with 12 h enrichment. Even though there was cross-reactivity among STECs, each STEC was able to be identified by recognizing unique immunosensor binding pattern.

Significance: This result indicates the developed immunosensor-based multiplexing array can be reliable method for simultaneous detection of multiple STECs. This array shows great potential to be adapted for automatic testing of food or environmental samples for the presence of STECs.

T3-08 Application of a Novel Four-Plex Quantitative PCR Assay for Quantification of *Escherichia coli* O157 on Cattle Hide and Carcass

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❖ ***Developing Scientist Competitor***

Introduction: We have previously developed and validated a multiplex (four-plex) quantitative PCR (mqPCR) to detect and quantify *E. coli* O157 in cattle feces. The assay targets genes that code for serogroup specific O157 antigen ($rfbE_{O157}$) and three major virulence factors, Shiga Toxins 1 and 2 (*stx*1 and *stx*2) and intimin (*eae*). Cattle hides are the major source of *E. coli* O157 contamination on carcasses at the time of slaughter. Although prevalence data have been reported, concentration data are an important component of quantitative microbial risk assessments. Although several real-time PCR assays have been developed to quantify *E. coli* O157, none have included *rfbE* in combination with *stx*1, *stx*2, and *eae*.

Purpose: Our objective was to validate a mqPCR for the quantification of *E. coli* O157 in cattle hide and carcass samples.

Methods: Pure culture sensitivity of the assay was determined with extracted DNA from serial ten-fold dilutions of *E. coli* O157 strain (ATCC 43894), positive for all four genes, cultured in Luria-Bertani broth. Cattle hide and carcass sponge samples (n = 78) were collected at a slaughter

plant. Samples, determined to be negative for *E. coli* O157 by PCR were inoculated with ten-fold serial dilutions of the same strain. Sensitivity of the mqPCR assay from spiked hide and carcass samples before and after six-hour enrichment was then determined.

Results: In pure culture, the minimum detection limit of the mqPCR assay was 2.2 x 10^3 CFU/ml. The detection limit of the assay for *E. coli* O157 with DNA extracted from cattle hide and carcass samples was identical to pure culture sensitivity (2.2 x 10^3 CFU/ml) for both sample matrices. After a six-hour enrichment, sensitivity increased to 2.2 x 10^0 CFU/m for carcass and hide samples.

Significance: The assay targeting the four genes is a sensitive and high-throughput method to quantify *E. coli* O157 in cattle hide and carcass samples.

T3-09 Colorimetric-SERS Dual Detection of Food Contaminants Using Aptamer-Gold Nanoparticle Conjugates

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❖ ***Developing Scientist Competitor***

Introduction: Gold nanoparticles (AuNPs) based colorimetric assay is gaining popularity as a rapid method for detecting small molecules due to its low cost and simple readouts. However, its application is limited due to nonspecific interactions with many compounds. Hence, it is necessary to develop an improved method for real application such as food analysis.

Purpose: The objective of this study was to develop a rapid, sensitive method to detect food contaminants by using highly specific aptamers coupled with AuNPs to perform colorimetric and SERS measurements.

Methods: Aptamers (ssDNA) specific to ampicillin and phorate were mixed with their target at varying concentration ratios (up to 1:100) and incubated for 10 min (n = 3). Next, 13 nm citrate-capped AuNPs was added and incubated for 5 min. Finally, NaCl was added. If no target was present, the aptamers would physically adsorb on AuNPs and prevent aggregation. However, if target was present, the aptamer would bind to target instead, causing aggregation of AuNPs (red to purple/blue). One µl of sample was deposited and dried on gold slide for Raman analysis. The absorbance measurements of the samples were analyzed between 450 - 750 nm. Total detection time was less than 40 min.

Results: SERS was more sensitive than colorimetric measurements and could detect as low as 100 nM. Sample spectra showed new peaks and target peak shifts, owing to molecular interactions between aptamer and target, which was confirmed using principal component analysis. Our results also suggest colorimetric measurements were not very sensitive possibly due to co-interaction between aptamer-AuNPs and aptamer-target. Method optimization may help to further increase sensitivity of the colorimetric assay.

Significance: This method can potentially be used for rapid screening of small molecules such as antibiotics and pesticides. We can also validate colorimetric measurements with SERS.

T3-10 Validation of a Gold Nanoparticle DNA-based Biosensor for the Detection of Non-PCR Amplified Bacterial Foodborne Pathogens in Solid Food Matrices

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Introduction: The prospect of bioterrorism in food has identified critical needs for detection. Our DNA-based biosensors, which utilizes gold nanoparticles (AuNPs) for signal amplification and magnetic nanoparticles (MNPs) for easy and clean separation, has been shown to detect non-PCR amplified genomic DNA targets (DNAt) from bacterial pathogens within liquid food matrices and is one step closer toward providing a new, simple, fast, reliable, and economical technology for detection.

Purpose: The purpose of this study is to further validate our DNA-based biosensor detection system by testing its ability to detect specific bacterial DNAt within solid food matrices such as egg yolks and spinach.

Methods: Non-PCR amplified genomic DNAt samples were extracted from food matrices spiked with *Salmonella* Enteritidis, *E. coli*, *Salmonella* Enteritidis and *E. coli*, or LB broth (control). DNA targets (50 ng/ml) were then hybridized into a sandwich-like structure consisting of MNPs/DNAt/AuNPs, of which both MNPs and AuNPs contained probes specific for the insertional element (*Iel*) of *Salmonella* Enteritidis. The sandwich structures were then placed onto screen-printed carbon electrodes to detect the voltammetric peaks of gold using differential pulse voltammetry (DPV). All samples were run in triplicate.

Results: The biosensor was able to detect non-PCR amplified *Salmonella* Enteritidis genomic DNAt from all food matrices that had been spiked with *Salmonella* Enteritidis compared to those spiked with another pathogen or control. For example, gold voltammetric peaks were higher ($P \leq 0.05$) in DNAt samples isolated from yolks spiked with *Salmonella* Enteritidis (4.4 x 10^{-5} A) or *Salmonella* Enteritidis and *E. coli* (3.2 x 10^{-5} A) compared to samples spiked with *E. coli* (1.6 x 10^{-5} A) or LB broth (6.5 x 10^{-6}A).

Significance: These findings suggest that our DNA-based biosensor can serve as a means to detect bacterial food pathogens from solid food matrices. Future work will determine if it can be used in a field non-laboratory setting.

T3-11 Evaluation of an Automated Most Probable Number System for Use in Measuring Bacteriological Quality of Grade "A" Milk Products: A Method Validation Study

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❖ ***Developing Scientist Competitor***

Introduction: The Food and Drug Administration (FDA) runs a long-standing milk sanitation program and uses Grade "A" Pasteurized Milk Ordinance (PMO) standards to maintain safety of Grade "A" milk sold in the U.S. The PMO requires Grade "A" milk samples be tested using validated total aerobic bacterial count methods approved by the National Conference on Interstate Milk Shipments (NCIMS).

Purpose: Conduct an interlaboratory method validation study to compare performance of the NCIMS-approved Petrifilm™ reference method to the alternative, automated MPN, TEMPO® method for total aerobic bacterial counts using new statistical approaches.

Methods: An interlaboratory method validation study was conducted concurrently with the FDA's annual milk proficiency test (PT) to compare method performance in five milk types: pasteurized, chocolate, cream, 2%, and raw. Eighteen analysts from 9 laboratories analyzed test portions from each sample in triplicate. Statistics including mean bias and matrix standard deviation (corrected for standard error) were calculated.

Results: Sample-specific bias of the alternative method for total aerobic count suggests that there are no large deviations within the population of samples considered. Based on analysis of 648 data points, mean bias of the alternative method across milk samples for total aerobic count was 0.013 (log) and the confidence interval for mean deviation was -0.066 - 0.009 (log). These results indicate that the mean difference between the selected methods is small and not statistically significant. Matrix standard deviation was 0.077 (log), showing there is a low risk for large sample-specific bias based on milk matrix.

Significance: An innovative multi-laboratory method validation study was conducted by integrating the study into the FDA's milk PT. Comparing the performance of an automated MPN to a reference method for aerobic count demonstrates how PT data and statistical methods including mean bias and matrix standard deviation can be used to compare method performance in a novel way.

T3-12 Accuracy and Precision of Analyst and Method Performance in Testing Indicator Organisms in Infant Formula Based on a Proficiency Study

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Introduction: Indicator microorganisms are widely used to assess the microbiological quality of foods. Measurement variability in microbiological testing could affect enforcement of regulations. Proficiency tests allow for statistical evaluation of the accuracy and precision of routine screening methods, analysts, and laboratories in order to quantify variability.

Purpose: Quantitatively analyze variability of analysts, laboratories, and methods from a proficiency test assessing the level of indicator organisms in infant formula.

Methods: Commercial liquid and powdered infant formula samples (LIF, PIF) were spiked at two different inoculum levels in duplicate with a cocktail of Gram positive and negative organisms. The inoculation procedures, mixing methods, and homogeneity/stability were verified by three independent trials according to ISO 17043 and ISO 13528. Aerobic Plate Count, Total Coliforms, Fecal Coliforms, *Enterobacteriaceae*, and *E. coli* were measured by 79 analysts/59 laboratories (LIF) and 19 analysts/19 laboratories (PIF).

Results: Homogeneity and stability of distributed samples, log-normal distributions, skewness, Youden plot analysis, and Analyst/laboratory z-scores were calculated according to ISO 13528 using ProLab®. Relative repeatability was 12.78 - 81.25% (LIF) and 31.03 - 105.37% (PIF). Relative reproducibility range was 31.03 - 105.37% (LIF) and 83.24 - 175.82% (PIF). Equivalency testing was calculated using Q-Hampel method and showed Petrifilm™ and traditional plate count methods were not equivalent for aerobic counts in LIF samples with a relative bias of 70.31%. MPN and Petrifilm™ had comparable performance for total coliform, *E.coli*, and fecal coliform counts in LIF samples with relative biases of -7.64%, 7.81%, and -0.5%, consecutively.

Significance: This study demonstrates the value of data from large, interlaboratory studies, such as proficiency tests, can be used in a novel way to measure accuracy and precision of methods, analyst's performance as well as overall performance of laboratories.

T4-01 Assessment of the Effect of Ingredients on Pathogen Survival in Cookie Dough

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Introduction: Cookie dough is often recognized as a potential vehicle for *Salmonella.* Food Safety concern for cookie dough has been further raised due to the recent *E. coli* O157: H7 outbreak associated with commercial product. It is believed that the nature of cookie dough of high fat and sugar contents and relatively low water activity can provide protection for pathogens to survive during storage.

Purpose: The goal of this study was to determine the survival of foodborne pathogens in cookie dough and assess the impact of common cookie dough ingredients on survival of pathogens.

Methods: Commercial cookie dough was inoculated with *Salmonella* Enteritidis and *E. coli* O157:H7 (10^6 CFU/g), stored at 4°C and -18°C, and the survival of pathogens was determined for 8 weeks. To determine the effect of ingredients on pathogen survival, cookie dough samples were prepared with various fat, sugar and salt contents, inoculated with *Salmonella* Enteritidis or *E. coli* O157:H7 (10^6 CFU/g), and their survival was determined for 8 weeks.

Results: After 8 weeks, 2.42 and 2.35 log reduction for *Salmonella* and 2.23 and 1.99 log reduction for *E. coli* O157:H7 were obtained for commercial cookie dough at 4**°**C and -18**°**C, respectively. Cookie dough prepared with seven different recipes of cookie resulted in reduction of both pathogens, ranging from 0.73 to 1.45 log CFU/g. Our results indicate that pathogens in cookie dough products can survive through their recommended storage conditions, and changing the content of a single ingredient hardly affects their survival rate.

Significance: Our study suggests that that refrigeration/freezing and monitoring the ingredients concentration do not reduce the survival of pathogens. Following good manufacturing practices is a fundamental step to eliminate initial introduction of pathogens in cookie dough, and, consumer education on risk of consuming raw cookie dough would be critical to eliminate future outbreaks.

T4-02 Analysis of Osmotic Stress on Methicillin-resistant *Staphylococcus aureus* (MRSA)

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Introduction: Understanding the stress of some of foodborne pathogens provides insight into how these organisms survive stress and subsequently cause disease in humans and animals. MRSA can tolerate high salt concentrations, a common extrinsic factor to control bacterial growth, and can be transmitted to humans through food.

Purpose: The purpose of this study was to define the transcriptional response of MRSA JE2 to osmotic stress and elucidate the role of the stress response regulator σ^B using RNA-Seq.

Methods: A nonpolar transposon was used to disrupt transcription of σ^B encoded by *rpoF*. The minimum inhibitory concentration (MIC) of NaCl was defined for wildtype and *rpoF*::TnT. RNA was extracted from early log phase (OD_{600} = 0.4) wildtype cells with and without exposure to 10% NaCl and *rpoF*::TnT cells exposed to 10% NaCl. RNA-Seq was performed on the Illumina HiSeq 2500 platform in triplicate. Reads were normalized and mapped to the MRSA FPR3757 genome using Tophat (v 2.0.11). The EdgeR package in R (v. 3.1.1) was used to identify differential expression (adj. *P*-value < 0.05) between wildtype cells with and without exposure to NaCL and wildtype and *rpoF*::TnT cells exposed to NaCl.

Results: The MIC for wildtype and *rpoF*::TnT exposed to NaCl was 13.8% and 9.4%, respectively. A total of 68 genes were differentially expressed when wildtype cells were exposed to NaCl, including up-regulation of virulence genes *asp23* and *rpoF*. Comparison of wildtype and *rpoF*::TnT exposed

to NaCl identified 371 differentially expressed genes and 16 ncRNAs. Virulence regulators *agr* and *sar* were upregulated in *rpoF*::TnT, while antibiotic resistance genes *femA*, *epiF*, and *epiP* were downregulated.

Significance: This study defines the global transcriptome of MRSA during osmotic stress and increases understanding of the σ^B regulatory network in *S. aureus*. Understanding stress response networks in MRSA may lead to the development of better control strategies and novel drug targets.

T4-03 *Lactobacillus plantarum* Isolates from Different Vegetables That Have Antifungal Activity against the Common Cheese Spoilage Mold *Penicillium commune* are Genetically Related

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Introduction: Molds are the most common cheese spoilage organisms which can lead to economic loss as well as raising public health concerns due to the production of mycotoxins. In a previous study, 897 lactic acid bacteria (LAB) isolated from different herbs, fruits and vegetables were screened for their antifungal activity and 12 were confirmed as having strong activity against the common cheese spoilage mold *Penicillium commune*. These 12 LAB were all identified by 16S rDNA sequencing as being *Lactobacillus plantarum*; however, it was not known if they are closely related *L. plantarum* isolates.

Purpose: This study aimed at determining if the 12 antifungal *L. plantarum* isolates were genetically related to each other and to 2 other *L. plantarum* strains (strain ATCC14917 from pickled cabbage and the commercial probiotic strain 299v).

Methods: Three different randomly amplified polymorphic DNA (RAPD) PCR primers (M13, P4 and P7) were tested for their ability to generate useful banding patterns using RAPD-PCR for the *L. plantarum* strains.

Results: It was found that one RAPD-PCR primer (M13) generated useful banding profiles with up to 9 bands visible between 750 bp and 3.5k bp. The other primers generated only 2 or 3 bands. All the 12 antifungal LAB isolates produced identical profiles as each other with the 3 primers and also to strain 299v, but were clearly different to strain ATCC14917.

Significance: These results demonstrate that the M13 primer is useful for genotyping antifungal *L. plantarum* isolates and that these antifungal *L. plantarum* isolates from different vegetables are likely to be closely related and therefore possibly share the same antifungal mechanism of action.

T4-04 Assessing Immunological Risk of *Listeria* Infection in the Aging Population Using a Susceptible Animal Model

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

Purpose: The goal of this study is to establish a murine model of oral LM infection that can be used as a surrogate for human foodborne Listeriosis in the aged population.

Methods: C57BL/6 (wild-type, WT) and IL17R-KO (Knock-Out) mice were orally infected with increasing doses of a murinized LM strain (Lmo-InlA^m) and monitored for body-weight loss and survivability. Tissues were collected and assayed for bacterial burden, histology, and cytokine response. Isolated splenocytes were assayed for cytokine mRNA response (IL-2R, TNF-α, IL-10 and IFN-γ) and protein by flow cytometry.

Results: When compared to WT mice, IL17R-KO mice are more susceptible to LM infection and showed increased tissue pathology, LM burden and a higher mortality rate. Old LM-infected KO-mice lost significantly (P = 0.001, ANOVA) more body-weight and had a higher bacterial burden in liver (P = 0.03) and spleen (P = 0.05) as compared to young mice. Uninfected, old KO-mice showed a higher baseline pro-inflammatory response when compared to young, uninfected mice. After infection, the pro-inflammatory cytokine (IFN-g and TNF-a) mRNA and protein in the liver or spleen were higher in the young mice compared to the old mice. Anti-inflammatory cytokine (e.g., IL-10), Treg ($CD4^+CD25^+$) cells, and T-cell activation marker, CD25 (IL-2Ra) expression in the old mice did not increase over baseline, suggesting cellular anergy and immunosenescence in the old mice.

Significance: These data suggest that IL17R-KO mice can be used as *in vivo* model to study oral Listeriosis. We further showed that older mice are more susceptible to LM infection due to slower and reduced pro-inflammatory response compared to young mice, resulting in a delayed clearance of the infection.

T4-05 Histo-blood Group Antigen Expressing Bacteria May Help with Human Norovirus Survival before Causing Infection

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Introduction: Human noroviruses (NoVs) bind to histo-blood group antigens (HBGAs) in a strain-specific manner, and an individual's susceptibility to NoVs correlates with his HBGA profile. Recently, it was reported that human NoV infection of B cells required the presence of HBGA-expressing enteric bacteria.

Purpose: This study aims to investigate if the HBGA-expressing bacteria have any protective role on NoV before causing infection.

Methods: Twelve bacteria strains were included belonging to *Enterobacter cloacae, Enterobacter aerogenes, Escherichia coli, Clostridium difficile, Bifidobacterium adolescentis, Bifidobacterium longum,* and *Campylobacter jejuni*. The HBGA expression of the bacteria as well as the binding of human NoV virus like particles (VLPs, GI.1 and GII.4 strains) to the bacteria were identified by flow cytometry using the corresponding antibodies. NoV VLPs pre-incubated with HBGA expressing or non-HBGA expressing bacteria were heated and detected by both porcine gastric mucin-binding assay and direct ELISA.

Results: The NoV-binding abilities of the bacteria correlated well with their HBGA expression profiles. Two HBGA expressing *E. coli* (LMG8223, and self-isolated strain 861, both GI.1 and GII.4 binders) and one non-HBGA expressing *E.coli* (ATCC 8739, neither GI.1 or GII.4 binder) were selected for the heat treatment test with NoV VLPs. As a result, compared with the same cell numbers of non-HBGA expressing *E. coli*, the presence of HBGA-expressing *E. coli* could always maintain higher mucin-binding ability (higher OD values of mucin-binding assay) as well as antigen integrity (higher OD values of direct ELISA) for NoV VLPs of both GI.1 and GII.4 after heat-treatment at 95°C for 2 min.

Significance: These results indicate that HBGA-expressing bacteria may protect NoVs during the food processing treatments and possibly in the human gastrointestinal tract before causing infection, which has to be further investigated in the future.

T4-06 Antibacterial Effect and Mechanism of High-intensity 405 nm Light Emitting Diode on *Bacillus cereus, Listeria monocytogenes*, and *Staphylococcus aureus* under Refrigerated Condition

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Introduction: Antibacterial effect of 405 nm light emitting diode (LED) on several pathogenic bacteria has been investigated, demonstrating that the LED could be useful to control bacterial growth. For food application, it is also necessary to evaluate its antibacterial effect on various foodborne pathogens at low temperature that simulates refrigerated storage condition. Moreover, its antibacterial mechanism on these pathogens should be elucidated well.

Purpose: The objective of this study was to investigate the inactivation of *Bacillus cereus, Listeria monocytogenes*, and *Staphylococcus aureus* by 405 nm LED illumination under refrigerated condition and to elucidate its antibacterial mechanism by examining bacterial membrane and DNA damage.

Methods: A 405 nm LED with irradiance of 18.2 mW/cm^2 illuminated bacteria in phosphate-buffered saline for 7.5 h (a total dose of 486 J/cm^2) at 4°C. The effect of LED illumination on bacterial membrane was determined using 4 - 7% NaCl and Live/Dead®Cell Viability assay. Comet assay and DNA ladder analysis were used to examine DNA degradation.

Results: The LED illumination inactivated 1.9, 2.1, and 1.0 log CFU/ml for *B. cereus, L. monocytogenes,* and *S. aureus*, respectively, exhibiting that *L. monocytogenes* was the most sensitive strain to the LED illumination. Regardless of bacterial strain, more than 90% of LED-illuminated cell populations became sensitive to NaCl within 4.5 h, while non-illuminated cells were still resistant to NaCl. A Live/Dead®assay clearly revealed that the LED illumination resulted in a loss of bacterial membrane integrity, whereas no DNA degradation was observed by both comet assay and DNA ladder analysis.

Significance: This study proposes the potential of 405 nm LED in controlling these Gram-positive pathogens in food matrix and suggests that the antibacterial mechanism of the LED illumination might be due to cell membrane damage rather than DNA degradation.

T4-07 Modeling the Growth Rate of *Pseudomonas fluorescens* as a Function of Residual Dioxygen Concentrations in Food Packages

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Introduction: Packaging protects foodstuffs from contact with the external environment and from air-borne contamination. Combined with a modified atmosphere, packaging also helps to prolong the shelf life of a food product and can also contribute to improving food safety. In industry, presence of residual oxygen concentrations exists in the packaging.

Purpose: The aim of this work is to study and model the impact of dioxygen concentrations on *Pseudomonas fluorescens* growth and assess whether residual oxygen concentration is sufficient for the development of aerobic microflora.

Methods: *Pseudomonas fluorescens* was cultured in BHI agar medium supplemented with 0.2% of glucose, 0.3% of yeast extract and rezazurin. All petri-dishes were prepared in advance, stored in an hypoxia laminar flow hood, inoculated and then stored at 25°C. To determine kinetics, 15 samplings were performed for given condition of O_2 enriched atmosphere. For each sampling time, agar medium was collected, diluted and plated to determinate bacterial population. Growth rate was estimated by fitting logistic primary model using delay and rupture.

Results: Growth rates were acquired for 9 concentrations of dioxygen between 0.1% to 6% for *Pseudomonas fluorescens*. With only 0.1% of dioxygen, the growth of pseudomonas is possible and an increase of the concentration of dioxygen resulted in an increase of growth rate of up to 5% O_2, value from which growth rate is stabilizing. Based on Zwietering gamma concept and Rosso cardinal model, this study integrates a new factor in predictive modeling which is the concentration of dioxygen.

Significance: This experimental protocol allowed performing data under statics residual dioxygen concentrations between 0.1% to 6% during several days. Taking into account the impact of residual dioxygen concentrations on bacterial growth in agar based media, will further allow the transfer of these models to simulate the growth of foodborne contaminants in packaged food.

T4-08 The Role of *Pseudomonas aeruginosa* DesB on Host Cell Infection

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❖ ***Developing Scientist Competitor***

Introduction: *Pseudomonas aeruginosa,* which is known as one of food spoilage bacteria, exhibits a variety of virulence traits. In our previous study, *P. aeruginosa* containing a mutant allele of *desB* (an aerobic desaturase-encoding gene) displayed decrease in elastase activity and motilities, and productions of pyocyanin and rhamnolipids compared to wild type *P. aeruginosa*, proving that DesB is required for full virulence of *P. aeruginosa*. However, the role of DesB on the relationship between *P. aeruginosa* and host cells has not been studied yet.

Purpose: The present study is aimed at investigating whether DesB contributes to the pathogenic activities in host cells, such as exotoxin production, hemolysis, cell invasion and intracellular replication.

Methods: For exotoxin production assay, HeLa cells were exposed to cell-free supernatant of wild type *P. aeruginosa* (WT) or its derived *desB* mutant, and exotoxins were indirectly quantified by cell viability assay. For invasion and intracellular replication assays, WT or *desB* mutant was inoculated in HeLa cells, and the efficiencies of invasion and intracellular replication of WT and *desB* mutant were compared. Hemolysis assay was performed by spotting overnight cultures of WT and mutants on blood-containing agar plate. In order to determine if *desB* controls the expression of *plcH*, a hemolytic phospholipase-encoding gene, expression of *plcH* in WT and *desB*mutant were compared by qRT-PCR.

Results: *desB* mutant had different efficiency in exotoxin production, invasion and intracellular replication in cells compared to WT. Furthermore, decrease in hemolysis was observed in *desB* mutant, but *plcH* expression had no difference between WT and *desB* mutant, indicating that reduced hemolysis in *desB* mutant is not attributed to *plcH*.

Significance: The results demonstrate that *P. aeruginosa* DesB have effect on pathogenesis-related behaviors in host cells, including exotoxin production, hemolysis, cell invasion and intracellular replication.

T4-09 *Listeria monocytogenes* from the British Columbia Food Chain Show Evidence of Co-selection of Cadmium and Benzalkonium Chloride Resistance

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❖ ***Developing Scientist Competitor***

Introduction: *Listeria monocytogenes* (*Lm*), a foodborne pathogen, causes rare but severe disease in at-risk populations. This pathogen is well suited to persistence in natural and food processing environments. Transferable genetic determinants that increase environmental fitness in *Lm*, including resistance to heavy metals and/or food processing sanitizers, are of concern.

Purpose: To evaluate *Lm* fitness in low cadmium environments and co-selection of cadmium and benzalkonium chloride (BAC) resistance in *Lm* isolated from the British Columbia food chain.

Methods: *Lm* sensitive (n = 30) or resistant (n = 17) to 10 µg/ml BAC were evaluated for resistance to 35 µg/ml cadmium chloride ($CdCl_2$) at 37°C and screened for known cadmium resistance determinants. *Lm* growth rates at 25°C in the presence of sub-lethal $CdCl_2$ (1 µg/ml) were determined. The effect of $CdCl_2$ in combination with low pH on growth rate was examined at 25°C. Transferability of resistance determinants was assessed through conjugation of co-resistant (BAC + cadmium) donor strains and a recipient cadmium sensitive derivative of *Lm* at 25°C. Transconjugants were confirmed by PCR and characterized for co-resistance.

Results: Forty strains were cadmium resistant, with 16 being co-resistant. Five cadmium resistant strains lacked known cadmium resistance determinants. In the presence of sub-lethal $CdCl_2$, sensitive strains showed an average 29% reduced growth rate ($P = 0.003$) compared to resistant strains. $CdCl_2$ and low pH significantly reduced the growth rate of cadmium sensitive strains ($P < 0.001$). Two of 16 co-resistant donor strains produced transconjugants. Transferred BAC and cadmium resistance determinants showed no increase in BAC resistance when combined with the BAC efflux pump *emrE* but restored growth in the presence of sub-lethal $CdCl_2$.

Significance: These findings suggest cadmium resistance benefits *Lm* fitness and environmental heavy metals may act as a selective pressure influencing co-resistance to common food processing sanitizers. Horizontal transfer of this co-resistance between *Lm* strains has not previously been reported.

T4-10 Effect of Probiotic on the Survival of Non-O157 Shiga Toxin-producing *E. coli* (STEC) Strains in African Fermented Weaning Food Products

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Introduction: Ogi is a fermented maize gruel in Nigeria and the commonest traditional fermented weaning food in many communities of West African while motoho is a fermented sorghum beverage commonly produced in Southern Africa countries. High prevalence of diarrheal diseases, among infants in developing countries coupled with the occurrence of pathogenic *E. coli* in fermented foods, is an indication of an underlying safety problem.

Purpose: In this study, the ability of probiotic bacteria to inhibit acid tolerant non-O157 STEC strains from environmental sources was investigated in ogi and sorghum motoho.

Methods: Ogi was processed by steeping the maize grains spontaneously and also with probiotic (*L. plantarum*-B411) for 72 h followed by souring (after wet milling and sieving) for 48 h while the probiotic (*L. plantarum*-FS2 and *P. pentosaceus*-D39) strains developed from ogi fermentation were used for the processing of sorghum motoho. All samples were inoculated with acid adapted (AA) or non-acid adapted (NAA) non-O157 STEC strains.

Results: The growth of AA and NAA non-O157 STEC strains were significantly ($P \leq 0.05$) inhibited in the spontaneous combined with probiotic fermented ogi, while AA non-O157 STEC strains were more significantly ($P \leq 0.05$) inhibited than NAA non-O157 STEC strains in the fermented sorghum motoho after 24 h.

Significance: Probiotic bacteria coupled with prior adaptation to acid i.e., backslopping are more effective in controlling the occurrence of environmental acid tolerant non-O157 STEC strains in traditional fermented weaning foods than the usual uncontrolled spontaneous fermentation.

T4-11 Comparison of First-order and Weibull Modelling of the Thermal Inactivation Kinetics of *E. coli* O157:H7, Non-O157:H7 *E. coli* and *Salmonella enterica* in Fettuccine Alfredo

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Introduction: Little is known about the thermal inactivation kinetics of the foodborne pathogens, Shiga Toxin-producing *Escherichia coli* (STEC) and *Salmonella* in pasta products, however there is a possibility that these organisms could contaminate and survive in undercooked pasta products.

Purpose: The objective of this study was to compare the thermal resistance of individual cocktails of O157 STEC, emerging big six non-O157 STEC, and *Salmonella enterica* in fettuccine with alfredo sauce using linear and Weibull models.

Methods: Five-strain cocktails with ca. 8 log CFU/ml were added to a 1:1 mixture of fettuccine pasta and alfredo sauce, stored for 30 min and placed into vacuum-sealed bags. Bags were immersed in a recirculating water-bath at 56°C, 58°C or 60°C (temperatures and come-up times were monitored), removed at various time intervals, and immediately cooled in an ice-bath. Stomached samples were serially diluted and spread-plated on Tryptic Soy Agar plates. Surviving bacteria from triplicate experiments were enumerated after 24 h at 37°C.

Results: Calculated *D*-values from the first-order model (56 to 60°C) were 6.40 ± 1.67 to 1.19 ± 0.19 min for O157 STEC, 7.02 ± 0.64 to 1.26 ± 0.24 min for non-O157 STEC and 3.75 ± 0.39 to 0.46 ± 0.03 min for *Salmonella*. Using the Weibull model, the t_D values needed to destroy 1 log (56 to 60°C) ranged from 5.28 ± 1.52 to 1.31 ± 0.41, 6.00 ± 0.59 to 1.49 ± 0.37, and 3.08 ± 0.54 to 0.37 ± 0.09 min for O157 STEC, non-O157 STEC and *Salmonella*, respectively. Using *D*-values and t_D values, the *z*-values were 5.47 ± 0.29, 5.36 ± 0.79 and 4.42 ± 0.33°C for O157, non-O157 and *Salmonella*, respectively, by the first-order model; and 6.62 ± 1.43, 6.60 ± 0.98 and 4.34 ± 0.14°C for O157, non-O157 and *Salmonella*, respectively, by the Weibull model.

Significance: Statistical analysis of these results show that the Weibull model is a better fit for describing the thermal inactivation of some pathogens (STEC in this study), but there was no difference between the two models in the case of *S. enterica.*

T4-12 Chemical, Physical and Morphological Properties of Bacterial Biofilms Affect Survival of Encased *Campylobacter jejuni* under Aerobic Stress

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❖ ***Developing Scientist Competitor***

Introduction: *Campylobacter jejuni* is a microaerophilic pathogen and leading cause of human gastroenteritis. The presence of *C. jejuni* encased in biofilms found in meat and poultry processing facilities may be the major strategy for its survival and dissemination in aerobic environment.

Purpose: In this study, *C. jejuni* multispecies biofilms formed with *Staphylococcus aureus*, *Salmonella enterica*, or *Pseudomonas aeruginosa* was characterized to explain multispecies biofilm's protection mechanism.

Methods: The mono- and multispecies *C. jejuni* biofilms are characterized by bio-chemical and bio-physical techniques, including Confocal Raman spectroscopy, atomic force microscopy, Fourier transform infrared spectroscopy, contact angle measurement and confocal laser scanning microscopy.

Results: After 4 days exposure to aerobic stress, no viable *C. jejuni* cells could be detected from monospecies *C. jejuni* biofilm. In contrast, at least 4.73 log CFU/cm^2 of viable *C. jejuni* cells existed in some multispecies biofilms. To elucidate the mechanism of protection mode, chemical, physical and morphological features of biofilms were characterized. Multispecies biofilms contained a higher level of extracellular polymeric substances with a more diversified chemical composition, especially for polysaccharides and proteins, than monospecies *C. jejuni* biofilm. Structure of multispecies biofilms was more compact and their surface was >8 times smoother than monospecies *C. jejuni* biofilm, as indicated by atomic force microscopy. Under desiccation stress, water content of multispecies biofilms decreased slowly and remained at higher levels for a longer time than monospecies *C. jejuni* biofilm. The surface of all biofilms was hydrophilic, but total surface energy of multispecies biofilms (ranging from 52.5 to 56.2 mJ m^{-2}) was lower than that of monospecies *C. jejuni* biofilm, leading to more resistance to wetting by polar liquids.

Significance: This knowledge can aid in developing intervention strategies to decrease the survival and dispersal of *C. jejuni* into foods or environment.

T5-01 A Comprehensive Needs Assessment of Retail Food Safety Practices of Farmers' Market Vendors in Pennsylvania

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❖ ***Developing Scientist Competitor***

Introduction: Farmers' markets remain a significant source of food for millions of Americans, with over 8,000 in operation in the United States. To ensure their continued success and to protect the public health of consumers, food safety risks at farmers' markets must continue to be evaluated and assessed.

Purpose: To investigate retail food safety risks at farmers' markets in Pennsylvania, a comprehensive needs assessment, utilizing concealed vendor observations, market manager focus groups, and surveys of vendors and Pennsylvania Department of Agriculture (PDA) inspectors, was conducted.

Methods: During a one year period, direct concealed observations of retail food safety behaviors and characteristics of farmers' market vendors (n = 42) in Pennsylvania were conducted using a customized, smartphone application (Food Safe Surveys). A paper-based questionnaire also was disseminated to vendors (n = 39) to evaluate self-reported food safety related behaviors. Lastly, a web-based questionnaire was disseminated to PDA inspectors (n = 47) and an in-person focus group (n = 8) of market managers was conducted to collect noncompliance inspection trends and market manager perceptions of food safety.

Results: Highlights of the observations (n = 102) revealed high risk behaviors including: improper or no hand washing associated with bare hand contact of Ready-to-Eat foods (29/102; 28%); inadequate storage of temperature-sensitive foods (31/102; 30%); and the absence of thermometers when required (62/102; 61%). Similar noncompliance behaviors were noted by PDA inspectors. In contrast, 48% (16/33) of vendors reported properly washing their hands when becoming contaminated at the market and 63% (15/24) of the vendors stated they use thermometers when required.

Significance: Through this multifaceted needs assessment, compelling inconsistencies between self-reported and observed retail food safety related behaviors at farmers' markets were identified. More importantly, this study has provided practical and relevant scientific data for use in the development of future farmers' market food safety training programs and outreach activities.

T5-02 Current Practices at Farmers' Markets in Florida and Their Effect on Food Safety

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Introduction: The number of farmers' markets has increased with growing interest in fresh produce consumption and support of local economy. Gaining popularity of farmers' markets also raises safety concern for food sold at farmers' markets.

Purpose: The goal of this study was to assess food safety risks associated with farmers' markets by market survey and microbiological testing of food samples from farmers' markets.

Methods: A total of 25 markets in North and Central Florida were visited surveyed from April to October of 2014 for current market conditions and vendor practices, including the availability of hand washing facility, animal presence, waste management, and separation of items sold at markets. Additionally, 130 baked goods samples were collected from selected 17 markets and analyzed for the presence of total coliform, generic *E. coli*, and *Staphylococcus aureus*.

Results: This study showed that only 20% of the markets have restrooms equipped with hand washing facility and 72% did not have any bathroom or hand washing facility on site. Majority of markets (64%) allowed animals in the market, and only 14% of vendors used gloves or other tools while handling food. Of 130 baked goods samples, one sample was confirmed positive for *S. aureus* by both selective plating and PCR. Also, 0.8% (n = 1) and 18% (n = 24) were positive for generic *E. coli* and total coliform, respectively.

Significance: Our study suggests that there is significant lack of food safety practices at farmers' markets, and food safety can be improved by educating vendors about best food safety practices. Knowledge from this study will be useful in developing effective food safety education programs for farmers' markets vendors and managers.

T5-03 Investigation of Food Safety Practices and Standard Operating Procedures in North Carolina Food Pantries

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❖ ***Developing Scientist Competitor***

Introduction: Since 2008, food insecurity affects approximately 14.5% of the U.S. population, with many relying on food pantries as a regular source of sustenance. An estimated 2,500 emergency food providers exist in North Carolina either partnering with nationally-sponsored food banks, or run as independent, community-driven entities. Food pantries in North Carolina exist outside of inspection requirements, without formal guidelines, compulsory food handling training, or pantry-specific food safety guidance.

Purpose: Given the lack of data regarding food pantry operating procedures, this study set out to capture and analyze how the pantries address food safety risks.

Methods: To complete a mixed-methods analysis, on-site interviews and observations were carried out in 105 urban, rural, and suburban food pantries in 12 North Carolina counties. Food pantry practices were evaluated against a modified version of the 2009 U.S. FDA model Food Establishment Inspection Report, alongside additional data on operating procedures, volunteer management, and managerial ability to respond to a recall.

Results: Data analysis reveals gaps in food safety knowledge and practice. Less than 40% (n = 32) of pantries had protocols in place for ill volunteers and just 25% reported written standard operating procedures. Through paired *t*-tests, significant differences in risk management activities between the food bank partners (n = 87) and independent pantries (n = 18) were revealed. Food safety training was required at 81% of the food bank partners received compared to just 35% of the independent pantries ($P < 0.01$). While 71% of pantries stationed in metropolitan areas reported knowledge of recalls, only 53% of those in non-metro areas do so ($P < 0.10$).

Significance: This research reveals how pantry managers currently take steps to mitigate the risk of foodborne illness. The policy implications of the findings suggest the creation of food safety guidance specific to food pantries and, potentially, regulatory change that would require food handling training.

T5-04 Effect of Abusive Storage Temperatures on the Survival and Growth of *Escherichia coli* O157:H7 in Leafy Green Vegetables Sold at Local Egyptian Markets

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Introduction: Leafy green vegetables have been increasingly recognized as an important vehicle for the transmission of *Escherichia coli* O157:H7.

Purpose: The study compares the survival and growth behavior of *E. coli* O157:H7 on damaged portions of the most widely consumed raw salad vegetables in the Egyptians' diet (arugula, green cabbage, leek, lettuce, radish, and spinach) stored at different abusive temperatures.

Methods: Damaged leaves were inoculated with a four-strain cocktail of approximately 10^5 CFU *E. coli* O157:H7 per leaf. Total aerobic microorganisms and surviving populations of *E. coli* O157:H7 were periodically enumerated after different storage times. The pH, moisture content, total phenolic content (TPC), total flavonoid content (TFC), and anti-oxidant capacity (AOC) for control un-inoculated leaves were determined. Data were analyzed with One-Way ANOVA and post hoc least significance difference.

Results: Storage of damaged leaves at 8°C for 4 days led to different outcomes of surviving *E. coli* O157:H7 populations possibly due to interference of background microorganisms. High TPC, TFC, and AOC in spinach leaves stored at 15°C for 3 days resulted in pathogen growth inhibition. Insignificant ($P > 0.05$) differences in population counts on damaged arugula leaves stored at 15°C and room temperature were owed to antibacterial activities of glucosinolates and ascorbic acid induced at elevated temperatures. Increased population counts on damaged leek and arugula held at room temperature for 3 days by 4 and 1.7 log units, respectively, was associated with high moisture retention (96%) and increase in final pH (6.82).

Significance: This is the first study conducted in Egypt on the influence of abusive storage temperatures on microbiological quality and safety of salad vegetables. In regard to Egypt's warm climate, considerations must be given by health authorities and Food Safety Agency to the provided data that match conditions of retail display and consumer home storage, to be implemented for risk assessment and development of intervention measures that remove or limit the growth of foodborne pathogens in leafy green category should contamination inadvertently occur at any stage of the production chain.

T5-05 Prevalence Survey for Norovirus and Hepatitis A Virus in Fresh Australian Leafy Greens and Berries at Retail

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Introduction: Norovirus (NoV) and Hepatitis A virus (HAV) are significant food safety hazards. In recent years several international foodborne NoV and HAV outbreaks have occurred with leafy vegetables and berries being the most common foods implicated.

Purpose: The aim of this survey was to determine prevalence of NoV (GI and GII) and HAV in Australian grown fresh berries (strawberries and blueberries) and leafy greens (packaged and loose) at retail during 2013-14.

Methods: Sample size (n = 152 berries and n = 150 greens) was calculated based on the provision of a probability of 0.95 of detecting at least one positive if at least 2% of the samples were contaminated. NoV GI, NoV GII and HAV were determined by quantitative RT-PCR (ISO/TS 15216). *E. coli*, used as a fecal indicator, was determined by the MPN method (AS 5013.15).

Results: No HAV was detected in any of the samples. NoV GI was detected (5 to 50 copies/25 g) in three loose leafy green samples (2%). NoV GII was detected (1,000 copies/25 g) in one loose leafy green sample (0.67%), which had also tested positive for NoV GI. The estimated prevalence of *E. coli* (≥ 3 CFU/gm) was 0.66% and 10.67% in berries (1/152) and leafy greens (16/150), respectively. None of the virus positive samples were positive for *E. coli*, suggesting it is a poor indicator for the risk of enteric virus contamination.

Significance: No NoV and HAV were detected in packaged product at retail, suggesting good sanitary and process controls throughout the Australian berry and leafy green production chains. Despite a low prevalence of NoV, loose leafy greens may pose a higher risk to NoV related foodborne illness. The point of contamination was not determined in this study but may be associated with consumer handling.

T5-06 Influence of Maturity, Source, Handling and Processing on the Safety of Canned Ackees (*Blighia sapida*)

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Introduction: Ackee (*Blighia sapida*), Jamaica's national fruit, is widely consumed by Jamaicans and traded internationally. Known to contain the natural toxin hyproglycin A (HGA), the product was prohibited from the US until 2000 when scientific evidence and HACCP-based production controls assured the USFDA of its safety. While fully mature ackees have very low HGA, occasional unexplained high levels cause periodic concerns that need attention.

Purpose: The perceived vs. actual maturity, sourcing and handling of ackees are possible causes of higher than expected levels of toxin in the fruit. Studies were undertaken to evaluate the impact of these variables on the HGA content of commercially canned Jamaican ackees.

Methods: Unprocessed and canned ackees were selected from three processing plants across the country and assessed to determine their HGA content using reversed phase high performance liquid chromatography (RP-HPLC). Samples represented different reaping and selection practices and different stages of actual vs. perceived maturity. The appearance of the fruit was recorded photographically, the study repeated three times and both uncooked and thermally processed (cooked) canned fruit were assessed.

Results: Hypoglycin A decreased as the maturity increased from a mean of 2,412 ppm for Stage 4 (immature) ackees to 162 ppm for Stage 7 (mature) uncooked ackees. Hypoglycin A levels were lower ($P < 0.05$) in cooked ackees than in uncooked ackees, but were found to be higher for mature (Stage 6 and 7) cooked ackees at 117 ppm and 111 ppm, respectively, than had been previously reported.

Significance: The finding of consistently higher toxin levels in the fruit across the island showed a systemic increase in HGA in Jamaican canned ackees over typically levels at the same stage of maturity, indicating the need to differentiate perceived and actual maturity levels. The findings also confirmed that cooking reduces the HGA content of the fruit.

T5-07 Quantitative Analysis of Handwashing Signs and Poster Guidance

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Introduction: Handwashing is an important part of preventing microbial cross-contamination. The US FDA Model Food Code states that handwashing sinks require a handwashing sign or poster to be clearly visible to any employees washing their hands.

Purpose: This research analyzes current handwash guidance by collecting and reviewing existing handwashing signs and posters, and subjects them to a quantitative analysis.

Methods: An Internet search compiled a comprehensive database of handwashing sign and posters. Keywords used in the search included: handwash, sign, poster, soap, and hand hygiene. A Google search was followed by a targeted search of US state and county health department websites. Instructions for lather time, rinse time, overall wash time, water temperature, pre-moistening of hands, drying method, technique, and total number of handwashing steps were recorded. The requirements for inclusion were minimal; the sign needed only to mention or show a picture of handwashing.

Results: Eighty-one unique handwashing posters or signs were identified. Every sign had at least one step, with the highest number of steps being thirteen. Thirty-seven (47%) signs indicated a specific lather time, with the average time being 18 s. No signs suggested more than 20 s lather, and none suggested less than 10 s lather. Twenty-four signs (30%) recommended using warm water. Two signs recommended using 100°F (37.8°C) water and one recommended using "hot" water. Sixty-two signs (77%) made a recommendation to dry hands in some manner and fifty-three (65%) suggested using a paper towel. Forty-one (51%) signs suggested one or more handwashing techniques, with the most common recommendations being to target between the fingers and the fingernails.

Significance: Our analysis reveals that handwashing sign and poster suggestions can vary quite widely. Lack of consistent signage may contribute to a lack of handwashing consistency and compliance.

T5-08 Deep Cleans Reduce Persistence of *Listeria monocytogenes* in Retail Delis

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Introduction: *Listeria monocytogenes* is commonly found in the retail deli environment, where it can persist over long periods of time and potentially cross-contaminate Ready-to-Eat foods. Previous studies by our group determined daily interventions failed to significantly reduce *L. monocytogenes* persistence in retail delis.

Purpose: The goal of this study was to determine the effect aggressive cleaning strategies have on *L. monocytogenes* persistence.

Methods: We recently performed an aggressive deep clean SSOP in nine delis. Samples were collected from ten food contact surfaces, fifteen non-food contact surfaces, and three transfer points immediately before and after each deep clean, then once monthly for three months. Pulsed-field Gel Electrophoresis (PFGE) was used to type 107 *L. monocytogenes* isolates collected. Isolates from the same store and sharing the same AscI and ApaI fingerprints were considered the same strain. A strain was defined as persistent if detected from the same store in at least three month's sampling.

Results: Three of eight strains previously identified as long-term persistent (i.e., in two separate six-month sampling studies from July 2010-October 2011) became transient after execution of the deep clean SSOP. Four additional strains previously identified as short-term persistent (i.e., persistent in only one six-month sampling study) were absent post deep clean, and one short-term persistent strain became transient.

Significance: Deep cleans were moderately effective at reducing or eliminating long-term persistent strains of *L. monocytogenes.* Additional development is needed to remove all persistent *L. monocytogenes* from the deli environments.

T5-09 Managing Condensation on Overhead Surfaces Using Microcapillary Film

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Introduction: Hot pressurized water is commonly used to clean food processing equipment, causing condensation to form on cool surfaces. The presence of moisture is associated with an increased risk of microbial proliferation. We have developed an adhesive-backed film containing open

microchannels for use on overhead surfaces. A capillary force generated by the microchannels increases the surface to volume ratio of condensate droplets, thereby increasing the evaporation rate.

Purpose: The purpose of this study was to evaluate the evaporation rate of condensate on surfaces covered with microcapillary film relative to control surfaces.

Methods: The study was designed to model condensation on pipes, where water droplets accumulate in a "drip line" by migrating to the lowest point. A two inch wide film containing capillary channels was applied lengthwise along the bottom of a 2 inch ID steel pipe cooled to 45°F. Condensation was formed using a commercial steam cleaner until steady state dripping was achieved on both surfaces. After removal of the humidity source the time to droplet disappearance was measured.

Results: The average time to droplet disappearance of condensate on the film was 31 min (+/- 5) compared to 298 min (+/- 152) for the hanging drops on the control surfaces (n = 64). The variability observed in the evaporation rate on control surfaces was caused by the volume difference in hanging drops. Larger droplets took significantly longer to evaporate (maximum = 520 min) than smaller droplets (minimum = 45 min).

Significance: The use of microcapillary film has been demonstrated to increase the evaporation rate of condensate. The use of this film may be beneficial in managing condensation on overhead surfaces in food processing facilities.

T5-10 The Hygienic Design of Manual Cleaning Equipment

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Introduction: Thanks in no small part to 3A in the USA and the European Hygienic Engineering Design Group (EHEDG) many food manufacturers appreciate the benefits of using hygienically designed production equipment as it is quicker and easier to clean, and minimizes the risk of product cross-contamination by microbes, allergens, foreign bodies etc. This in turns maximizes food safety and quality, reduces the risk of expensive product rejection or recall, and minimizes food waste. However, when it comes to the equipment used to clean food production equipment, very few cleaning tools are developed with good hygienic design in mind. Consequently, their use can jeopardize all of the above.

Purpose: To investigate the hygienic design of food industry cleaning equipment and propose improvements for the future.

Methods: Drilled and stapled, resin set, and fused filament food industry brushware were investigated, with regard to hygienic design, using microscopy and UV sensitive lotion (as a contaminant). These brushware types were also assessed against 3A/EHEDG and European Brushware Federation (FEIBP) hygienic design criteria, and the requirements of key food safety and quality standards.

Results: All existing brushware had hygienic design issues, as indicated by the presence of residual 'contamination' and crevice, and various levels of non-conformance to established hygienic design criteria, and safety and quality standards.

Significance: These investigations indicate that much of the cleaning equipment used in food manufacturing environments is generally of poor hygienic design. Given the constant drive to improve food safety and quality food manufacturers need to minimize the risk of product contamination through all possible means, including the use of hygienically designed cleaning equipment. As a long established provider of professional cleaning equipment to the food industry Vikan have developed a range of cleaning equipment in line with hygienic design criteria that will help in this regard.

T5-11 Screening and Prioritizing Conventional and Emerging Disinfection By-products Developed in Fresh and Fresh-cut Produce during Chlorine-based Disinfection

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❖ ***Developing Scientist Competitor***

Introduction: Chlorine-containing disinfectants are widely used for cleaning and sanitization in food processing environments. One of the main concerns is the reactivity of chlorine with organic substance to generate disinfection by-products (DBPs) with potential hazards. The side reactions that lead to DBP formation can consume disinfectants and thus lower the efficacy in inactivating pathogens.

Purpose: The purpose of this study is to prioritize DBPs based on screening of their concentrations that can be formed after typical chlorine disinfection processes of produce, including lettuce and strawberries.

Methods: About 50 g of the cut produce were placed into a 200 ml DI water as blank wash and 200 ml NaOCl solution (200 mg/l as Cl_2) as process water, and agitated on a shaker for 30 min at 25°C room temperature. After 30 min, the process water was analyzed for chlorine consumption, and by four analytical methods to screen for the occurrence of around 40 different DBPs including trihalomethanes, haloacetic acids, halonitriles, halonitromethanes, haloacetamides, haloquinones, nitrosamines, and others.

Results: When washed by DI water, 10 and 14 DBPs (out of a total of 27 target DBPs), were detected in wash water from lettuce and strawberries, respectively, at 0.01-0.21 µg/l and 0.03-0.89 µg/l. After washing by 200 mg (as Cl_2)/l NaOCl solution, 22 DBPs were detected in wash water from lettuce at 0.18-1014 µg/l, and 22 DBPs were detected at 0.18-671 µg/l in the case of strawberries. The formation concentrations of some DBPs were also found to be pH dependent.

Significance: The results indicate that residues of chlorine and DBPs are present in produce obtained from the market. Treatment by chlorine can generate a range of conventional and emerging DBPs in produce, and these DBPs may remain in the produce or leach into the wash water.

T6-01 Understanding Differences in Recent U.S. Cost of Foodborne Illness Estimates

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Introduction: Cost of illness estimates play an important role in food safety policy and education. They are required for approval of federal major regulations. They help convey the importance of food safety to policy and consumer audiences.

Purpose: In fall 2014, USDA Economic Research Service (ERS) published new cost of foodborne illness estimates. These estimates differ significantly from other recent cost of foodborne illness estimates due to methodology and scope. The purpose of this presentation is to present ERS cost of foodborne illness estimates and to examine differences between these estimates and those recently published by FDA and others. This presentation will give professionals working on food safety a clearer understanding of the methodological and empirical issues at play in these different estimates.

Methods: Cost of foodborne illness estimates were based on pathogen-specific disease CDC incidence estimates (Scallan et al. 2011), FoodNet data and other scientific literature. Deaths are valued using U.S. value of statistical life estimates. Medical treatment costs and the value of lost time are used to approximate WTP to reduce risk of non-fatal illnesses.

Results: ERS estimates WTP to prevent domestically acquired foodborne illnesses caused by 15 leading pathogens at over $15.2 billion. These pathogens cause over 95% of domestically acquired foodborne illness in the U.S. for which a pathogen cause can be identified. Other recent estimates for all domestically acquired foodborne illness are as high as $51 billion. Methodological differences are an important driver of differences between estimates.

Significance: USDA agencies will likely use ERS cost of foodborne illness estimates in their regulatory analysis, internal decision analysis and consumer outreach efforts. It is important for food safety professionals to understand how and why these estimates differ from other available estimates.

T6-02 An Environmental Scan of Food Safety Educational Initiatives Targeted at Consumers in the United States

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❖ ***Developing Scientist Competitor***

Introduction: In-home food safety practices can be influenced through messages provided by food safety educators. These messages are communicated to reduce risky practices that could potentially lead to foodborne illness. This information is a necessary step to a needs assessment to better describe the current population of food safety educators, their programs, and sources of information and materials.

Purpose: Although many agencies, organizations, and educational outlets provide the public with food safety messages, there has not to date been a systematic collection of a landscape of whom is providing what information to what audiences.

Methods: An online survey was developed with questions. The survey was distributed to the Partnership for Food Safety Education's BAC Fighter (self-subscribed health and food safety educators) and food safety educators through Internet searches expanded through a snowball participant selection process. Food safety educators from different sectors such as academia, federal government, food retail, non-profits, public health agencies, and schools (K-12) were targeted.

Results: Ninety percent (n = 469) of educational programs were delivered in-person, while 36% were delivered through online sources. Almost half of food safety educators (48%, n = 397) did not measure or were unaware if others measured impacts of their food safety outreach programs. When survey participants were asked whom their programming/outreach programs (n = 469) were designed to reach, children, parents of children and the elderly were ranked highest, while less than 15% of these programs targeted ethnicity based populations and farmers' market vendors.

Significance: Improvements can be made for educating consumers about food safety by addressing the gaps identified within the data gathered. These improvements may include greater usage of program evaluation and additional educational materials for underserved populations.

T6-03 Identifying Food Safety Education Needs for Ontario's (Canada) Youth: An Analysis of Key Informant Interviews

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Introduction: Youth are a unique audience for food safety education, given that they can have riskier eating behaviors, and that food handling is a common youth job opportunity. Before food safety education can be successfully delivered to this audience, their specific education and training needs must be determined.

Purpose: To determine the most important food safety education needs for high school students in Ontario, Canada. Specifically our goal was to prioritize messages from the Ontario Ministry of Health and Long-Term Care's existing, standardized food handler training program, and identify additional messages unique to this demographic.

Methods: We conducted 19 semi-structured key informant interviews with experts in food safety and youth education. Interviewees were given the standard training material prior to the interview, and were asked about the need for food safety education in youth, to prioritize the content from the standard training material based on youth's needs, and to identify any other priority training messages for youth. We identified implicit and explicit priority training needs via thematic analysis.

Results: Food safety education in youth was considered important due to reported perceptions of invulnerability, low understanding of risks associated with foods, the 'second weaning' phenomenon, and the need to instill good practices before bad habits are established. Priority education messages were: hand hygiene; cross contamination; temperature (emphasizing reheating, leftovers, lunches, and snacks); and microbiology (emphasizing how food gets contaminated and how anyone can get sick). Other unique education needs included travelling with food, and sharing of food and drink.

Significance: Ontario's standard food handler training program, developed mainly for commercial food handlers, aligns well with the education needs of the province's youth, particularly if risks important to youth (e.g., packed lunches) can be highlighted.

T6-04 The Healthy Baby, Healthy Me Food Safety Education Program for Pregnant Women: Results of an Education Intervention.

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Introduction: Foodborne illnesses conservatively costs between 1 and 3 billion dollars yearly. Just one food safety education program can save over $400K of that cost each year. To have the greatest impact on the public health, food safety education should be targeted to those at greatest risk of foodborne illness. This program focused on pregnant women.

Purpose: This intervention study developed, implemented, and evaluated educational impact of a food safety education curriculum for low-income pregnant women.

Methods: Low-income, English- and Spanish-speaking women in Ohio and Colorado (n = 580) participated in an 8-lesson curriculum that was either a Usual education program with minimal food safety information or an Enhanced program that emphasized food safety education. Lessons focused on *Listeria monocytogenes* and *Toxoplasma gondii* (Healthy Baby) and *Salmonella* species and *Campylobacter jejuni* (Healthy Me), and taught pathogen control behaviors and food selection for illness prevention. Lessons were taught by peer-educators in the two states. Either single participants or small groups of 2 - 3 participants were taught in homes or community centers equipped with kitchen facilities. Pre-and Post-education knowledge and behavior assessment was completed in the participant's home

Results: Knowledge of foodborne pathogens improved for both groups post-education, and significantly for the Enhanced curriculum participants. Additionally, food safety behaviors significantly improved for the Enhanced group. Across curriculum groups, English-speaking participants reported greater improvement post education in handwashing, pre-rinsing vegetables and avoiding risky foods than did Spanish-speaking participants. Spanish speakers were more likely to promptly refrigerate leftovers post-education than English speakers.

Significance: This comprehensive, randomly-controlled intervention demonstrates that enhanced food safety education improves both knowledge and behaviors related to foodborne pathogens that have high public health impact in the United States.

T6-05 Cognitive, Behavioral and Microbial Analysis of Older Adult Consumers' Domestic Risk Factors Associated with Listeriosis

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Introduction: In Europe, Listeriosis associated with older adult consumers has reportedly increased three-fold (1990s-present), with majority of international incidence and mortality associated with older adults. Listeriosis is predominantly sporadic and associated with refrigerated extended shelf life Ready-to-Eat (RTE) foods; therefore, consumer implementation of temperature control, 'use-by' date adherence and ensuring maximum two days storage after opening is essential to ensure food safety. Older adults are reported to be frequent consumers of RTE foods; however, data on older adults' domestic storage practices of RTE foods are lacking.

Purpose: Qualitative and quantitative research methods were used to determine older adults' domestic storage practices of RTE foods to evaluate the potential impact on *L. monocytogenes* and Listeriosis risk.

Methods: One hundred older adults (≥ 60years) participated in the mixed-methods study involving a 'model kitchen' food preparation session; self-complete questionnaire, computer-assisted personal-interview, home-kitchen microbiological survey and domestic refrigerator time-temperature profiling. Study findings informed development of laboratory re-enactment using RTE foods spiked with *L. monocytogenes* to determine potential impact of storage malpractices on food safety.

Results: Although knowledgeable of some recommended practices, older adults' self-reported and actual behavioral data indicate such practices are not always implemented. Many older adults failed to express positive attitudes towards such practices; additionally, the majority of refrigerators operated at temperatures exceeding recommendations (≤ 5.0°C). Identified common malpractices, particularly prolonged storage of RTE foods and inadequate refrigeration temperatures were widespread; re-enactment of which determined *L. monocytogenes* growth was significantly greater ($P < 0.001$) than following recommendations, thus potentially making RTE foods unsafe for consumption and increasing the risk of listeriosis.

Significance: This study utilized novel data collection methods and increases our understanding of older adults' domestic RTE food storage practices which have been microbiologically determined to increase the potential risk of Listeriosis. Findings may inform the development of targeted consumer food safety education.

T6-06 Evaluation of a National Pilot Produce Safety Train-the-Trainer Workshop and Curriculum

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Introduction: The Produce Safety Alliance (PSA) has developed a science-based, on-farm food safety curriculum and Train-the-Trainer program for produce safety educators to assist fresh fruit and vegetable growers in meeting the training requirements in the Food Safety Modernization Act's (FSMA) Produce Safety Rule.

Purpose: There are an estimated 189,000 total fruit and vegetable growers in the United States and although not all of them are subject to the Produce Safety Rule, all growers should have food safety knowledge since many are required by their buyers to implement food safety practices. To keep growers informed of the regulation and competitive in the marketplace, a qualified group of trainers is needed.

Methods: Thirty-seven prospective trainers, including produce growers, industry members, extension educators, and regulatory personnel, attended the two-day Train-the-Trainer pilot and were asked to complete an extensive evaluation that reviewed the curriculum's content, supplemental resources, trainer pre-requisite knowledge, and the trainer certification process.

Results: Pilot attendees overwhelmingly agreed (27/29) that the two-day training format for trainers was appropriate in length while 86% (25/29) agreed that the provided teaching notes were sufficient. To most effectively cover the content, 73% (19/26) of the attendees plan to train as a team and 64% (14/22) plan to incorporate a second day of training for growers to focus on writing a farm food safety plan. Participants also identified the need for funding to conduct training, a readily available technical assistance network, and the need for prospective trainers to have a basic understanding of the proposed regulations prior to attending a Train-the-Trainer workshop.

Significance: Developing a cadre of competent trainers along with appropriate educational materials will help growers comply with the final regulations while reducing produce safety risks on the farm. This pilot evaluation process will also be conducted for the one-day grower pilot training in January 2015.

T6-07 Evaluation of a Multi-day Good Agricultural Practices Training and Farm Food Safety Plan Writing Workshop

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Introduction: In December of 2009, personnel from the National Good Agricultural Practices (GAPs) Program, Cornell Cooperative Extension, and New York State Department of Agriculture and Markets began multi-day GAPs trainings for produce growers to help them understand GAPs and develop a written farm food safety plan. Implementing GAP practices reduces produce safety risks and a plan is required if the farm needs a third-party audit to meet buyer demands.

Purpose: Trainings have been attended by 689 individuals representing over 350 farms and fruit and vegetable processors. Given the time, effort, and expense of both attending and conducting the trainings, there was a desire to assess training impact and value.

Methods: Short-term evaluations were collected at the end of each training day. In the spring of 2014, 80 past participants, each representing a different farm, were asked to complete a 20-question survey to assess the progress they had made on their plans and audits, costs they had incurred, and impact on their market access.

Results: Evaluations indicated that 13% (39/289) of participants had a written farm food safety plan before coming to the training and by the end of the second day, 48% (230/478) of participants report having 50 - 100% of their farm food safety plans written. Of those responding to the long-term survey, 63% (50/80) reported having finished a written farm food safety plan and 38% (30/80) have completed a third-party audit. Forty-three percent

(35/80) of growers reported maintaining sales valued from $14,000 to $2,000,000, while 16% (14/80) of growers reported expanded sales valued at $15,000 to $300,000.

Significance: The multi-day GAPs trainings are helping growers develop written farm food safety plans, implement practices to reduce microbial risks, successfully pass audits, and maintain market contracts as well as expand market access.

T6-08 The Efficacy of a Food Safety Intervention When Implemented by Health Department Sanitarians in Response to Violations Recognized during Inspection

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Introduction: Restaurant food handlers who lack food safety certification often have low levels of food safety knowledge. This knowledge deficit occurs despite institutional or certified manager responsibilities for teaching these uncertified personnel. Sanitarians may be an underutilized source of food safety training for uncertified food handlers.

Purpose: We determined the preliminary efficacy of an educational intervention for non-certified food handlers when implemented by sanitarians in response to real-time identification of significant violations recognized during inspection.

Methods: Eligible restaurants were those where a health department sanitarian identified a violation involving time/temperature abuse or a hygiene issue during a routine inspection. Seven health department sanitarians from a convenience sample of four Illinois counties performed a pre and 1-week post food safety knowledge survey on the first 1 to 3 volunteering non-certified food handlers at each of these restaurants. The intervention was an educational illustrated booklet. Sanitarians addressed knowledge gaps identified on the pre survey in real-time.

Results: There were 35 restaurants meeting the eligibility criteria including 57 food handlers. Baseline food handler knowledge scores included 20 - 49% correct (21.1%), 50 - 69% correct (56.1%), 70 - 79% correct (15.8%), 80 - 89% correct (3.5%), and ≥ 90% correct (3.5%). The mean post-intervention food safety knowledge score increased substantially compared to the mean pre-intervention score (58% to 78%, n = 57, $P < 0.05$). The most substantial rise in knowledge occurred for the question about how long to wash one's hands (38.6% vs 80.7% correct, $P < 0.0001$).

Significance: Our study demonstrated preliminary efficacy of an educational intervention when implemented by sanitarians in response to selected violations recognized during inspection. These data support the need for additional study examining the use of sanitarians to perform targeted food handler training during restaurant inspections.

T6-09 Thinking Outside the Box: Traditional and Non-traditional Recommendations to Improve Consumer Food-handling Behavior

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Introduction: In the last 20 years there have been many efforts to improve consumer food-handling behavior, from traditional education programs to national ad-campaigns, new kitchen gadgets and technology, and better instructions on food labels. Despite these initiatives many consumers still lack motivation and knowledge necessary to practice safe food-handling at home.

Purpose: The goal of this presentation is to share information from an assessment of education and risk communication strategies currently utilized (and under-utilized) with respect to their ability to influence consumer behavior.

Methods: Published literature related to consumer food safety, education, and risk communication was systematically reviewed. In-depth interviews with food safety educators and key stakeholders were conducted to gain insight about the background and goals of ongoing programs. A set of recommendations based on all findings was created to enhance future efforts to improve consumer food safety.

Results: Consumers lack adequate knowledge of several food safety topics, such as recommended cooking temperatures and how to use a meat thermometer. Much more often, however, consumers fail to handle food safely at home for other reasons, including lack of convenience, lack of obvious cues or reminders, competing cultural and social norms, or low perceived risk. Currently there are few initiatives that address these other issues – most focus entirely on improving consumer food safety knowledge.

Significance: Although knowledge is critically necessary, it is not sufficient to guarantee consumers will handle food safely at home. Campaigns to improve consumer knowledge should focus specifically on the few topics where knowledge is inadequate. Furthermore, additional efforts are necessary to address the many other internal and external factors that influence behavior, in order to motivate individuals who already possess adequate food safety knowledge. This tailored approach may not only improve behavioral outcomes, but also help program managers allocate valuable resources and funding more strategically.

T6-10 Working Knowledge and Communication Practices of Public Health Officials in Response to Norovirus Outbreaks in Schools

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❖ ***Developing Scientist Competitor***

Introduction: Norovirus is the leading cause of viral gastroenteritis in schools. School administrators often turn to public health departments for their expertise during gastroenteritis outbreaks, making public health officials and school administrators important players in managing outbreaks.

Purpose: The objective of this study was to investigate communication messages and methods of local health officials to school risk managers (principals, janitorial staff, nurses) about prevention and infection management of norovirus.

Methods: Norovirus-related communications by local public health officials (n = 127) and their level of engagement with schools were evaluated using a 26-question online survey. Participants were selected via a convenience sample of North Carolina health departments and asked a combination of open-ended, Likert scale, and importance-ranking tasks. Questions focused on interactions with schools, knowledge of norovirus prevention, recommended norovirus control measures, education methods currently used in schools, and preferences for new education programs.

Results: When asked about norovirus control measures, many participants said they would recommend cleaning with bleach (93%) and excluding sick food handlers (97%, n = 102), but 22% (n = 102) would also recommend quaternary ammonium compounds for sanitizing, which are not effective against noroviruses. Nearly a quarter (23%, n = 102) reported recommending commercially available alcohol-based hand sanitizers to schools as an effective control measure. Only 31% of participants (n = 127) had been contacted by a school about norovirus, and few participants (15%, n = 118) said they had provided training to school staff about norovirus.

Significance: This study suggests that public health officials generally provide correct information to schools, though there is also an indication that some of this information is incorrect, and not evidence-based. In practice, these inaccuracies may lead to prolonged norovirus outbreaks in schools, but they also highlight areas where better knowledge transfer to the public health community is needed.

T6-11 Evaluating the Evidence-base of Outbreak Management and Clean-up Guidelines Available to Schools Experiencing Norovirus Outbreaks

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Introduction: An estimated 75% of absenteeism in schools is due to illness or injury, posing an educational impact on students and economic impact on families, and schools. It is estimated that norovirus causes nearly a quarter of school gastrointestinal outbreaks. Current materials provided to schools may not be effectively relaying accurate information. Public health officials and outbreak reports suggest that while evidence-based norovirus infection control guidelines specific to schools exist, the best management practices are not always used.

Purpose: The project aim was to evaluate currently available norovirus management literature directed at schools for technical content and evaluate accuracy of the information. The project was carried out to provide insight into gaps in communication and evaluate the need for future development of materials based on the best available science.

Methods: Artifacts, defined as publicly accessible online guidelines and instruction documents (n = 63), were found using online searches such as 'school norovirus guidelines,' and 'school norovirus prevention and control.' Content analysis was employed to evaluate document source and themes. Coders identified agreement with U.S. Centers for Disease Control and Prevention evidence-based best practices and captured erroneous recommendations (omission and commission).

Results: There were inconsistencies and inaccuracies in the material and instructions provided to schools. Of the analyzed documents, 24% gave vague instructions void of specific compounds, PPE use or distance around a vomit event. Preventative cleaning was mentioned in 60% of documents; only 14% mentioned that commercially available, alcohol-based hand sanitizers are not effective. Twenty-four percent of documents said individuals could return after symptoms cleared; 30% did not mention absenteeism.

Significance: The results suggest a need for more evidence-based and more detailed materials targeted towards schools control and prevention methods. Targeted, clear instructions with varying formats may increase compliance and decrease the frequency and/or severity of norovirus outbreaks.

T7-01 Produce Outbreaks, United States, 1998–2013

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Introduction: Fresh fruits and vegetables are increasingly recognized as sources of foodborne disease outbreaks in the United States. Implementation of the U.S. Food Safety Modernization Act (FSMA) requires a better understanding of the epidemiology of outbreaks attributed to raw produce.

Purpose: Describe the frequency and characteristics of outbreaks attributed to raw produce.

Methods: We reviewed outbreaks (≥ 2 persons with a similar illness) attributed to the consumption of produce likely consumed raw, reported to CDC's Foodborne Disease Outbreak Surveillance System during 1998–2013. We analyzed the number of outbreaks, outbreak-associated illnesses, hospitalizations, and deaths over time, and etiologic agents. Implicated produce items were further categorized for analysis.

Results: During 1998–2013, 17,374 foodborne disease outbreaks were reported; 972 outbreaks (10% of outbreaks with a food reported) were attributed to raw produce, resulting in 34,674 (17%) outbreak-associated illnesses, 2,315 (25%) hospitalizations, and 72 (31%) deaths. Over time, raw produce outbreaks accounted for an increasing proportion of outbreaks with a food reported, from 8% during 1998–2005 to 14% during 2006–2013. Among the 778 outbreaks caused by a single etiologic agent, norovirus (418, 54%), *Salmonella enterica* (167, 21%), and Shiga Toxin-producing *Escherichia coli* (74, 10%) were most commonly reported. Among outbreaks attributed to a single produce category (n = 612), raw vegetable row crops (235 outbreaks, 38%), fruits (216, 35%), and seeded vegetables (66, 11%) were most frequently implicated. The most common etiologic agent-produce categories reported were norovirus in vegetable row crops (119 outbreaks), norovirus in fruits (93), and *Salmonella enterica* in fruits (51).

Significance: The proportion of all foodborne outbreaks attributed to raw produce has increased. Outbreaks caused by norovirus and *Salmonella* suggest that contamination of raw produce during food preparation, as well as during harvest and processing, are important. Implementation of FSMA could result in fewer outbreaks attributed to raw produce.

T7-02 Persistence and Internalization of *Listeria monocytogenes* in Romaine Lettuce

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Introduction: *Listeria monocytogenes* (LM) has been implicated in various outbreaks involving fresh produce. While no LM outbreaks have been linked to romaine lettuce, the number of LM recalls specific to lettuce is increasing. Understanding potential of persistence and internalization of LM on and within romaine lettuce will aid in determining food safety risk.

Purpose: To determine the persistence of LM on romaine lettuce from a brief seed contamination event through the harvest period studying various LM strains and cultivar combinations, as well as different growth conditions. Additionally, to examine potential internalization of LM into the hypocotyl of romaine under simulated field conditions.

Methods: Persistence of 3 LM strains on 3 romaine lettuce cultivars was assessed independently by inoculating seeds with an initial inoculum of approximately 8 log CFU/ml. Seeds were grown on various soil types (e.g., standard potting mix) or sterile soft-top agar for up to 60 days. For internalization studies, seeds were inoculated with a LM strain expressing green fluorescent protein (GFP). Three plants were fixed, paraffin embedded, and sectioned; localization was studied using standard immunohistochemistry techniques.

Results: Plants grown on sterile soft-top agar maintained between 4.4 to 7.8 log CFU/g LM after a 60 day period, while LM dropped below the limit of detection (2 log CFU/g) by day 45 in plants grown in soil. Cultivar and LM strain differences were not significant. A total of 539 LM cells were internalized in all major tissue types with the majority localizing in the pith followed by cortex, xylem, phloem and epidermis.

Significance: Romaine lettuce can support growth and internalization of LM, which could serve as a vehicle of LM transmission to consumers warranting the need to improve current produce safety strategies.

T7-03 Persistence and Internalization of *Salmonella* on/in Organic Spinach Sprout: Exploring the Contamination Route

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Introduction: Germinated seeds/sprouts have been associated with *Salmonella* contamination.

Purpose: The effects of contamination route, including seed and water, on persistence and internalization of *Salmonella* in organic spinach cultivars- Lazio, Space, Emilia and Waitiki were studied.

Methods: Spinach seeds (1g) were contaminated with *S*. Newport using 10 ml of *S*. Newport-sterile water suspension overnight. Contaminated water for germination was prepared by resuspending and diluting an overnight culture (40 ml) of *S*. Newport in sterile water. For testing both routes, contaminated or non-contaminated seeds were placed in a germination pouch, irrigated with sterile or contaminated water (15 ml) and germinated in the dark at 25°C. After 5 days, germinated sprouts were analyzed for *S*. Newport population and internalization. Germinated sprouts were potted in soil, grown in a plant growth incubator for 4 weeks and leaves, stems and roots from 3 plants of each cultivar were sampled for *Salmonella* population by plating. Surface-sterilized plants were analyzed for internalized pathogen following enrichment. Potting soil and water runoff were sampled for *Salmonella* after 4 weeks of plant growth.

Results: Contaminated seeds and irrigation water had *S*. Newport populations of 7.64±0.43 log CFU/g and 7.12±0.04 log CFU/ml, respectively. Sprouts germinated using contaminated water and seeds had *S*. Newport populations of 8.09±0.04 and 8.08±0.03 log CFU/g, respectively. Populations of *S.* Newport in leaves, stem and roots of spinach plants were as follows: contaminated seed- 2.82±1.69, 1.69±0.86, and 4.41±0.62 log CFU/ml; contaminated water- 3.56±0.90, 3.04±0.31, and 4.03±0.42 log CFU/ml of macerated tissue suspension, respectively. Internalization was observed in spinach grown from contaminated seed and in sprouts germinated with contaminated water. *S*. Newport populations of 2.82±0.70 log CFU/g and 1.76±0.46 log CFU/ml were recovered from soil and water runoff, respectively.

Significance: The contamination of spinach during germination can result in persistence, internalization and environmental reintroduction of *Salmonella*.

T7-04 On-farm Evaluation of the Prevalence of Human Enteric Bacterial Pathogens during the Production of Melons in California and Arizona

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Introduction: Following the 2011 outbreak of *Listeria monocytogenes* on cantaloupe a widely held speculation was the ubiquitous prevalence of *Salmonella enterica* and *Listeria monocytogenes* on cantaloupe and other melons.

Purpose: To provide a seasonal characterization of the microbiological safety of the cantaloupe supply from a major domestic production region.

Methods: A multi-grower/handler study was conducted over three seasons with samples taken after all pre-shipment handling, whether field or facility-packed. Honeydew and watermelon (n = 36 - 60) and cantaloupe (n = 200) per packed lot were tested, between 2011 and 2014, for contamination. Full rind composites (1 composite = 3 fruit) were enriched in Universal Pre-enrichment Broth with 0.05% Tween for 18 h at 35°C. For *Salmonella*, aliquots were then selectively enriched in tetrathionate broth at 42°C for 6 h followed by M Broth for 18 h at 37°C. For *Listeria*, samples were enriched in *Listeria* Enrichment Broth with supplements at 35°C for 48 h. For each lot, controls were inoculated with ~ log 1 and ~ log 2 CFU/ composite (n = 5 or 10 composites). Aliquots of enriched samples were frozen in glycerol and retained for confirmation if positive by Roka Atlas system. Confirmation for *Salmonella* or *Listeria* on XLT4 or CHROMListeria, and by PCR.

Results: Overall, 4870 fruit were tested of which 4,312 were cantaloupe. Seven verified *Salmonella* were detected (sv. Poona, Newport, Heildelberg – two each, and one Agona), all from cantaloupe. Nineteen *Listeria* spp. were detected; no *L. monocytogenes* were detected. When applied individually all control enrichments were positive. When *Salmonella* and *Listeria* were combined, 95% and 100% detection was observed for *Listeria* and *Salmonella*, respectively, at log 2 and 83% and 100% at log 1.

Significance: The overwhelming outcome of this survey was for a general absence of detection of *Listeria* spp., no detection of *Listeria monocytogenes*, and highly isolated detection of *Salmonella.*

T7-05 Effect of Postharvest Practices on Cantaloupe Colonization by *Listeria monocytogenes*

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Introduction: Listeriosis is a life-threatening foodborne disease that creates significant challenges to food industry. During 2011 *Listeria monocytogenes* cantaloupe outbreak in several Listeriosis cases the onset of symptoms took place within 24 h after consuming the fruit, suggesting that victims ingested an extremely high pathogen dose. We hypothesized that *L. monocytogenes* can infiltrate cantaloupes in the process of hydro-cooling and colonize the fruit internally.

Purpose: Evaluate the effect of hydro-cooling on *L. monocytogenes* ability to infiltrate into cantaloupes and develop large bacterial population in edible portions of the fruit.

Methods: Cantaloupes, both freshly harvested and purchased from a local grocery marker, were used in the study. Fruit temperatures were calibrated to 40°C for 24 h. Hydro-cooling was conducted at 6°C for 40 min by a submersion of cantaloupes into water containing Acid Blue 9 dye (100 mg/ml) and 3 Jensen farm outbreak *L. monocytogenes* strains (6 log CFU/ml). After hydro-cooling fruits were stored at 3°C. On day 1, 7, and 18 after hydro-cooling, 3 - 5 fruits of each cultivar were surface disinfected by a submersion in 0.5% sodium hypochlorite solution for 15 min. Different areas of fruit mesocarp (variable depths and distances from stem scar) were aseptically extracted and analyzed for bacteria internalization.

Results: Dye uptake by cantaloupes in the process of hydro-cooling demonstrated that from the stem scar water is distributed within the fruit through the vascular system primarily in hypodermal mesocarp, and reaches the calix. Water influx spreads through the mesocarp *via* secondary xylem vessels. Under experimental conditions *L. monocytogenes* internalized into cantaloupes during hydro-cooling. Populations of internalized *L. monocytogenes* colonized edible portions of the fruit reaching 8 log/g within two weeks after hydro-cooling at storage temperature of 3°C.

Significance: Identification of postharvest practices leading to melon contamination will allow significant reduction in foodborne Listeriosis associated with consumption of cantaloupes

T7-06 Baseline Assessment of the Prevalence of *Salmonella* and *Listeria* on Cantaloupe and in Select Melon Production Environments in Arizona

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Introduction: Contamination of melons by pathogens such as *Salmonella* and *Listeria monocytogenes* can cause outbreaks and recalls.

Purpose: To understand the natural prevalence of key foodborne pathogens and indicator microorganisms on cantaloupes and associated environmental samples from Arizona production fields.

Methods: Samples including cantaloupe, adjacent soil, water, rhizosphere biomass and air were collected from seasonal commercial fields during 2013. Site assessments of fields were also conducted. For microbiological analysis, cantaloupe rind composites (1 composite = 3 fruit) and environmental samples were enriched in Universal Pre-enrichment Broth (UPB) with 0.05% tween for 18 h at 35°C. For *Salmonella*, aliquots were then selectively enriched in tetrathionate broth at 42°C for 6 h followed by M Broth for 18 h at 37°C. For *Listeria*, the samples were selectively enriched in *Listeria* Enrichment Broth at 35°C for 48 h. Enterococci and coliform populations were determined by plating on ECC CHROMagar® and m-*Enterococcus* agar. Aliquots of all enriched samples were frozen in glycerol and stored for culture confirmation if judged as positive by Roka Atlas system testing. Presumptive positive samples were confirmed for *Salmonella* or *Listeria* by streaking on XLD or MOX, by PCR and biochemical testing.

Results: A total of 315 cantaloupe composites, 53 root, 54 soil, 22 air and 12 water samples were tested. Analysis of enrichments by the combined methods indicated that 7/315 and 6/315 fruit rind composites were positive for *Salmonella* serovars and *Listeria* spp., respectively. *L. monocytogenes* was not detected. Two soil and 3 water samples were positive for *Salmonella*. One root sample was positive for *Listeria* spp. The average coliform and enterococci counts on fruit rinds were 4.45 ± 0.53 and 2.49 ± 0.58 log CFU/g, respectively. Surveys of farms indicated no unusual features.

Significance: The results indicate that while vehicles of pathogen contamination on the field during cantaloupe cultivation might exist, detection of pathogens remains rare and challenging.

T7-07 Effect of Thermal and Non-thermal Treatments on Bacterial Populations on Melon Rind Surfaces

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Introduction: Consumers demand for fresh produce has led to higher incidence of foodborne illness arising from consumption of cantaloupes contaminated with bacterial pathogens. To solve this food safety problem, the produce industry and research institutions are working to develop a better sanitizer treatment that can eliminate or minimize bacterial transfer to fresh-cut pieces during preparation.

Purpose: In this study, we evaluated the effect of sanitizer treatments on microbial reduction on cantaloupe rind surface. Also, changes on cantaloupe rind surface as a result of treatments on the melons were evaluated.

Methods: Whole cantaloupes were purchased from a wholesale distributor and were inoculated with *Salmonella* spp. at 4.52 log CFU/cm^2. The melons were treated as follows; water washed, 1,000 ppm chlorine, 3.5% hydrogen peroxide, and heated 3.5% hydrogen peroxide at 80°C. All treatments were for 5 min and the rind surfaces were examined with scanning electron microscopy and bacterial inactivation due to treatments was determined using plate count and XLT4 agar plates.

Results: Total mesophilic bacteria on cantaloupe rind surface before inoculation averaged 4.8 log CFU/cm^2. After treatments with chlorine and unheated hydrogen peroxide, mesophilic aerobes were reduced to 2.2 and 1.9 log CFU/cm^2 while *Salmonella* was reduced to 1.9 and 1.6 log CFU/cm^2, respectively. The surviving population for *Salmonella* spp. on cantaloupe rind after treatment with heated 3.5% hydrogen peroxide was below detection. Changes on cantaloupe rind surfaces were observed and were dependent on the type of treatment. Internal flesh of all treated cantaloupes was not affected, probably due to the thickness of the melon rind. Microbial populations on fresh-cut cantaloupe pieces prepared from heat treated melons were significantly lower than untreated, chlorine, and 3.5% hydrogen peroxide treated melons

Significance: Treating whole melon surfaces with heated 3.5% hydrogen peroxide before fresh-cut preparation will improve the microbial safety and quality of the prepared fresh-cut pieces.

T7-08 Evaluating the Effect of Cover Crops on the Survival and Growth Dynamics of Foodborne Bacterial Indicators in Soil and on Cantaloupes Grown Organically

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Introduction: Growers may minimize cantaloupe contamination with foodborne pathogens through implementation of Good Agricultural Practices (GAPs). However GAPs focus on exclusion of foodborne pathogens and say little about the impact of production system on contamination. Cover cropping is practiced for seasonal soil protection, nutrient management and control of phytopathogens. The impact of cover crop systems on foodborne pathogens remains unexplored.

Purpose: We assessed the survival of bacterial foodborne pathogen surrogates in five cover crop/green manure systems – rye, hairy vetch, crimson clover, rye combinations and bare ground, in the production of cantaloupe in organic and transitional-organic fields.

Methods: Cover crops arranged in a randomized complete block design were inoculated with non-pathogenic *Escherichia coli* and *Listeria innocua* using a handheld sprayer in fall 2013 and 2014. Cover crops were tilled in spring and cantaloupe 'Arava' and 'Sivan' seedlings were transplanted. Soil and fruit samples were collected, and bacteria enumerated using an MPN method. Data were analyzed using a mixed model with repeated measures.

Results: In fall 2013 - spring 2014, cover crop was a factor for *E. coli* ($P = 0.043$) but not for *L. innocua*. Both species declined over time ($P < 0.0001$). In fall 2014, time was again a factor for *E.coli* in both organic and transitional fields ($P < 0.0001$), and cover crop by week was only a factor in the transitional field ($P < 0.0001$). Cover crop by week was a factor for *L. innocua* in organic ($P = 0.0018$) and transitional ($P < 0.0003$) fields. Bacterial survival on 'Sivan' varied by fruit size and green manure treatment for *L. innocua*; conversely, *E.coli* was mostly absent. 'Arava' fruit weight was lower in bare ground than in hairy vetch or hairy vetch plus rye plots ($P = 0.0001$).

Significance: These data reveal that cover crop/green manure treatment and time may impact the survival of foodborne indicator bacteria in fields and subsequent dispersal onto cantaloupes.

T7-09 Practical Validation of Surface Pasteurization of Cantaloupe

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Introduction: Commercial adoption of surface pasteurization for whole cantaloupes was initiated following outbreaks of *Listeria monocytogenes* and *Salmonella* linked to cantaloupes without the benefit of adequate validation data.

Purpose: In response, hot water pasteurization, in combination with antimicrobials, was evaluated on cantaloupe under laboratory and commercial packing conditions for effectiveness against rind-attached and planktonic bacteria.

Methods: A recirculating laboratory-scale wash system was used to establish process parameters. Ultimately, twenty cantaloupes were harvested and received four treatments: a heated wash (65°C, 45 s), a 30 ppm peroxyacetic acid (PAA) spray, or a heated wash followed by PAA spray, with untreated melons providing initial populations of native microbiota. Melons inoculated with *Listeria innocua* and an attenuated *Salmonella* Typhimurium were similarly treated. At a commercial facility, melons were collected every 30 min prior to heated wash, following wash (~64°C), following heated wash and a 50 ppm PAA spray, and after final pack. Wash water was quantified for bacterial populations.

Results: In laboratory studies, application of PAA alone did not significantly reduce ($P > 0.05$) populations of heterotrophs, coliforms, or *Enterobacteriaceae* on melon rinds (3.6, 2.1, and 2.6 log CFU/cm^2) and was ineffective following the heated wash. All treatments more effectively reduced inoculated strains (2.3 - 4.3 log CFU/cm^2) than native microbiota (-1.1 - 2.2 log CFU/cm^2). Under commercial packing, similar populations ($P > 0.05$) of coliforms, *E. coli*, and Enterobacteriaceae (2.5, 1.5, and 2.5 log MPN/100 ml) were maintained in the wash water throughout packing. While the heated wash, with and without PAA spray, were equally as effective against attached bacteria, samples of the final product contained ~1.9 log CFU/cm^2 greater populations (n = 40).

Significance: Although a pasteurization treatment may enhance reductions of attached bacteria and prevent build-up in the recirculating water, care must be taken to prevent re-contamination of melons during final packing.

T7-10 A Meta-analysis of the Effect of Sanitizing Treatments on *Escherichia coli* O157:H7 Counts in Fresh Produce

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Introduction: Because washing fresh produce constitutes a critical control point, several reports are found that quantify *Escherichia coli* O157:H7 concentrations in fruits and vegetables before and after washing with different sanitizing agents.

Purpose: The objective of this study was to summarize the effect of sanitizers on the mean log-reduction of *E. coli* O157:H7 on fresh produce, and to explain between-study variability by incorporating study characteristics (type of produce, sanitizer, concentration, treatment time and temperature) to a mixed-effects meta-analysis model.

Methods: After the extraction of log-reduction values, sample size and study characteristics from the 40 primary studies considered appropriate for meta-analysis, a mixed-effects linear model was fitted to the data, considering mean log-reduction as the response variable, type of sanitizer, sanitizer concentration, temperature and time as the fixed-effects explanatory variables, and the interaction study×produce as subject of the random effects.

Results: For most of the sanitizers, the meta-analysis model showed that the log-reduction is greater as the sanitizing concentration, washing temperature and time increase ($P < 0.001$), and that reductions appear to be lower for leaf vegetables. Averaging over all types of fresh produce, the lowest log-reductions are attained by regular treatments with ozone gas (1.63 log CFU) and hydrogen peroxide (1.98 log CFU). Treatments with ozonized water, organic acids (citric acid, lactic acid, tartaric acid and malic acid) and chlorine reach comparable mean log-reductions in the range of 2.0 to 2.6 log CFU. A significantly higher bactericidal effect is obtained by calcium hypochlorite and peroxyacetic acid (3.2 log CFU), and chlorine dioxide gas and slightly acidic electrolyzed water (3.5 log CFU). Acidified sodium chlorite is by far the sanitizer that reaches the highest log-reduction (5.1 log CFU).

Significance: The resulting meta-analysis model has the capability to provide overall log-reduction estimates for *E. coli* O157:H7 when using a given sanitizer and sanitizing treatment.

T7-11 Post-harvest Reduction of Coliphage MS2 from Romaine Lettuce during Simulated Commercial Processing with and without a Chlorine-based Sanitizer

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Introduction: Foodborne outbreaks associated with fresh produce have become increasingly problematic in the United States. However, knowledge about where contamination occurs in the supply chain, particularly for viruses, is extremely limited.

Purpose: The purpose of this study was to assess the efficacy of a chlorine-based sanitizer against MS2 coliphage, as a surrogate for enteric viruses on fresh-cut Romaine lettuce during simulated commercial production.

Methods: Batches of Romaine lettuce (6 kg) were submerged for 15 min in 80 liters of water containing coliphage MS2 at 10^9 - 10^{10} PFU/ml and then processed using a small-scale commercial leafy green processing line. A commercial chlorine-based sanitizer (XY-12, Ecolab) was added to ~800 liters of water adjusted to pH 7.0 - 7.5 to obtain 25 ppm free chlorine with sanitizer-free water serving as a control. Triplicate 50 g lettuce samples were collected after inoculation, mechanical shredding, flume washing (2 min), shaker table dewatering, and centrifugal drying. Viruses on the lettuce were recovered in Tris-glycine buffer and determined as plaque-forming units with *Escherichia coli* Famp serving as the host in a double agar overlay assay. An unpaired two sample *t*-test was used to compare virus removal during the various stages of processing.

Results: The inoculated lettuce contained MS2 at 10^4 to 10^6 PFU/g before processing. The greatest reduction in MS2 occurred between shredding and flume washing, ranging 0.45 to 1.02 log PFU/g and 0.56 to 0.98 log PFU/g for sanitizer and sanitizer-free treatments, respectively. Numbers of MS2 virus remained relatively stable following flume washing with or without 25 ppm free chlorine. Total reductions of 0.98 and 0.79 log PFU/g were seen after processing with and without the sanitizer, respectively, with no statistical difference observed between the two treatments ($P > 0.05$).

Significance: These findings suggest that currently recommended commercial production practices are unable to effectively decrease virus on leafy greens during commercial processing.

T7-12 Prevalence and Diversity of *Salmonella enterica* spp. in Irrigation Water, Poultry Litter and Amended Soils on the Eastern Shore of Virginia

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Introduction: As one of the major agricultural regions plagued by recurring tomato associated foodborne outbreaks, the Eastern Shore of Virginia (ESV) is an ideal location for environmental field studies to examine foodborne pathogen contamination on produce.

Purpose: Therefore, a study was performed to investigate the prevalence and diversity of *Salmonella* spp. in irrigation water, poultry litter and amended soils on ESV.

Methods: Irrigation pond water and well water, poultry litter, and soils were sampled from four vegetable farms, three chicken farms, and four pairs of representative farms with or without chicken litter fertilization on ESV, respectively. The most probable number (MPN) method was used to detect *Salmonella* spp. in the samples. Presumptive *Salmonella* colonies were confirmed by the cross-streaking method. Molecular serotyping was carried out to determine the *Salmonella* serovars.

Results: Spatial (pond location) and temporal (month) differences were observed in the presence of *Salmonella* in surface pond water. The prevalence of *Salmonella* spp. in four tested ponds from farms A, B, C and D were 14.8, 11.1, 18.5 and 35.2%, respectively. *Salmonella* levels were significantly higher in spring and fall, compared to in winter ($P < 0.05$). While the prevalence of *Salmonella* spp. in well water samples was low, several well water samples did test positive for *Salmonella* in Nov 2013/2014 and Dec 2014. Most houses of the three tested chicken farms were *Salmonella* positive during the study period, except for the period from Nov 2013 to Jan 2014. *Salmonella* was found to survive up to four months in poultry litter amended soils from the tested farms.

Significance: This research reported the contamination of *Salmonella* spp. in relationship to irrigation water sources and chicken manure used for vegetable production. These data provided important information on the prevalence of *Salmonella* in a major agricultural region for food safety risk assessment.

T8-01 Reduction of Aichi Virus by Sodium Metasilicate and Calcium Hypochlorite in Suspension

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❖ ***Developing Scientist Competitor***

Introduction: Aichi virus (AiV) is a newly emerging virus responsible for gastroenteritis outbreaks worldwide. Currently, there is limited data in literature on effective methods to control the spread of AiV. Therefore, improved chemical methods are being researched to inactivate AiV and to prevent transmission and reduce viral illnesses worldwide. Sodium metasilicate and calcium hypochlorite are known for their antimicrobial properties and used in industry. Hence, their effects against AiV need to be explored.

Purpose: The objective of this research was to determine the ability of sodium metasilicate (SMS) and calcium hypochlorite ($Ca(ClO)_2$) to inactivate AiV in suspension at room temperature.

Methods: AiV at ~ 5 log PFU/ml was treated with equal volumes of SMS at 5% and 10%, $Ca(ClO)_2$ at 0.02% and 0.2%, or phosphate buffered saline (pH 7.2 as control) at room temperature for 0.5, 1, and 5 min. At each time-point, treated viruses were initially serially diluted in cell-culture media containing 10% fetal bovine serum (FBS), followed by dilutions in 2% FBS. Viral infectivity was assessed by plaque assays in duplicate using Vero host cells. Data obtained from three replicate treatments were statistically analyzed.

Results: AiV at 5 log PFU/ml was reduced to non-detectable levels after 5 min with both 5% and 10% SMS, but showed insignificant reduction after 0.5 min with both 5% an 10% SMS and 0.59 ± 0.37 log PFU/ml reduction after 1 min with only 10% SMS ($P > 0.05$). However, $Ca(ClO)_2$ at both tested concentrations of 0.2% and 0.02% reduced AiV to non-detectable levels after 15 s.

Significance: This study showed that $Ca(ClO)_2$ at 0.02% could more effectively and rapidly reduce AiV in suspension than 5% or 10% SMS to control the spread of AiV. Thus, $Ca(ClO)_2$ shows potential for application in industry to control AiV spread.

T8-02 Cocktails of Plant-produced Colicins for Efficient Control of Major Pathogenic Strains of *Escherichia coli*

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Introduction: Enteropathogenic *Escherichia coli* (EPEC), such as *E. coli* O157:H7, cause over 200,000 illnesses at a cost to the EU and U.S. economies of approximately €2 billion annually. Many of these illnesses have been linked to consumption animal products or, lately, organically grown vegetables. Post-harvest intervention strategies with efficacy against the many different strains of EPEC are needed. Colicins are antimicrobial proteins produced by and effective against certain *E. coli* strains. Several colicins have been shown to be highly effective against EPEC strains and in reducing EPEC populations in both live animals and on animal-derived products. However, strain-to-strain variation in sensitivity to different colicins suggests that a mixture of several different colicins would be needed to be efficacious against a broad range of EPEC.

Purpose: To determine the efficacy of colicins with different modes of action against the "Big 7" EPEC strains.

Methods: Colicins M, E7, N, E1, and E3 were expressed in a highly efficient green-plant expression host. This system provides high yields (up to 5 kg active protein per ton of fresh green biomass) and very low manufacturing costs. Antimicrobial activity against pure cultures was determined in both soft-agar overlay assays and by enumeration from broth culture.

Results: Col M had the highest ($P < 0.01$) specific activity against O26, O45, O111, and O157. Col M was not however efficacious against O121. Of the colicins tested, only Col E7 and Col N had significant activity against O121. Mixtures of Col M and Col E7, at a total quantity of 5 mg of colicin/kg of food product, caused significant ($P < 0.01$) reductions (2 - 6 logs) in EPEC from a variety of foods.

Significance: A cocktail of colicins would be a highly effective intervention strategy for the reduction of EPEC on a variety of food items.

T8-03 Antimicrobial and Synergistic Potential of t-cinnamaldehyde Nano-emulsion Combined with Commercially Available Antimicrobials against Methicillin-resistant *Staphylococcus aureus*

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Introduction: MRSA has emerged as an epidemic crisis in clinical, veterinary medicine and food safety worldwide. t-cinnamaldehyde is well known as a natural antimicrobial and a good source of combination therapy. However, its application is limited due to high minimum inhibitory concentration (MIC) and insolubility in water. Nano-emulsion technology may offer a solution.

Purpose: The present study was aimed to evaluate antimicrobial and synergistic potential of t-cinnamaldehyde nano-emulsion combined with commercially available antimicrobials against Methicillin-Resistant *Staphylococcus aureus.*

Methods: The t-cinnamaldehyde nano-emulsion was formulated using ultrasonication and tween 80 as emulsifier. Antimicrobial efficacy and synergistic potential of t-cinnamaldehyde nano-emulsion was evaluated against hospital-acquired (HA) and community-acquired (CA) MRSA strains using broth-dilution test and checkerboard assay.

Results: The t-cinnamaldehyde nano-emulsion showed minimum inhibitory concentration (MIC) of 312.5 µl/l against both CA and HA MRSA strains. Synergism analysis with commercial antimicrobials such as cefoxitin, tetracycline, erythromycin, ciprofloxacin, and vancomycin showed additive interaction with cefoxitin, tetracycline, erythromycin.

Significance: Antimicrobial nano-emulsion of t-cinnamaldehyde and its combinations with commercial antimicrobials offer alternatives to control MRSA in hospital, community and food processing settings.

T8-04 Grape Seed Extract against Human Noroviral Surrogates in Model Food Systems and Simulated Gastric Conditions

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❖ ***Developing Scientist Competitor***

Introduction: Grape seed extract (GSE) is reported to have antiviral activities against human norovirus surrogates (feline calicivirus (FCV-F9) and murine norovirus (MNV-1)) *in vitro*. Further studies in model food systems and under gastric conditions are needed to understand the application of GSE as an antiviral agent.

Purpose: The objectives of this study were to determine the antiviral effects of GSE at 37°C in (1) apple juice (AJ) and 2% milk as model food systems; and (2) under simulated gastrointestinal conditions against FCV-F9 and MNV-1.

Methods: FCV-F9 and MNV-1 at ~5 log PFU/ml were treated with GSE at 2, 4 or 8 mg/ml prepared in apple juice (AJ; pH 3.6), 2% milk, simulated gastric fluid (SGF; pH 1.5) or simulated intestinal fluid (SIF; pH 7.5), and AJ, 2% milk, SGF, SIF, malic acid (pH 1.5 and 3.0) and phosphate buffered saline (pH 7.2) over 24 h at 37°C. Virus infectivity of triplicate treatments was evaluated using plaque assays in duplicate and data were statistically analyzed.

Results: GSE at 1 mg/ml in AJ reduced FCV-F9 to undetectable levels after 5 min, while 4 mg/ml GSE in milk reduced FCV-F9 by 1.07 ± 0.03 log PFU/ml after 24 h. GSE at 1 mg/ml in AJ reduced MNV-1 to undetectable levels after 1 h, while 1, 2 and 4 mg/ml GSE in milk all reduced MNV-1 by merely 0.3 log PFU/ml after 24 h. Both viruses did not survive in SGF after 1 h. GSE at 1 mg/ml in SIF reduced FCV-F9 to undetectable levels after 1 h and MNV-1 by 1.03 ± 0.04 log PFU/ml after 1 h.

Significance: Reduced antiviral activity of GSE was observed in milk compared to apple juice. These results indicate that time-released GSE encapsulation may be needed for use as antivirals or application in food systems to cause optimal foodborne viral reduction.

T8-05 Application of Antimicrobial Agents via Commercial Spray Cabinet to Inactivate *Salmonella* on Skinless Chicken Meat

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Introduction: *Salmonella* is a food safety concern for raw poultry products. The need for new innovative antimicrobials and applications is essential towards reduction of *Salmonella* in poultry and poultry products.

Purpose: The aim of this study was to determine the efficacy of three antimicrobial compounds against *Salmonella* on raw chicken meat when applied individually and in combination through a commercial spray cabinet.

Methods: Raw chicken thigh meat inoculated with 5 logs CFU/g of *Salmonella* Typhimurium ATCC 53647 (ST) passed through a spray cabinet being sprayed with 5% lauric arginate (LAE), 0.8% vinegar solution (VS), near neutral electrolyzed water (NNEW) and deionized water (DW). Then analyzed treated and untreated inoculated samples: (1) at 0, 15, 30, 45 and 60 s exposure time intervals, (2) in 4°C storage at 0, 1, 2, and 3 d after a 60 s exposure, and (3) in 4°C storage at 0, 1, 2, and 3 d after a 30 s combination exposure to each LAE and VS. Analysis of variance (ANOVA) and Tukey test were used for determining mean significant differences ($P < 0.05$). The experiment was carried out in duplicates for each replicate (n = 3 x 2).

Results: In comparing individual antimicrobials, 60 s treatment time resulted in the highest reduction of ST with LAE achieving the highest reduction (2.07 log) followed by VS, NNEW and DW (0.63, 0.56 and 0.53 logs, respectively). At 3 d LAE significantly ($P < 0.05$) reduced ST 1.28 logs. The combination, VS then LAE had a significantly ($P < 0.05$) higher reduction than using LAE followed by VS (1.61 and 0.93 logs, respectively).

Significance: The results of this study suggest that LAE is a viable compound to reduce ST on raw chicken meat and the order of application of antimicrobial agents plays a vital role.

T8-06 Modeling the Survival of Unstressed, Acid, Cold, and Starvation Stress Adapted *Listeria monocytogenes* in Ham Extract with Hops Beta Acids (HBA) and Sensory Evaluation of HBA on Ready-to-Eat Meat Products

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Introduction: Hops beta acids (HBA) are used to provide flavor in beer and has been approved by the USDA-FSIS to control *Listeria monocytogenes* on Ready-to-Eat (RTE) meats. Currently, no publication addressed the antilisterial activity of HBA in food systems particularly with stresses adapted cells, and sensory efficacy of HBA's application on RTE meats.

Purpose: This study aims to investigate the efficacy of HBA against unstressed, or different stresses adapted *L. monocytogenes* in ham extract, and the consumers' acceptability of HBA applied on RTE meats.

Methods: Unstressed, acid, cold, or starvation-stress adapted *L. monocytogenes* was inoculated (1.3 - 1.5 log CFU/ml) into 10% ham extract without (control) or with HBA (4.44 or 10.0 µg/ml). Survival/growth of the pathogens during storage (7.2°C, 26 days) was periodically monitored on tryptic soy

agar plus 0.6% yeast extract and PALCAM. Sensory evaluations (30 participates, 9-point hedonic scale) were performed with ham, turkey breast and frankfurters dipped into 0.05, 0.11 and 0.23% HBA solution for 2 min. Data (2 repeats/3 samples per repeat) were analyzed using the mixed model procedure of SAS, DMFIT, and USDA-Integrated-Predictive-Modeling-Program software.

Results: Ham extracts without HBA (control) supported rapid growth of unstressed and stresses adapted cells with growth rate of 0.26 - 0.44 log CFU/ml/day and lag phase of 0 - 0.18 days. HBA inhibited unstressed *L. monocytogenes* growth by showing slower ($P < 0.05$) growth rate (0.24 - 0.29 log CFU/ml/day) and longer ($P < 0.05$) lag phase (3.49 - 12.98 days) than the control. Acid, cold, or starvation-stress adapted cells showed cross-protection to HBA with higher ($P < 0.05$) growth rate (0.44 - 0.71 log CFU/ml/day) and similar or shorter ($P < 0.05$) lag phase (0 - 5.44 days) than the unstressed cells. The HBA did not ($P > 0.05$) affect the sensory attributes of RTE meats.

Significance: Results should be useful for developing operation protocols of HBA to control *L. monocytogenes* during post-lethality processing of RTE meats.

T8-07 Inhibition of Biofilm-forming Shiga-Toxigenic *Escherichia coli* Using Bacteriophages Isolated from Beef Cattle Environment

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Introduction: Shiga-toxigenic *Escherichia coli* (STEC) are important foodborne pathogens that can form biofilms in the food production environment. They have been implicated in a number of foodborne outbreaks associated with meat and fresh produce. It is therefore crucial to devise effective control strategies. Ubiquitous and host-specific bacteriophages can be an attractive alternative to control STECs in the food industry.

Purpose: Isolate and characterize bacteriophages from beef cattle environment and evaluate their efficacy against biofilm-forming STEC.

Methods: Fecal (n = 20) and water (n = 67) samples were collected from cattle operations and enriched in NZCYM broth for isolation of bacteriophages. Isolated phages were tested for lytic-activity against STECs (O157:H7, O121, O145, O111, O103, O126, O45), using spot-on-lawn assay. Phages were purified and their morphology observed under transmission electron microscope (TEM). Physiological characteristics were determined for temperature sensitivity (40 to 90°C for 60 min) and pH (0.7 to 11 for 24 h) and storage stability (4, -20 and -80°C for 3 mo.). Effectiveness of bacteriophages against STEC-biofilms on food contact surfaces (stainless steel/high density polyethylene coupons) was also evaluated.

Results: Several phages were isolated that exhibited inhibition towards one O121, one O145 and seven O157:H7 strains. All O157:H7-specific-phages were also effective against O45 and O145. The O121-phage and three O157:H7-phages were identified by TEM as *Myoviridae* family with a short contractile tail. The other four O157:H7-phages belonged to *Siphoviridae* family with a long non-contractile tail and the O45-phage belonged to *Tectiviridae* family with no tail. All phages were very stable at 40-60°C but lost activity at 90°C after 10 minutes, except the O121-phage. The bacteriophages also survived acidic and basic pH range and 3 mo. storage.

Significance: Bacteriophages specific for STECs, with high lytic-activity, pH and thermal stability and that could reduce biofilm formation, could be used as bio-control agents in the meat and produce industry.

T8-08 Rechargeable Antimicrobial Coatings for Food Processing Equipment

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Introduction: Reducing the microbial contamination of food contact and non-contact materials remains a significant opportunity to improving food quality and safety. Yet, many antimicrobial coating technologies suffer from high cost, poor long-term effectiveness, and/or limited commercial translatability.

Purpose: In this work we describe the design of polymeric antimicrobial coatings which incorporate N-halamine antimicrobial moieties which regenerate antimicrobial activity with exposure to halogenated sanitizers.

Methods: Using layer-by-layer deposition and coat-cure technologies, alternating polyamine and polyacrylic acid (or polyanhydride) polymers were deposited onto stainless steel, polyethylene, and polypropylene materials. Both dip and spray-on application techniques were explored. Material chemistry was characterized by Fourier transform infrared spectroscopy, colorimetric halamine quantification assays, and water contact angle. Antimicrobial activities were characterized by submerging coupons of coated substrates in an aqueous suspension of *Listeria monocytogenes* for varying contact periods. Experiments were performed in quadruplicate (n = 4) on at least two independent days to verify repeatability of results. Significance was determined using the general linear model followed by Tukey's pairwise comparisons using a confidence interval of 95%.

Results: We have introduced sufficient N-halamine functionality in these polymeric antimicrobial coatings to inactivate greater than 5-log cycles of *Listeria monocytogenes*. Materials were able to regenerate antimicrobial activity after 100 rechlorination cycles, and prolonged exposure to both acidic and alkali clean-in-place chemical agents. Tailoring the chemistry of the polymers used in the nanoscale coating process resulted in retained antimicrobial character even after chlorine dissociation from the surface.

Significance: Such rechargeable antimicrobial polymer coatings can aid in improving food safety by reducing cross-contamination of microorganisms from food processing equipment.

T8-09 Inhibitory Effects of Phytochemicals on Quorum Sensing, Biofilm Formation and *in vivo* Virulence of Foodborne Pathogens

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❖ ***Developing Scientist Competitor***

Introduction: Recent studies have demonstrated that virulence and spoilage regulated phenotypes in foodborne bacteria are cell density dependent phenomenon regulated at the molecular level by the mechanism of quorum sensing. Quorum-sensing regulated biofilms in food-processing environments are a major concern as they are chronic sources of microbial contamination, leading to food spoilage and transmission of diseases. Therefore, quorum-sensing inhibitors can be used as novel intervention strategies for enhancing food safety.

Purpose: The present study investigated the efficacy of sub-inhibitory concentrations of phytochemicals in inhibiting quorum sensing regulated phenotypes, biofilm formation and *in vivo* virulence in foodborne pathogens.

Methods: The phytochemicals used in the study were trans-cinnamaldehyde, curcumin, quercetin, phellandrene, caryophellene and berberine against the foodborne bacteria *Pseudomonas fluorescens*, *Salmonella enterica* serovar Typhimurium, *Escherichia coli* and *Vibrio parahaemolyticus*.

Quorum-sensing inhibition was determined by using the biosensor strains of *Chromobacterium violaceum, P. aeruginosa* PA01 and molecular docking studies. Antibiofilm activity of phytochemicals was examined by microtiter plate assays and fluorescence microscopy. *In vivo* efficacy of the phytochemicals was assessed using *Caenorhabditis elegans* model.

Results: The phytochemicals used in the study were capable of inhibiting quorum-sensing, preventing biofilm formation and inactivating mature biofilms of foodborne pathogens in the concentration ranging from 1.8 to 62.5 µg/ml. Caryophyllene and phellandrene inhibited biofilm formation by reducing metabolic activity and exopolymeric substance production at 0.019 µg/ml and 0.038 µg/ml, respectively ($P < 0.05$). *In vivo* virulence studies revealed that the tested phytochemicals had a protective effect on *C. elegans* fed on the foodborne pathogen *Salmonella enterica* serovar Typhimurium.

Significance: Results suggest that the phytochemicals capable of inhibiting biofilm formation and virulence production in foodborne pathogens could potentially be used to control biofilms in food processing environments and incorporated in packaging films to enhance food safety.

T8-10 Gaseous Chlorine Dioxide and Biocontrol Microbes for Control of *Salmonella enterica* on Tomatoes

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Technical

Introduction: *Salmonella enterica* is one of the most common foodborne pathogens associated with produce contamination and a food safety concern. Therefore, reduction of contamination by *Salmonella* and other foodborne pathogens at post-harvest is of considerable concern.

Purpose: To evaluate the effects of gaseous chlorine dioxide in combination with *Pseudomonas chlororaphis* and *P. fluorescens* (biocontrol microbes) for reduction of *Salmonella enterica* on tomatoes at post-harvest.

Methods: *P. chlororaphis* was spot-inoculated on stem-scars of tomatoes and was then subsequently exposed to *S. enterica* serovars Montevideo and Typhimurium. Tomato samples (n = 6 per treatment) were stored for 24 or 48 h prior to evaluation. In a comparative assay, tomato (stem scars) inoculated with *Salmonella* Montevideo and *Salmonella* Typhimurium serovars and stored at 13°C, were exposed to gaseous chlorine dioxide at 0.4 mg/l for 2 or 4 h in separate experiments (90 % RH, 13°C). The *Pseudomonas* strain was assessed on *Pseudomonas* Agar F while *Salmonella* serovars were quantified on XLT-4 medium.

Results: The reductions of *Salmonella* Montevideo and *Salmonella* Typhimurium populations on tomatoes following 4 h chlorine dioxide gas treatment were 4.6 and 5.3 log CFU/g, respectively. In comparison, the populations of these pathogens (5.42 log CFU/g) on the untreated control tomatoes were significantly ($P < 0.05$) higher than that of the treated tomatoes. Similar results were obtained with the 2 h chlorine dioxide gas treatment. When tomatoes were inoculated with *P. chlororaphis* preemptively as antagonistic biocontrol, the reductions of *Salmonella* serovars averaged 2.6 log CFU/g while the untreated control had 5.8 log CFU/g of *Salmonella* on produce.

Significance: The application of gaseous chlorine dioxide and sequential application of biocontrol microbes such as *P. chlororaphis* was effective in reducing populations of *Salmonella* on tomatoes.

T8-11 Inhibitory Activity of Plant-derived Antimicrobials against *Lactobacillus brevis* on Organic Leafy Greens

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Introduction: Fresh-produce has become an important part of the regular diet, with an increased trend towards organic produce consumption, including leafy greens. However, controlling the growth of spoilage microorganisms on organic greens during transportation and storage is challenging. *Lactobacillus brevis* is a food-spoilage microorganism resulting in economic losses to the fresh-produce industry. Antimicrobials are routinely used during flume-washing, however, synthetic decontaminants are prohibited for organic fresh-produce. Plant-derived antimicrobials are therefore being considered as alternatives.

Purpose: Evaluate plant-derived antimicrobials during flume-tank-washing of organic leafy greens to reduce *Lactobacillus brevis*.

Methods: Carvacrol, cinnamaldehyde, and oregano and cinnamon essential oils (EO) were tested at 0.5% concentration. Hydrogen-peroxide, water and phosphate buffered saline (PBS) were used as controls. Organic baby spinach and romaine and iceberg lettuce were inoculated with *Lactobacillus brevis* (10^6 CFU/g), washed with antimicrobials for 2 minutes, and stored at 4°C for 3 days. Surviving bacteria were enumerated on MRSA on day 0, 1, and 3. Wash waters were enumerated for *L. brevis,* and their pH recorded before and after washing leaves. Reduction in bacterial populations (as log CFU/g) were compared using ANOVA ($P < 0.05$).

Results: Carvacrol and oregano EO were the most effective ($P < 0.05$) on all leafy greens, reducing *L. brevis* populations to undetectable levels immediately after washing on day 0. Cinnamon EO reduced populations by 1.0 - 1.5 logs by day 3 in all leafy greens. On leafy greens washed with cinnamaldehyde and hydrogen-peroxide, majority of reduction was seen on day 0 and 1 but by day 3, an increase in bacterial populations was observed, except in baby spinach where a 1.0-log reduction was observed on all three days. No growth was detected from wash waters for any of the treatments except PBS and cinnamon EO.

Significance: Plant-derived compounds and essential oils have the potential to inhibit spoilage microorganisms such as *L. brevis* on organic leafy greens.

T8-12 Microbial Safety of Cold Cuts and Fate of *Listeria monocytogenes* ATCC 7466 in Chicken Cold Cuts Prepared with Antimicrobials

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Introduction: Ready to eat foods contain high levels of sodium chloride because it performs various functions in meat including the inhibition of foodborne pathogens, however, high intakes of this salt may lead to numerous health implications. *Listeria monocytogenes*is is however known for its prevalence in RTE foods especially deli meats.

Purpose: The objective of this work is to determine the microbiological quality of cold cuts from selected retail outlets in Durban and furthermore look into sodium chloride substitutes that may be used to inhibit *Listeria monocytogenes* in cold cuts.

Methods: Cold cuts were purchased from four retail outlets in Durban, South Africa and analysed for Aerobic bacteria (AB) , Aerobic sporeformers (ASF), Anaerobic sporeformers (AnSF), *E. coli* and *Listeria monocytogenes.* To determine the effect of salts and acid on *Listeria monocytogenes*, chicken cold cuts were prepared with 2% sodium chloride, 2% potassium chloride, 2% calcium chloride and 2% acetic acid respectively. Samples were inoculated with 10^8 cfu/ml of *Listeria monocytogenes* ATCC 7644 stored at 4°C and 7°C for 5 days and *Listeria*count was carried out every 24 h.

Results: The mean values of AB, ASF and AnSF in the sampled cold cuts were 5.32 log cfu/g, 1.12 log cfu/g and 0.97 log cfu/g respectively; 91.6% of the cold cuts tested were positive for *Listeria* and 41.6% were positive for *E. coli.* It was found that 2% Acetic acid had the most effective inhibition

whereas 2% Potassium chloride was the least effective against *Listeria monocytogenes* under both storage temperatures. Acetic acid showed a significant difference (P < 0.05) in the inhibition of *L. monocytogenes*however there was no significant difference between inhibition with the other antimicrobials.

Significance: This work shows that the sampled cold cuts constitute a public health risk however acetic acid may be successfully used to reduce sodium levels and inhibit pathogens in cold cuts.

T9-01 Time Since Irrigation and Rain Events is Significantly Associated with an Increased Prevalence of *Listeria monocytogenes* in Spinach Fields in New York State

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❖ ***Developing Scientist Competitor***

Introduction: As rain and irrigation events have been associated with an increased prevalence of foodborne pathogens in produce production environments, quantitative data are needed on the effects of various environmental and temporal factors on the risk of produce contamination after rain and irrigation events.

Purpose: This study was conducted to identify environmental and temporal factors associated with *L. monocytogenes* and *Listeria* spp. isolation from produce production environments following rain and irrigation events.

Methods: Two spinach fields, one with a high and one with a low expected risk for *L. monocytogenes* isolation, were sampled 24, 48, 72 and 144-196 h following irrigation and rain events. Expected field risk was a function of a field's proximity to water and roads. Environmental and temporal factors were evaluated for their association with *L. monocytogenes* and *Listeria* spp. isolation using a generalized linear mixed model. Differences in allelic type diversity between samples were calculated using the Shannon-Weiner Index and T-Hutcheson tests.

Results: The risk for *L. monocytogenes* and *Listeria* spp. isolation from soil samples was highest during the 24 h immediately following irrigation and rain events (OR = 23.4 and OR = 7.59, respectively). Additionally, *L. monocytogenes* and *Listeria* spp.-positive isolates associated with irrigation events were significantly less diverse than those associated with precipitation events (*P* = < 0.001), suggesting that irrigation water may be a point source for *L. monocytogenes* and *Listeria* spp. contamination.

Significance: These findings suggest that small changes in management practices, such as treating irrigation water or waiting 24 h after rain or irrigation events to harvest crops, may reduce the risk of *L. monocytogenes* of fresh produce.

T9-02 Use of Geographic Information Systems to Predict the Risk of *Listeria monocytogenes* Contamination in Produce Fields

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Introduction: Technological advancements, particularly in geographic information systems (GIS), have made it possible to develop models to predict the risk of pathogen contamination in produce production environments. Yet, few studies have examined the validity and robustness of such models.

Purpose: A study was performed to test a set of hierarchical rules associated with a previously developed model to predict the prevalence of *Listeria monocytogenes* in New York State produce farms.

Methods: Predictive risk maps were developed for four enrolled produce farms. The expected prevalence of *L. monocytogenes* was determined for each field based on the hierarchical rules: proximity to water, roads, and pastures, and available soil moisture (AWS). Drag swab samples were collected from a subset of plots assigned to each risk category. Chi-square tests were used to evaluate whether each rule accurately predicted *L. monocytogenes* prevalence. Multivariable analyses were performed to test the effect of factors on the likelihood of *L. monocytogenes* isolation from each risk category.

Results: The overall prevalence of *L. monocytogenes* was 12% (128/1,056). Specifically, *L. monocytogenes* was detected in 24% (43/176), 8% (21/264), 17% (23/132), 5% (7/132), and 10% (34/352) of samples collected from risk categories near water, near roads, low AWS, high AWS, and near pastures, respectively. The expected prevalence of *L. monocytogenes* (previous model) was not significantly different from the observed prevalence of *L. monocytogenes* for risk categories: near water (*P* = 0.72), high AWS (*P* = 0.28), and near pasture (*P* = 0.54). Additionally, multivariable analyses showed that proximity to water and pasture were significantly associated with isolation of *L. monocytogenes* (OR = 3.8 and 2.4, respectively) from drag swab samples.

Significance: These findings suggest the risk of *L. monocytogenes* contamination on-farms is not uniform; instead risk of contamination is driven by environmental factors specific to each on-farm location. Thus, GIS offers growers' science-based-data on pathogen risk to tailor GAPs.

T9-03 Spatial and Temporal Factors Affecting Prevalence of *Salmonella* and STEC in Wild Birds and Rodents in Proximity to CAFOs and Vegetable Fields in the Southwest Desert

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Introduction: Vegetable fields are sometimes located in close proximity to concentrated animal feeding operations (CAFOs). Cattle housed in CAFOs may be reservoirs of *Salmonella* and Shiga Toxin-producing *Escherichia coli* (STEC). However, little is known about the potential for wildlife that live at the CAFOs to transfer pathogens to nearby produce fields.

Purpose: To determine the prevalence of *Salmonella*, *E. coli* O157, and non-O157 STECs in cattle housed at CAFOs in the southwest desert, and in wild birds and rodents that frequent those CAFOs. We also examined movement patterns of wildlife to determine if they could transfer pathogens from the CAFO to nearby leafy green fields, thereby posing a risk to human health.

Methods: We collected fecal samples from cattle (n = 300), wild birds (n = 383) and rodents (n = 775) each season from summer 2013 to winter 2014. Cattle feces were collected from pen floors. Wild birds and rodents were live-captured, tagged, and swabbed to collect fecal material. Wild birds were fitted with radio transmitters and rodents with ear tags to determine movement between animal and vegetable production areas. Pathogens were isolated by pre-enrichment followed by IMS (STECs), selective plating, and PCR confirmation.

Results: CAFO cattle show a significant peak in the prevalence of *Salmonella* (16.5%), *E. coli* O157 (52.5%) and non-O157 STEC (17.0%) during fall compared to spring and summer (*P* = 0.019). Rodents and birds trapped at the CAFO do not show a significant seasonal difference in pathogen prevalence, but movement data do indicate that birds traveled regularly between the CAFO and produce fields.

Significance: Rodents at CAFOs likely pose a minimal pathogen risk to nearby fresh produce fields. However, birds have a greater home range and travel regularly between feeding and roosting sites. There is a need for practical and cost-effective approaches to co-manage agriculture with the surrounding natural environment.

T9-04 Differential Expression of *E. coli* O157:H7 Virulence Genes in Model Ready-to-Eat Produce Microenvironment during Temperature Drop and Refrigeration

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Introduction: The contamination and persistence of pathogens in ready-to-eat produce have emerged as a significant food safety and public health concern. Viable produce-borne pathogens cope with several stresses during production and storage of the commodities. During such stressful exposures (such as temperature fluctuations, low-temperature storage), pathogens must change physiologically to adapt to the new environment. Understanding the implication of this stress-induced adaptation on pathogenicity of the organisms is important.

Purpose: This study aims to evaluate the differential virulence gene expressions of *E. coli* O157:H7 at 25°C and 4°C by real-time PCR and *in vitro* bioassay in bacteriological medium and simulated produce microenvironment.

Methods: Rifampin-resistant *E. coli* O157:H7, strain EDL933 grown at 37°C was exposed to 25°C for 1 h, 4°C for 1 h, and 4°C for 10 min in lettuce leaf lysates or Luria-Bertani medium. QRT PCR utilizing comparative critical threshold method was performed to evaluate the expression of selected genes including virulence, heat- and cold-shock protein, etc., with reference to selected housekeeping genes. Adhesion of treated bacterial cells to mammalian cells (MAC-T) was quantified by plating-based method and Giemsa staining.

Results: Bacterial cold-shock protein (*cspA*) gene was found to be up-regulated at both the temperatures (25°C and 4°C) as compared to expression at 37°C ($P < 0.05$). The results also revealed that two key virulence genes *stx1A* and *stx1B* were up-regulated significantly ($P < 0.05$) upon cold-shock treatments; but another virulence gene *eae* was down-regulated. MAC-T cell adhesion assay revealed a temperature-dependent reduction in attachment of cold shocked *E. coli* cells.

Significance: Cold-shock resulted in the reduction in attachment of *E. coli* to epithelial cells while promoting higher levels of Shiga Toxin gene expressions at molecular level. The present study reveals the role of cold-shock (refrigeration) on the potential severity of sublethally injured *E. coli* O157:H7 infections associated with ready-to-eat produce.

T9-05 Variation in Gene Expression and Chlorine Resistance among Enterohemorrhagic *E. coli* on Pre-harvest Lettuce

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Introduction: Illnesses linked to consumption of fresh produce have increased in the last several decades, with produce now responsible for 25% of all foodborne outbreaks. In the field, foodborne pathogens such as enterohemorrhagic *E. coli* (EHEC) are capable of surviving on produce over time, yet little is known about how the pathogen adapts to this environment.

Purpose: The goals were to characterize the physiological state of EHEC on pre-harvest lettuce, and determine if the stresses experienced by EHEC on pre-harvest lettuce impact its ability to survive subsequent decontamination treatments.

Methods: Greenhouse-grown Romaine lettuce was spray inoculated with one of 4 different EHEC strains (O157:H7, n = 2; O26:H11, n = 2) and incubated in the greenhouse for 5 days. On days 1, 3, and 5, lettuce leaves were washed for 2 min in sterile water or 50 ppm chlorine. EHEC were enumerated after washing by plating on MacConkey agar. Changes in EHEC gene expression were measured over time by extracting RNA from inoculated lettuce and using RNA-seq.

Results: One day post-inoculation, all 4 strains had an average 1.0 ± 0.3 log CFU/g lower recovery from chlorine-washed lettuce vs. water-washed lettuce. Three and five days post-inoculation, the O157:H7 spinach outbreak strain had an average of 0.4 ± 0.2 log CFU/g lower recovery from chlorine washed lettuce, while the other three strains remained at ~ 1 log CFU/g. Analysis of RNA-seq data indicated significantly higher transcript levels for genes encoding proteins involved in responding to oxidative stress for the O157:H7 spinach strain on day 3 post-inoculation ($P > 0.05$).

Significance: Adaptation of EHEC to stresses on pre-harvest lettuce can lead to cross-protection to subsequent stresses. Assessing physiological changes that occur during this adaptation can provide a greater understanding of stresses the pathogen is experiencing, and may be used to develop control measures for this pathogen in the food supply.

T9-06 Wash-Line Effectiveness in Reducing Surviving *Salmonella* from Field-inoculated Navel Oranges and Preventing Cross-contamination in a Pilot Postharvest System

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Introduction: In anticipation of proposed federal preventive control compliance standards, the California citrus industry proactively supported a study of wash-line sanitation efficacy against a field-applied pathogen surrogate.

Purpose: The log reduction and cross-contamination potential of an attenuated *Salmonella enterica* sv. Typhimurium was assessed on oranges during passage through a pilot immersion and high pressure wash line.

Methods: Oranges were spray-inoculated under field conditions with attenuated *Salmonella* Typhimurium (~6 log CFU/orange). Oranges were harvested three days post-inoculation, separated into groups (n = 30) and immersed in water (21°C), 200 ppm free chlorine (pH 7.0, 21°C and 26°C), or combined chlorine and 1.0% sodium bicarbonate (pH 8.0, 26°C). Additional samples (n = 30) were treated with a high pressure wash (150 psi) containing the same disinfectants or a combined high pressure wash and immersion treatment. Following disinfection, oranges passed through a wax cabinet and dryer at 49°C (2 min residence time). Individual fruit were quantified and enriched for *Salmonella.* Additionally, a mix of inoculated (n = 185) and uninoculated (n = 110) oranges were passed through the high pressure wash line (200 ppm free chlorine, 1.0% sodium bicarbonate) to assess cross-contamination.

Results: At harvest, oranges contained 2.70 log CFU/orange of *Salmonella*, with post-treatment reductions of 1.12 - 1.45 log CFU/orange. 76.7-86.7% of samples were positive for *Salmonella* following immersion treatments, while the high pressure wash with disinfectants resulted in 43.4 - 60% positive samples. The combined high pressure wash and immersion treatment was the most effective, with 33.3% of samples positive. In the cross-contamination study, 45.9% of inoculated oranges remained positive post-disinfection, while 4.5% of uninoculated oranges became contaminated during treatment.

Significance: The sequential chemical and mechanical wash-line treatments significantly reduced the surface-adhering populations of applied *Salmonella* indicating a substantial log-reduction may be achieved. However, low numbers of persistent *Salmonella* indicate continual improvements in wash-line design and process controls would be prudent in full commercial handling.

T9-07 The Efficacy of a New Fresh Produce Wash (*First Step+10*) at Inactivating Foodborne Pathogens in Rinse Water as Well as on Cut Apples, Cherry Tomatoes, Cantaloupe Rind, and Spinach

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Introduction: Produce washes may prevent cross-contamination of foodborne pathogens in flume tank waters and inactivate pathogens on fresh produce.

Purpose: To determine the efficacy of a new commercial product, *First Step+ 10*, at inactivating foodborne pathogens in rinse water.

Methods: The BS EN 1276 (2009) method was used to assess survival of *Salmonella, E. coli* O157:H7 and *Listeria monocytogenes* in a quantitative suspension test with product concentrations of 0.5, 0.6, 0.8, 1.0, 1.2, and 1.6%. An organic interference substance (bovine albumen) (BA) was added to the suspensions to create "CLEAN" conditions (0.03% BA) or "DIRTY" conditions (0.3% BA). Fresh produce was inoculated with pathogens and treated with the produce wash (5 min).

Results: The diluted produce wash solutions were tested against *E.coli* O157:H7 per BS EN 1276 under both "CLEAN" and "DIRTY" conditions. In 5 min, the produce wash inactivated more than 5 log CFU of *E.coli* O157:H7 under both CLEAN and DIRTY conditions. Various concentrations of diluted produce wash were inoculated with four *Salmonella* serovars (*viz*., Stanley, Montevideo, St. Paul and Newport). Following BS EN 1276, the produce wash achieved a ≥ 6.6-log inactivation of four strains of *Salmonella* in 5 minutes contact time under both CLEAN and DIRTY conditions for all dilutions tested. A cocktail of five *Listeria monocytogenes* strains was tested against the produce wash. Using BS EN 1276, under CLEAN conditions, 0.5% to 1.0% produce wash achieved > 6-log reduction of *Listeria monocytogenes* in 5 min contact. Under DIRTY conditions, 0.8% and 1.0% produce wash achieved more than a 5-log reduction, and 0.5% and 0.6% produce wash inactivated > 4.0 log CFU/ml. Wash waters also inactivated up to 3.59 log CFU of pathogens on fresh cut produce.

Significance: This antimicrobial produce wash is capable of preventing cross contamination in wash waters and inactivating foodborne pathogens on fresh produce.

T9-08 Field Assessment of Pathogen Surrogate Survival on Navel Oranges Following a 'Contaminated' Irrigation Event

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Introduction: In anticipation of proposed federal agriculture water standards, a multi-year field assessment of pathogen surrogate survival on preharvest fruit was conducted.

Purpose: Assess the impact of inoculum type, solar UV exposure, and time on persistence of surface-inoculated bacteria under conditions of full environmental exposure.

Methods: Navel oranges (n = 150) were inoculated with *Salmonella* Enteritidis using dry (Feclone- and chalk-based) and liquid inoculum and exposed to shade or sun for 20 d. Whole oranges were quantified for *Salmonella* 0, 3, 6, 11, and 20 days post-inoculation (DPI). Oranges (n = 500) were similarly inoculated under field conditions with attenuated *Salmonella* Typhimurium c3985 and generic *Escherichia coli*. Oranges were assessed for presence of target microorganisms at 0, 5, 12, and 19 DPI. Additional oranges (n = 110) were spray-inoculated with attenuated *Salmonella* Typhimurium (target 6 log CFU/orange) and processed 17, 27, and 49 DPI for spatial surface mapping of stress-adapted survivors. Each rind was dissected into 5 latitudinal sections and enriched for *Salmonella* presence.

Results: Extended survival of *Salmonella* Enteritidis was observed for shaded oranges, with 40 and 20% of the samples positive for dry inoculum (Feclone- and chalk-based, respectively) and 20% for wet inoculation, whereas only Feclone-based inoculation had 10% positives 20 DPI with solar UV exposure. Under field conditions, generic *E. coli* declined faster than attenuated *Salmonella* Typhimurium for both inoculation methods. Twenty-one DPI, *E. coli* was present in 1.7 and 2% of samples, while *Salmonella* was present in 15% and 12.2% for wet and dry inocula, respectively. Spatial mapping of *Salmonella* Typhimurium 49 DPI revealed greatest survival at the pedicel and stylar ends, with 30 and 25% positive samples, respectively.

Significance: Bacterial pathogen populations from irrigation water are likely to experience a log-die-off exceeding the proposed federal allowance if fruit contact occurs. Regardless, extended survival of low populations persisting into postharvest handling is possible.

T9-09 Efficacy of Alcohol-based and Soap-based Hand Hygiene Interventions on Farmworker Hands Soiled during Harvest

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Introduction: While the FDA guidelines recommend soap and water for hand hygiene of produce handlers, alcohol-based hand sanitizer (ABHS) may be useful in soap and water scarce situations.

Purpose: Our goal was to compare the effect of two soap-based and two ABHS-based hygiene interventions on the microbial load and soil (defined as matter removed from hands during sampling) on farmworker hands after produce harvesting.

Methods: One hundred eighty-one farmworkers, after produce harvesting, were randomly assigned to soap-based groups (antimicrobial or pumice soap), ABHS-based groups (label-use or two-step), or no-hygiene control. We measured each intervention's ability to reduce the microbial load (coliforms, *E. coli,* and *Enterococcus*) and soil (A_{600nm} of hand rinsate). $P < 0.05$ was considered significant.

Results: With no hand hygiene, farmworkers' hands were heavily soiled (geomean $A_{600nm} = 0.46$) and contaminated with high coliform (geomean 3.4 log CFU/hand) and *Enterococcus* (geomean 5.3 log CFU/hand) levels after 1 - 2 hours of harvesting tomatoes. Farmworkers' hands across all intervention groups had significantly less *Enterococcus* and *E. coli*, but not coliforms, compared to those in the control group. No significant differences in microbial loads were observed across any soap- or ABHS-based groups. The two-step ABHS intervention had lower levels of coliforms and *Enterococcus* than the pumice soap and label-use ABHS interventions, and was the only intervention to have significantly fewer samples with *E. coli* than the control group. Farmworkers' hands across all intervention groups had significantly less soil compared to those in the control group; soap-based interventions were better at removing soil from hands than ABHS-based interventions.

Significance: ABHS was equally effective as recommended practices (handwashing with soap) at reducing indicator organisms on farmworkers' hands, and was more effective when used with the two-step ABHS technique. Based on these results, ABHS can be viewed as an effective hand hygiene solution, even on soiled hands.

T9-10 Microbial Water Quality for Frost Protection: Implications for Strawberry Safety and Quality

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Introduction: The microbial quality of water used for frost protection/irrigation of strawberries is critical to minimize the risk of pathogen contamination. The newly proposed microbial water standard within FSMA precludes water to be in direct contact with produce if the geometric mean (GM) and statistical threshold value (STV) of samples exceed 126 CFU/100 ml and 410 CFU/100 ml of generic *E. coli,* respectively.

Purpose: To study the effect of sampling frequency, depth, and timing relative to disturbance events on the presence of indicator (*E. coli* and *Enterococcus* spp.) and pathogenic (*Salmonella* and STEC) organisms on surface waters used for frost protection/irrigation.

Methods: Over 12 weeks, 216 one-gallon water samples were collected from surface water used for frost protection/irrigation. Every 2 weeks, samples were taken at 36 locations at 0, 5, 10, and 15 m away from the irrigation water intake and at 3 depth ranges; 0.5 - 1, 1.75 - 2.25, and 3 - 3.5 m below the water surface. Water was filtered using a modified Moore swab system and quantitative/qualitative recovery of indicator and pathogenic microorganisms was achieved via selective/differential culturing methods and probe-based PCR systems.

Results: *Salmonella* and STEC were recovered from multiple water sampling events despite all 216 one-gallon samples having populations of generic *E. coli* on average 3 fold below ($P < 0.05$) the proposed GM and STV. Significant increases in the number of positive samples for *Salmonella* was determined on sampling dates that coincided with rain events ($P < 0.05$), but marginally for indicator microorganisms. *Salmonella* was detected on samples coming from depths between 1 and 2.25 m. Although, marginally higher populations of generic *E. coli* and *Enterococcus* ($P < 0.05$) were observed between 3 - 3.5 m. Yet these populations were 3 fold below the GM and STV proposed by FSMA.

Significance: Our findings suggest that the newly proposed microbial water standard within FSMA needs to be reassessed to ensure pathogen free water and fresh produce safety.

T9-11 Dynamic Models to Predict the Fates of *Staphylococcus aureus* and Pathogenic *Escherichia coli* in High Risk Vegetables

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❖ ***Developing Scientist Competitor***

Introduction: As fresh vegetable consumption has been increased for a healthy diet, foodborne outbreaks linked to fresh vegetables such as lettuce, sprout, and water celery have also been increased.

Purpose: This study developed dynamic models to describe the growth pattern of *Staphylococcus aureus* and pathogenic *Escherichia coli* in lettuce, sprout, and water celery under fluctuating temperature.

Methods: Five-strain mixtures of *S. aureus* and *E. coli* were inoculated in lettuce, sprout, and water celery at 3 log CFU/g. Bacterial populations were enumerated on mannitol salt agar (*S. aureus*) and *E. coli*/Coliform count plate (*E. coli*) at periodic intervals during storage at 4°C (336 h), 10°C (288 h), 15°C (288 h), 25°C (96 h), and 30°C (96 h). The Baranyi model was then fitted to the *S. aureus* and *E. coli* growth data to calculate maximum specific growth rate (μ_{max}; log CFU/g/h) and lag phase duration (λ; h). Temperature effects on the parameters were analyzed by the square root model (μ_{max}) and a polynomial equation (λ). *S. aureus* and *E. coli* growth in vegetables were simulated under dynamic temperature. The predicted values from dynamic temperature were compared with observed values, and root mean square error (*RMSE*) was calculated to evaluate the model performance.

Results: *E. coli* and *S. aureus* growth were observed only on lettuce and water celery at 10 - 30°C. The μ_{max} values of the pathogens on lettuce and water celery increased ($P < 0.05$) from 0.002 to 0.428 log CFU/g/h as temperature increased, and λ for the bacteria ranged 1.49 to 52.16 h, depending on product and temperature. h_0 ($\mu_{max} \cdot \lambda$) values were used to simulate *E. coli* and *S. aureus* growth under changing temperature. *RMSE* values were 0.3 - 0.5.

Significance: The developed models should be useful in describing *E. coli* and *S. aureus* growth patterns on lettuce and water celery under dynamic temperature.

T9-12 Attachment Ability of Shiga Toxin-producing *Escherichia coli* to Alfalfa, Lettuce, Tomato and Fenugreek Seeds

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Introduction: Outbreaks of Shiga Toxin-producing *Escherichia coli* (STEC) infection have been linked to the consumption of contaminated fresh produce such as lettuce, and alfalfa and fenugreek sprouts. In several incidences, contaminated seeds were suspected as the source of the responsible pathogens.

Purpose: To compare the attachment abilities of different STEC strains to vegetable seeds with different surface integrity properties and chemical treatment practices.

Methods: Two different levels of nalidixic acid (NA) resistant cells of O157:H7 (F, K and H) and O104:H4 (G) strains were co-incubated with mechanically damaged and intact, as well as chemically treated and untreated, alfalfa, fenugreek, tomato and lettuce seeds at room temperature for 5 h. Contaminated seeds were rinsed with sterile water then soaked overnight in phosphate buffered saline at 4°C. Seed soaking solutions were plated onto tryptic soy agar (TSA) as well as TSA and sorbitol MacConkey agar supplemented with NA. Pathogen attachment rate was expressed as the ratio of the number of attached cells to number of inoculated cells. Fisher's least significant difference test was used to determine the significance of difference among the tested pathogens on different vegetable seeds.

Results: Overall, the number of attached STEC cells was the highest (10.4%) on lettuce seed, followed by tomato (4.8%), alfalfa (2.5%) and fenugreek (1.8%) seeds. The ability of STEC cells to attach to the damaged seeds (6.4%) was significantly higher ($P < 0.05$) than to intact seeds (3.3%) whereas no significant difference was observed between pathogen attachment to chemically treated *vs*. untreated seeds. Strain K displayed the best attachment ability on the tested seeds (12.5%), followed by F (5.2%), H (1.5%) and G (0.2%).

Significance: This work highlights the difference in attachment ability of STEC strains on various vegetable seeds. The rate of STEC attachment was influenced by seed surface integrity and chemical treatment practice.

T10-01 Evaluation of Microbial Populations in Chicken Ceca Raised on Pasture Flock Fed with Commercial Prebiotics via Sequencing (Illumina MiSeq)

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Introduction: Prebiotics are non-digestible carbohydrate dietary supplements that stimulate the growth of one or more beneficial bacteria in the gastrointestinal tract of the host. These bacteria can inhibit colonization of pathogenic bacteria by producing antimicrobial substances such as short chain fatty acids and competing for niches with pathogens within the gut. Pasture flock chickens are generally raised outside with fresh grass, sunlight and air, which is a different growth conditions with conventional chickens.

Purpose: The purpose of this study was to evaluate the difference of microbial populations in ceca fed with commercial prebiotics derived from brewer's yeast cell wall via Illumina MiSeq platform.

Methods: A total of 147 day-of-hatch naked neck chickens were distributed into 3 groups consisted of 1) C, control (no prebiotic); 2) T1, Biolex® MB40; 3) T2, Leiber®ExCel, consistently supplemented prebiotics during the experimental period. At 8 weeks, a total of 15 birds from each group were randomly selected and extracted ceca for DNA extraction. The Illumina MiSeq platform based on 16s rRNA gene was applied for microbiome analysis.

Results: Both treatments had limited impact on the microbial populations at the phylum level, with a slight decrease in the number of *Bacteroides* for both treatments and an increase of proteobacteria for the Biolex® MB40 treatment. Both treatments resulted in significant decreases of members of the *Bacteroides* genus (53% to 43%), which include species that can be detrimental. This decrease was accompanied with a significant increase of *Phascolarcobacterium* and *Faecalibacterium*, both *Firmicutes* associated with health benefits. The Leiber® ExCel treatment also induced an increase in *Ruminococcus*, which is another beneficial genus.

Significance: According to the development of next generation sequencing (NGS), microbiome analysis based on 16s rRNA gene is useful to evaluate the prebiotics effects on poultry gut health as well as pathogenic bacteria prevalence.

T10-02 Multidrug Resistant Clones of *Salmonella* Infantis from Broiler Chickens

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Introduction: The emergence of multidrug resistant (MDR) strains of *Salmonella* Infantis in broiler chickens has become a public health concern worldwide. However, molecular characteristics and the evolutionary processes that promote its spread are not entirely known.

Purpose: The purpose of this study was to provide a comprehensive assessment of the diversity of MDR *Salmonella* Infantis (n = 50) from broiler chickens exhibited resistance to at least three antimicrobials during 2012-15 in Turkey.

Methods: Isolates were tested by *Xba*I PFGE, 7-gene MLST and plasmid profiling by PFGE and conventional gel electrophoresis. Integron associated gene cassettes (*ant(3")-Ia, qacEΔ1* and *sul1*) and 21 regions of antimicrobial resistance markers were determined by traditional PCR. Molecular characteristics of the representative clinical isolates from human patients of serovars Paratyphi B, Typhimurium, Kentucky, Enteritidis, Othmarschen and Typhi were compared with the results obtained from Infantis isolates.

Results: The emerging Infantis strains revealed a single genetic clone by PFGE fingerprinting which resulted in three distinct PFGE profiles of > 95 % similarity. The findings were supported by MLST analysis, since all the strains presented the same MLST sequence type, ST32. Most strains harbored plasmids in variable size, while the majority of the isolates had nalidixic acid–streptomycin–sulfonamide–tetracycline resistance type. *tetA*, *aadA1* and *sul1* regions were associated with tetracycline, aminoglycoside and sulfonamide resistance, respectively.

Significance: Our results suggest the recent emergence of serovar Infantis is an outcome of a clonal dissemination, while molecular characteristics of class 1 integrons and the plasmids represent a possible explanation for the dissemination of the emergent strains.

T10-03 *Salmonella* Presence and Numbers on Skin Parts of Turkey Carcasses

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❖ ***Developing Scientist Competitor***

Introduction: Turkey skin parts (i.e., drumstick, thigh, and wing) are commonly utilized as a source of fat in ground products. Based on our previous data, *Salmonella* prevalence and mean number in turkey neck skin was 42% with 2.5 log MPN/sample. Although, neck skin is not currently used in ground turkey production, other skin parts are used and potentially sources of contamination.

Purpose: The objective of this study is to compare the prevalence and numbers of *Salmonella* in skin of turkey parts (i.e., drumstick, thigh, and wing) at post-chill.

Methods: In collaboration with a commercial turkey processor, a total of 20 turkey flocks predicted to be highly contaminated with *Salmonella* are being sampled. From each flock, 15 samples per part type are collected at post-chill and tested for *Salmonella* using most probable number (MPN) and enrichment methods.

Results: Thus far, samples from 15 flocks have been collected and tested. *Salmonella* prevalence in skin of drumstick, thigh and wing was 15.1%, 18.7%, 25.3%, respectively ($P < 0.05$). Mean *Salmonella* numbers per gram of skin was 0.21 MPN/g (drumstick), 0.17 MPN/g (thigh), 0.34 MPN/g (wing), which were not significantly different ($P > 0.05$).

Significance: Based on the preliminary results, *Salmonella* was present on turkey skins at a significant prevalence. It may contribute to *Salmonella* contamination of ground turkey. *Salmonella* numbers on skin samples was low. In other words, when a sample was *Salmonella*-positive, the pathogen load (numbers) was low.

T10-04 Biofilm Formation and Antimicrobial Resistance among Most Prevalent Poultry-associated *Salmonella* Serotypes (MPPSTs) Isolated from the US Poultry and Poultry Products

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Introduction: Poultry-associated *Salmonella* serotypes are the important cause of foodborne illnesses in humans. Antibiotic resistance among *Salmonella* poses a significant public health concern. *Salmonella* also produces biofilm on food and food-processing surfaces which may further aid in resistance to antibiotics, disinfectants and various sanitizers during poultry meat processing.

Purpose: Determine the antimicrobial resistance and the biofilm forming ability of most prevalent poultry-associated *Salmonella* serotypes (MPPSTs) isolated specifically from US poultry.

Methods: A total 145 MPPSTs isolated from US poultry including *Salmonella* Kentucky (57), Enteritidis (41), Typhimurium (14), Heidelberg (13), Mbandaka (10), Seftenberg (4) and three strains each of Montevideo and Infantis were tested for their ability to form biofilms and susceptibility to 15 antibiotics belonging to penicillin, quinolone, cephalosporin, aminoglycoside, sulfonamide and tetracycline classes. Additionally, one strain of each MPPST was tested for its resistance to chlorine (commonly used carcass sanitizer) using a new custom designed laboratory model that uses chicken meat extract (CME) medium and simulates carcass chilling, an important pathogen control step in poultry meat processing.

Results: Overall, 133 out of 145 (92%) isolates were resistant to at least two classes of antimicrobials tested. A total of 81 out of 145 (56%) isolates formed biofilm on polypropylene surface. Irrespective of concentration of CME, the amount of free chlorine decreased from 50 ppm to 3 - 5 ppm in < 5 min. In general, as the CME concentration increased, the pH of the chlorinated water and the survival of different MPPST strains also increased. At lower CME concentrations (3%), serotypes Kentucky and Mbandaka survived until 5 min; serotypes Heidelberg survived until 30 min whereas Montevideo, Typhimurium, Infantis, Seftenberg and Enteritidis survived until 90 min post-inoculation.

Significance: Multi-drug resistance among MPPST is common. Several *Salmonella* strains produce biofilms which could further enhance resistance to antimicrobials and also to carcass sanitizers and disinfectants during meat processing. MPPSTs also differ in their susceptibility to chlorine and that the level of CME in immersion chilling is an important contributing factor in *Salmonella* survival.

T10-05 How Belgian Broiler Slaughterhouses Can Improve Their Ability to Control the Level of *Campylobacter* Carcass Contamination under Routine Processing – Risk Factor Identification

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Introduction: Decrease of *Campylobacter* contamination on broiler carcasses plays a significant role in the reduction of the public health risk of campylobacteriosis. Physical and chemical treatment of poultry can successfully lower the *Campylobacter* contamination although these interventions are not in the line with consumers' preferences and acceptability as well as in some cases needs authorities' authorization. As an alternative to physical or chemical treatments, improvement of technical aspects of the slaughter process might reduce *Campylobacter* counts on broiler carcasses.

Purpose: The aim of this study was to elucidate the slaughter process and batch characteristics associated with *Campylobacter* counts on carcasses during slaughter of *Campylobacter*-positive broiler batches in Belgian slaughterhouses.

Methods: Quantitative data describing *Campylobacter* carcass contamination was collected during the slaughter of 28 *Campylobacter*-positive broiler batches in six slaughterhouses. Additionally, batch and slaughterhouse specific information were collected and their association with *Campylobacter* counts after five processing steps (bleeding, plucking, evisceration, washing, chilling) was studied using multilevel mixed-effects negative binomial model.

Results: Reduction of *Campylobacter* colonization level and optimization of transport and holding time might result in a lower broiler carcass contamination across the slaughter line. Additionally, incorrect setting of plucking, evisceration and cloaca cutter machines, low scalding temperatures, dump based unloading system and electrical stunning were identified as risk factors associated with an increase of *Campylobacter* counts on broiler carcasses at selected processing steps.

Significance: These data revealed existing variations of the routine broiler slaughter practices and batch characteristics contributing to lower carcass contamination. Therefore based on the results obtained, practical and economical achievable modifications of the slaughter process can be applied in order to improve the slaughterhouse ability to control *Campylobacter* carcass contamination.

T10-06 Domestic Handling of Chicken Carcasses: Quantification of *Campylobacter* Species Cross-contamination

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Introduction: Cases of campylobacteriosis are mostly linked to the consumption of contaminated poultry and attributed mainly to incorrect handling practices adopted by consumers during food preparation.

Purpose: The aim of the study was to collect qualitative and quantitative data by simulation in a domestic setting of cross-contamination of *Campylobacter* spp. from raw chicken meat to utensils used during food preparation.

Methods: Tests were performed on naturally contaminated skin-on raw chicken carcasses. Three different cross-contamination scenarios were simulated. Scenario 1 mimics the condition in which hands, knife and cutting board used to cut the chicken first and the plate used to place a chicken wing before cooking were not washed after use, and were used as such to cut a tomato. Moreover in Scenario 1, chicken's washing with rinsing water was added as an additional step before handling. Scenarios 2 and 3 mimic the intermediate and best case conditions, respectively, in which hands and the above-mentioned utensils were rinsed or washed (20 seconds at 40°C) with water and soap after use.

Results: Scenario 1 showed that the surfaces that were most contaminated were the cutting board (9/12 samples) and hands (9/12), followed by plate (8/12) and knife (7/12). *Campylobacter* was detected and quantified, respectively, in 6 and 4 out of 12 tomato samples which were cut on the contaminated surfaces. Chicken's washing before manipulation didn't reduce the quantity of *Campylobacter* spp. on the skin. Rinsing and washing of contaminated hands and kitchen utensils before handling other foods (e.g., tomato) eliminated in most samples the microorganism. However, due to the low contamination level (10-100 CFU/g) of the chicken, it was not possible to state which, among these two hygienic practices, was more effective.

Significance: The study confirms the importance of cross-contamination in the epidemiology of foodborne *Campylobacter* cases and of consumer education as a preventive measure.

T10-07 Searching for Suitable Indicator Viruses of Fecal Contamination for Pork Carcass Processing

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Introduction: There are concerns about the zoonotic transmission of viruses through undercooked pork products. Enteric viruses are shed in fecal material and can potentially contaminate carcasses during meat processing operations. There is a lack of information on suitable indicator viruses for pathogenic enteric viruses in the meat processing chain.

Purpose: The study compared the incidence and levels of contamination of hog carcasses with F- coliphages (FC), porcine teschovirus (PTV), and porcine adenovirus (PAdV) at different stages of the dressing process to assess their potential as indicator viruses of fecal contamination.

Methods: One hundred swab samples (100 cm^2) were collected from random sites on hog carcasses at 4 different stages of the dressing process and from meat cuts on 10 separate occasions over the span of a year from 2 pork processing plants (1000 samples in total, 500/plant). Numbers of viable FC were determined by plaque assay and the numbers of genome copies (GC) of PTV and PAdV were determined by q(RT)-PCR.

Results: For both processing plants, FC and PAdV were detected in 100% of samples and PTV was detected in 97% of samples after bleeding with a mean of 3.1 log plaque forming units (PFU)/100 cm^2 for FC and mean GC ranging between log 5.0 - 5.4 for PAdV and PTV. FC, PAdV and PTV were detected in 32%, 0%, and 3% of pork cuts, respectively, for plant 1 and detected in 20%, 3%, and 22% of pork cuts, respectively, for plant 2. Maximum numbers of FC, PAdV, and PTV were 2.2 - 4.0 log units lower on pork cuts than on eviscerated carcasses for plant 1 and 0.1 - 0.7 log units lower for plant 2.

Significance: Numbers of viable F-coliphages are high enough to trace through the carcass dressing process. Consumers are at risk when consuming undercooked meat contaminated with pathogenic enteric viruses.

T10-08 Prevalence, Antibiogram and Biofilm Formation of *Campylobacter coli* and *Listeria monocytogenes* from Pork Carcasses in Selected Slaughter Slabs in Oyo State, Nigeria

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Introduction: *Campylobacter coli (CC)* and *Listeria monocytogenes (LM)* are foodborne pathogens of significant public health and economic concerns.

Purpose: The study determined the prevalence, antibiotic sensitivity profile and biofilm-forming abilities of *CC* and *LM* isolates along the processing lines of swine slaughtered for human consumption.

Methods: A six-month study (May–October, 2014) isolated *CC* and *LM* and estimate the contamination level along pork processing line. Three hundred swabs (2 cm^2 area) and tissue samples were collected during the processing (Rectal swab before slaughtering, Skin swab before scalding/singeing, Skin swab after scalding/singeing, Pool of skin swab from cranial part, Pool of skin swab from caudal part, Swab of small intestine, Knife swab, Table swab, Liver tissue, and Kidney tissue). Scalding and singeing were the two processing methods considered in this study. Antibiotic sensitivity was assessed using the disc diffusion method. Biofilm mass was determined over a 5-day period using the Crystal Violet Binding Assay.

Results: Overall prevalence of *CC* and *LM* was 29% and 14%, respectively. High aerobic plate counts (log 7 CFU/cm^2 - log 8 CFU/cm^2), higher than the < log 6 CFU/cm^2 stipulated by International Food Standard Agencies. *Campylobacter* count (4.14 - 5.77 mean log CFU/cm^2 for scalding process and 0.995 - 5.93 mean log CFU/cm^2 for singeing process) and *LM count* (1.76 - 4.24 mean log CFU/cm^2 for scalding process and 0.995 - 3.27 mean log CFU/cm^2 for singeing process) were within the international limit (log 2 CFU/cm^2 - log 7 CFU/cm^2) stipulated by International Food Standard Agencies. Generally, *CC* strains were very resistant to all the antibiotics tested, while *LM* was moderately sensitive to all antibiotics used. All strains produced biofilm; however, biofilm forming abilities was not significantly different ($P > 0.05$) between strains.

Significance: The biofilm forming abilities of these strains will lead to persistence of these pathogens in food and food processing environment.

T10-09 Determination of Sources of *Escherichia coli* on Beef by Multiple-locus Variable-number Tandem Repeat Analysis

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Introduction: The microbiological condition of beef carcasses has been greatly improved in recent years, likely as a result of well controlled dressing process. Some plants at least in North America can produce most dressed carcasses essentially free of *Escherichia coli*. However, outbreaks of verotoxigenic *E. coli* (VTEC), particularly of the serotype O157 still occur. It is generally assumed that *E. coli* on final products are deposited on the meat during dressing process.

Purpose: The objective was to determine the origin of *E. coli* on cuts and trimmings.

Methods: *E. coli* were enumerated in samples obtained from 160 carcasses entering the breaking facility before work and during each of three breaks throughout the day, from the conveyor belt before work and after each break, and from cuts and trimmings when work commenced and after each break. Selected *E. coli* isolates were genotyped by MLVA.

Results: *E. coli* was recovered from 7 (< 5%) chilled carcasses, with total numbers being mostly ≤ 1.0 log CFU/160,000 cm^2. The log total numbers of *E. coli* recovered from the conveyor belt, cuts and trimmings were mostly between 1 and 2 log CFU/80,000 cm^2. MLVA of 327 isolates revealed 80 genotypes, with 37 (46%) each containing one isolate. However, 27% of the isolates were of genotypes that were recovered from more than one day. Of isolates recovered from cuts, 49%, 3% and 19% were of the genotypes that were found among isolates from the belt, carcasses, and both the belt and carcasses, respectively. A similar composition was also found for *E. coli* isolates from trimmings.

Significance: This study unequivocally identified conveying equipment to be the main source of contamination of cuts and trimmings from carcasses that carried very few *E. coli* and persisting strains of *E. coli* in beef breaking facility.

T10-10 Analysis of RTE Test Results as a Function of *Listeria monocytogenes* (*Lm*) Sanitation Control Alternative

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Introduction: Federal regulation 9CFR430 establishes four alternatives for controlling *Lm* for establishments producing Ready-to-Eat (RTE) products. Alternative (Alt) 1 uses both a post-lethality treatment (PLT) that reduces or eliminates microorganisms on the product as well as an antimicrobial agent or process (AMAP) that suppresses or limits the growth of *Lm*. Alternatives 2a and 2b use either a PLT or an AMAP, respectively. Alternative 3 only uses sanitation measures.

Purpose: FSIS evaluated the effects of each alternative on percent positive rates using *Lm* RTE program data for product samples, food contact surface (FCS) samples and non-food contact environmental samples for years 2005–2012.

Methods: FSIS tested product samples for *Lm* using FSIS's Microbiology Laboratory Guidebook. Results were compared to the interventions applied at the establishment.

Results: The analysis ranked the effectiveness of the different alternatives as follows: Alt1 > Alt2a ≈ Alt2b > Alt3 (overall percent positive rates were 0.21%, 0.3%, 0.27% and 0.42%, respectively). The results indicate that sanitation alone was less effective than some forms of intervention (PLT and/or AMAP) for controlling *Lm* contamination in both products and FCS. Statistically significant differences ($P < 0.05$) were observed between alternatives

2b and 3 for product and FCS samples, while the difference between environmental samples tested under the two alternatives approached statistical significance ($P < 0.08$). A smaller number of samples were collected and tested under alternatives 1 and 2a (less than 5% of all samples collected) compared to alternatives 2b and 3.

Significance: The results suggest that PLT and/or AMAP are more effective than sanitation alone in controlling *Lm*.

T10-11 Characterization of *E. coli* O157:H7 Strains Isolated from "High Event Period" Beef Trim Contamination

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Introduction: A "High Event Period" (HEP) is defined as a time period in which commercial plants experience a higher than usual rate of *E. coli* O157:H7 contamination of beef trims. Our previous studies suggested that instead of being a direct result of bacteria on animal hides, in-plant biofilm formation and sanitization resistance may play critical roles in HEP contamination. Therefore, further characterization of HEP *E. coli* O157:H7 strains, including biofilm formation under plant conditions, sensitivity to sanitization, the presence of resistant genes and plasmids are required to test the above hypothesis and to understand the molecular basis for the phenomenon.

Purpose: To characterize phenotypes of biofilm formation and sanitizer resistance of HEP *E. coli* O157:H7 strains and to determine any potential molecular mechanisms for the observed phenotypes.

Methods: A subset of 45 *E. coli* O157:H7 strains obtained from 14 HEPs and a group of 47 *E. coli* O157:H7 "Diversity Control Panel" strains were compared for biofilm formation on solid surfaces, minimal inhibitory concentration (MIC) of common sanitizers, antibiotic resistance profiles, and the presence of sanitizer resistant genes and the virulence plasmid *pO157*.

Results: Compared to the control panel strains, the HEP strains demonstrated higher potency of biofilm formation on materials commonly used in meat plants under fabrication room conditions. The HEP strains also exhibited significantly higher MIC ($P < 0.05$) to common sanitizers. Even though no difference was observed in the presence of QAC resistant genes between the two strain groups, HEP strains overall harbored higher copy numbers of the *pO157* plasmid which has been associated with bacterial optimal survival and efficient colonization in the host and in the environment.

Significance: These data suggest a potential role of the plasmid *pO157* in biofilm formation and sanitization resistance by HEP *E .coli* O157:H7 strains, which may contribute to meat contamination.

T10-12 Prevalence of *Salmonella* Species in Oregon's Exempt Poultry Processing Operations

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Introduction: Microbiological data for the prevalence of *Salmonella* spp. in poultry processed in facilities that operate under exemption from continuous inspection is lacking on state and national levels. This data is essential to identify best practices to reduce risk of foodborne illness.

Purpose: Understand prevalence and spread of *Salmonella* spp. in Oregon's exempt poultry operations.

Methods: Oregon's exempt poultry facilities (n = 16) were asked to participate in a *Salmonella* testing study. Five agreeable processors were randomly selected, and dressed carcasses (n = 15 - 16) were tested for *Salmonella* using a modified USDA-MLG method. Two facilities were selected for further environmental and carcass sampling.

Results: The initial round of carcass testing revealed substantially different *Salmonella* prevalence: 3 processors: 0 detectable *Salmonella* (n = 15); 1 processor: 1/15 positive; 1 processor: 10/16 positive. The facility with highest prevalence (A) and one facility with no detectable *Salmonella* (B) were selected for sampling during active processing. Facility A had very high rates of *Salmonella* in live birds (16/30 positive feces; 17/20 positive crops). Facility A also had positive carcasses (16/20), equipment/tools (6/7), and processing water (9/24). For facility B, positive samples were only detected in fecal samples (2/30) and a single crop (n = 20). All other samples from B [carcasses (n = 20), equipment/tools (n = 6), processing water (n = 33)] were negative for *Salmonella*.

Significance: *Salmonella* prevalence in poultry from Oregon's exempt poultry processors ranged from very high (> 60%) to very low (0/15). The processing facility that produced carcasses with no detectable *Salmonella* had few birds carrying *Salmonella*, but processing practices were successful at preventing spread to carcasses. The facility with high rates of *Salmonella* in carcasses also had an exceptionally high rate in live birds, which was not mitigated by processing. On-farm production practices should be evaluated to identify best practices to mediate the spread of *Salmonella* in live birds.

T11-01 Trends in Foodborne Illness in the United States; 1996-2013

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Introduction: Current statistical methods for evaluating national foodborne illness surveillance data over time make pair-wise comparisons between the most recent surveillance year and one or more baseline periods. This avoids the problem of specifying the model form for trend (e.g., linear, exponential, polynomial) but cannot distinguish trends in foodborne illness from year-to-year variability.

Purpose: The analysis examines trends in U.S. foodborne illness without specifying a model form for trend.

Methods: The analysis considers FoodNet (Foodborne Diseases Active Surveillance Network) cases and population data for 1996-2013 for *Campylobacter*, *Listeria*, *Salmonella*, *Shigella*, STEC O157, *Vibrio*, and *Yersinia*. To control for the addition of states to the FoodNet surveillance system over time, available data from the original five states (CA, CT, GA, MN, OR) for 1996-2012 are analyzed separately. The trend analysis uses B-splines, a flexible, semi-parametric regression method.

Results: *Campylobacter*, *Listeria*, STEC O157, and *Yersinia* exhibit early decreases in reported incidence followed by no trend. *Shigella* steadily decreased in reported incidence over time. *Salmonella* and *Vibrio* exhibit increasing trends in reported incidence. The results are insensitive to whether the data from all states or the original five states are considered.

Significance: The findings indicate a lack of evidence for continuous reduction in foodborne illnesses in the U.S. during 1996-2013.

T11-02 Global and Regional Incidence and Mortality of Diarrheal Diseases Commonly Transmitted through Food: Estimates from the WHO Foodborne Epidemiology Reference Group

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Introduction: Diarrheal diseases are major contributors to disease burden worldwide, particularly in children. Although the global disease burden due to diarrheal diseases has been estimated, comprehensive estimates of the incidence and mortality due to specific etiologies are not available.

Purpose: The objective of this study was to provide estimates of the global and regional incidence and mortality of diarrheal diseases caused by nine pathogens that are commonly transmitted through foods.

Methods: We abstracted data from systematic reviews and national estimates of foodborne diseases and, depending on mortality rates of the country, applied either a national incidence estimate or a modified Child Health Epidemiology Reference Group (CHERG) approach to estimate the etiology-specific incidence and mortality of diarrheal diseases, by age and region.

Results: The nine diarrheal diseases assessed caused 1.8 billion (95% uncertainty interval [UI] 1.1 – 3.3 billion) cases and around 599,000 (95% UI 472,000 – 802,000) deaths worldwide in 2010. The largest number of cases were caused by norovirus (677 million; 95% UI 468 – 1,153 million), ETEC (233 million; 95% UI 154 – 380 million), *Shigella spp.* (188 million; 95% UI 94 – 379 million) and *G. lamblia* (179 million; 95% UI 125 - 263); the largest number of deaths were caused by norovirus (213,515; 95% UI 171,783 – 266,561), enteropathogenic *Escherichia coli* (121,455; 95% UI 103,657 – 143,348), enterotoxigenic *E. coli* (73,041; 95% UI 55,474 – 96,984) and *Shigella* (64,993; 95% UI 48,966 – 92,357). There were marked regional differences in incidences and mortality for several diseases. Nearly 40% of diarrheal disease cases and 43% of diarrheal disease deaths occurred in children under five years of age.

Significance: Diarrheal diseases caused by nine pathogens commonly transmitted through food are responsible for a large disease burden, particularly in children. These etiology-specific burden estimates can inform efforts to reduce foodborne diarrheal diseases.

T11-03 Potential Transmission and Persistence of Antimicrobial Resistance (AMR) *Salmonella* after Application of Swine Manure in the Environment

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Introduction: Land application of animal manure is an important source of fertilizer. However, the presence of pathogens in soil and their occasional transmission to human and animal has become a topic of public health concern during the past few years.

Purpose: The objective of this study is to determine the transmission of *Salmonella* due to swine manure application in the environment.

Methods: At the different time points of application: day 0, 7, 14, and 21, the soil and lagoon samples were collected representing swine farms in North Carolina (n = 3) and Iowa (n = 7). A total of 1,000 soil samples (NC = 300; IA = 700) and 100 lagoon samples (NC = 30; IA = 70) were included in this study. Antimicrobial susceptibility (AST) was characterized using Sensititre® with a panel of 15 antimicrobial drugs. PCR was performed to identify the resistant determining genes. Genotypic characterization was done using pulse field gel electrophoresis (PFGE).

Results: Overall *Salmonella* prevalence was 11.73% (129/1100). The prevalence in soil and lagoon were 9.8% (98/1000) and 31% (31/100), respectively. Decrease in prevalence of *Salmonella* in the area from Day 0 to Day 21 was observed over time. We identified 8 serotypes including Anatum (17.14%) and Litchffield (5.71%) in IA, while Altona (20%), Johannesburg (1.43%) Muenster (22.86%), Worthington (14.29%), Mbandaka (7.14%), and Uganda (14.29%) in NC. Sixty-eight percent of *Salmonella* isolates were MDR with the most frequent antibiotic resistance against Streptomycin (66.7%), sulfisoxazole (56.5%), and tetracycline (52.2%). Streptomycin resistance was encoded in 39.3% of the isolates by the *addA2* gene, while *sul1* gene (22.6%) was detected in sulfisoxazole resistance. *TetA* (17.24%) and *TetB* (27.6%) were also observed. According to PFGE pattern, we detected clonal relatedness among *Salmonella* recovered from lagoon and soil at multiple time points with relatively close geographic proximity.

Significance: Our study highlights the potential role of manure application on *Salmonella* persistence and transmission in the environment.

T11-04 Isolation and Characterization of *Escherichia coli* from Swine at the Farm, Lairage and Slaughter

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Introduction: Correlating on farm use of antimicrobials using post-slaughter antimicrobial resistance data has been difficult. Studies using *E. coli* have been lacking.

Purpose: To determine if antimicrobial resistance patterns in *E. coli* observed in pigs upon arrival at the abattoir were reflective of those observed in ceca from the same cohort of pigs post-slaughter.

Methods: Pigs were sampled at a large, Midwestern abattoir, by convenience. Before placement, 10 random pen (lairage) swab samples were taken. At placement, 10 fecal swabs were taken from voided manure pats (arrival). After 2 h, pigs moved by group to processing; 10 ceca were randomly removed and 50 ml of cecal fluid (CF) was collected. Samples were transported on ice, swabbed onto blood agar/Tergitol-7, incubated at 35°C for 24 h, examined for typical *E. coli* phenotypes (rough, smooth, mucoid, and hemolytic), and 1 - 4 colonies of each phenotype was selected. Antimicrobial susceptibility testing (AST) was conducted using broth microdilution (TREK Sensititre™) per manufacturer's instructions and CSLSI or NARMS breakpoints.

Results: Samples were collected from 14 truck lots over eight dates representing n = 139 unique animals at arrival, n = 80 at lairage, and n = 140 post-slaughter (CF). Of the n = 359 samples, n = 345 were positive for *E. coli*; n = 138 arrival, n = 131 CF and n = 76 lairage. Isolates (n = 953) were recovered; n = 813 were classified by phenotype: n = 531 rough, n = 197 smooth, n = 62 mucoid, and n = 23 hemolytic. Only n = 736 isolates were recovered for AST. The top 3 AST patterns by location were Tetracycline (cecal), StreptomycinTetracycline (cecal), and AmpicillinStreptomycinTetracycline (arrival). AST patterns varied by phenotype and location. Four isolates (all CF) were resistant to nine antimicrobials and n = 33 (n = 21 arrival, n = 8 CF, n = 4 lairage) were pan-susceptible.

Significance: AST patterns were identified post-slaughter (CF) that were not identified at arrival and vice versa suggesting that *E. coli* AST surveillance post-slaughter may not be a surrogate for on-farm testing.

T11-05 Outbreak of *E. coli* O157:H7 Infections in Alberta, Canada, Caused by Exposure to Contaminated Pork Products

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Introduction: An outbreak of *E. coli* O157:H7 infections was investigated in Alberta, Canada, in 2014. Through epidemiological, microbiological and food safety investigations, contaminated pork products were confirmed as the cause of the illnesses.

Purpose: A multi-agency investigation was conducted to characterize the outbreak, identify and remove contaminated product from the market and recommend long-term prevention measures.

Methods: Public health investigators defined and interviewed cases, collected food specimens from case homes and conducted descriptive analyses to identify hypotheses. Federal food safety investigators led systematic traceback investigation of six hypothesized food sources. Federal, provincial and local food safety/public health agencies conducted inspections of implicated facilities. Food and environmental specimens were collected and analyzed at provincial and federal laboratories. *E. coli* O157:H7 isolates were identified using routine culture methods and were characterized by pulsed field gel electrophoresis (PFGE) and multi-locus variable number tandem repeat analysis (MLVA).

Results: This outbreak resulted in 119 illnesses including 22 (18.5%) hospitalizations and 6 cases of hemolytic uremic syndrome. Symptom onsets ranged from July 20 to October 6, 2014. The majority of cases reported consuming dishes containing pork at Asian-style restaurants in two geographically distinct cities during their exposure period. The outbreak strain, defined by sixteen PFGE patterns, was isolated from food and environmental specimens, including a pork carcass, whole pork cuts, finished products, and food and non-food contact surfaces.

Significance: Contaminated pork products produced and distributed in Alberta, Canada, were the source of the outbreak. Contamination of this novel vehicle persisted through cooking. The systematic approach used in the traceback investigation was effective in the absence of a leading hypothesis. The complexity of food distribution networks and impact of poor invoicing on ability to take action were highlighted.

Note: Preparation of the final outbreak investigation report is ongoing. If accepted, results reported in the abstract will be adjusted.

T11-06 Prevalence and Antimicrobial Resistance of *Salmonella* during Conventional and Organic Processing of Antibiotic-free Broilers

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❖ ***Developing Scientist Competitor***

Introduction: Product contamination by *Salmonella*, especially antimicrobial resistant strains, is a major food safety issue to the poultry industry.

Purpose: The purpose of this study was to determine the prevalence and antimicrobial resistance (AR) of *Salmonella* in carcass rinses and environmental samples obtained from a commercial plant that processes antibiotic-free broilers using conventional (CP) and organic (OP) processing methods.

Methods: During four separate processing days (two CP and two OP), five carcass rinses were collected from four processing steps (n = 20 for CP; n = 15 for OP): post evisceration (PE), post inside/outside bird washer (I/O), post water chill (PWC), and post air chill (PAC). Each day, 55 environmental samples were collected (49 for OP). Isolates of *Salmonella* were recovered according to the USDA-FSIS protocols and two isolates per positive sample (a total of 186 isolates) were analyzed for AR using the NARMS protocol.

Results: A downward trend in *Salmonella* prevalence was observed from PE to post chill for both CP (from 40% PE to 30% PWC and 20% PAC) and OP birds (from 100% PE to 70% PAC). However, for CP birds, an increase in prevalence was observed from PE (40%) to I/O (80%). One hundred thirty-nine isolates (74%) were resistant to at least one antimicrobial, and fourteen isolates (8%) were resistant to three or more. The most common resistance was to tetracycline (130 isolates, or 70%), and the second most common was streptomycin (50 isolates, or 27%). These data indicate a high prevalence of *Salmonella* throughout PE processing steps for both CP and OP birds, although a lower prevalence was observed post-chill.

Significance: The presence of several isolates resistant to commonly used antimicrobials highlights the need to control *Salmonella* populations during processing and demonstrates that multidrug resistant isolates can be present even in flocks raised without antibiotics.

T11-07 Characterizing the Risks Associated with Consumption of Raw Meat and Poultry Products

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Introduction: The U.S. Department of Agriculture, Food Safety and Inspection Service (FSIS) conducts foodborne illness investigations in response to reports of foodborne illness possibly associated with meat, poultry, or egg products. In 2014, a number of investigations related to the consumption of raw meat and poultry products prompted an epidemiological evaluation and subsequent review of policy.

Purpose: FSIS epidemiologists conducted this study to quantify and characterize foodborne illness investigations associated with uncooked meat and poultry products. The priorities were to identify risk factors, examine targets for risk communication, and explore policy options to positively impact public health.

Methods: Starting with the 2014 investigations, FSIS epidemiologists identified key words related to foodborne illness investigations involving consumption of uncooked meat and poultry products. Using these key words, the epidemiologists systematically searched FSIS investigation databases and primary investigator records. The investigations were characterized by year, pathogen, food product, reason for raw consumption, and location.

Results: Of the 19 foodborne illness investigations from 2005 through 2014 associated with consumption of raw meat or poultry products, 12 investigations were attributed to Shiga Toxin-producing *Escherichia coli,* six to *Salmonella,* and one to *Campylobacter jejuni.* Sixteen investigations involved beef products, two involved poultry, and one investigation involved both beef and lamb products. Investigations were grouped into broad categories: consumers tasting raw meat and poultry mixtures, “tiger meat” or “cannibal sandwich” consumption, and other cultural practices where products are customarily consumed raw.

Significance: Epidemiological analysis identified risk factors that could be conveyed in risk communication messages utilizing social media and traditional fact sheets. With this information, it may be necessary to re-examine policy, procedures, and research to prevent infections caused by consumption of raw meat and poultry products.

T11-08 Quantification of Six Major Non-O157 *Escherichia coli* Serogroups in Feces of Feedlot Cattle by Spiral Plating and Quantitative Real-time PCR Methods

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❖ ***Developing Scientist Competitor***

Introduction: Shiga Toxin-producing non-O157 *E. coli* of serogroups O26, O45, O103, O111, O121 and O145 are major foodborne pathogens. Cattle are a major reservoir and shed the organisms in the feces. Data on fecal concentration of non-O157 *E. coli* in cattle is limited because of lack of validated quantification methods.

Purpose: To evaluate the applicability of spiral plating method and multiplex real-time quantitative PCR (qPCR) to quantify six non-O157 *E. coli* serogroups in cattle feces.

Methods: Cattle fecal samples (n = 1,152) collected from eight commercial feedlots were suspended in *E. coli* broth. Diluted fecal suspensions were spiral plated onto selective chromogenic medium. Concentration (CFU/g) was determined by counting chromogenic colonies using a counting grid and testing ten randomly picked colonies individually by a multiplex conventional PCR targeting six serogroups and four virulence genes. Concentration of each serogroup was determined based on the proportion of colonies positive for the serogroup. DNA extracted from fecal suspensions was subjected to two qPCR assays: Assay 1 - O26, O103, and O111; Assay 2 - O45, O121, and O145. Fecal suspensions incubated at 40°C for 6 h were subjected to a culture method for the detection of six non-O157 *E. coli* serogroups.

Results: A total of 787 (68.3%) samples were positive by culture method for at least one of the six non-O157 *E. coli* serogroups, and of those 139 (17.7%) were quantifiable (> log 2.9) by spiral plating method and 493 (42.8%; > log 4) by qPCR assays. *E. coli* O103 was the predominant serogroup quantified by spiral plating method (8.7%) and qPCR (25.5%).

Significance: Spiral plating method can be used to determine the concentration of non-O157 *E. coli* serogroups and those that carry Shiga Toxin genes in cattle feces. The qPCR assays are more sensitive but allow quantification only at the serogroup level.

T11-09 Prevalence of *Listeria monocytogenes* in Slaughterhouses and Genetic Analysis among the Isolates Using Molecular Typing Methods

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Introduction: *Listeria monocytogenes* has been recognized as an important foodborne pathogen. The pathogen has been isolated from fresh meat and poultry, and it can be transmitted to processed meats. Hence, the prevalence of *L. monocytogenes* in slaughterhouses needs to be evaluated.

Purpose: The objective of this study was to investigate the prevalence of *L. monocytogenes* in slaughterhouses and to analyze the genetic correlations among the isolates.

Methods: One hundred feces samples from cecum and 103 surface samples from 43 cattle and 60 pig carcasses were collected from 7 slaughterhouses, and 11 human isolates were obtained from hospitals. *L. monocytogenes* were identified by amplifying *Listeria*-specific genes (*hly* and *prs*) by PCR and eventually confirmed by 16s rRNA sequencing. The presence of virulence genes such as *actA*, *inlA*, *inlB*, *plcB*, and *hlyA*were confirmed by PCR, and the serotypes were determined by multiplex-PCR and agglutination assay. Genetic correlations were also evaluated by the PFGE (pulsed-field gel electrophoresis) patterns formed by Asc I, and the PFGE patterns were compared among slaughter isolates and the slaughter isolate patterns were compared to the PFGE patterns from human isolates.

Results: Of 217 slaughterhouse samples, 12 samples (5.5 %; cattle: 4; pig: 8) were *L. monocytogenes* positive. All isolates had virulence-related genes such as *actA*, *inlA*, *inlB*, *plcB* and *hlyA,* and serotypes were generally1/2a, 1/2b, and 1/2c. Genetic correlations among slaughterhouse isolates mostly ranged 85% to 100%, and some of the isolates had genetic correlations with human isolates.

Significance: These results indicate that *L. monocytogenes* in slaughterhouse may be a potential source for human Listeriosis.

T11-10 Prevalence of *Campylobacter* in Integrated Mixed Crop-livestock Farms and Its Survival Ability in Post-harvest Products

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❖ ***Developing Scientist Competitor***

Introduction: Products from Mixed Crop-Livestock Farms (MCLFs) are commonly sold in farmers markets or road-side shops. Regulating the microbiological quality of these products is difficult because most small farm operations that commonly sell their products at farmers markets are exempt from USDA scrutiny under the Food Safety Modernization Act. *Campylobacter*, one of the most common causative agents of acute gastroenteritis, can contaminate these products.

Purpose: The purpose of this study was to investigate the ecological distribution of *Campylobacter* in MCLF environment and their products compared to conventional farms and their products at pre- and post-harvest level.

Methods: A total of 1287 samples at pre-harvest level and 1,281 samples at post-harvest level were collected from Maryland and the DC metropolitan area. *Campylobacter* was identified with biochemical tests and PCR. Antibiotic resistance of the isolates was determined with agar dilution method.

Results: A total of 222 *Campylobacter* isolates were identified. *Campylobacter* was detected in 11.16% and 3.6% of MCLFs and conventional farm samples, respectively, but none from produce-only farm samples. Antibiotic resistance was 2 - 3 times higher in conventional farms compared to MCLF isolates except tetracycline resistance, which was observed in 51.02% of MCLFs but none in conventional farm isolates. At post-harvest level, *Campylobacter* was isolated in 87.5%, 71.43% and 33.33% of whole chicken carcasses in farmers markets, organic and conventional retail supermarkets, respectively. Tetracycline resistance was found > 3 times higher in organic poultry meat isolates compared to conventional counterparts. No *Campylobacter* was detected in post-harvest produce samples due in part to the disability of *Campylobacter* to survive in absence of sufficient water activity.

Significance: This study reveals the higher risks of the MCLF environment and their products that are sold in retail and farmers markets. Higher tetracycline resistance in MCLF environment, farmers market and organic poultry carcass isolates warrants further investigation.

T11-11 Prevalence and Diversity of *Listeria* Species and *Listeria monocytogenes* in an Urban and Agricultural Source Watershed

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Introduction: Human Listeriosis cases are commonly associated with contaminated food, however, less is known about environmental sources of *Listeria monocytogenes*.

Purpose: This study examined the diversity of *Listeria* spp. and characterized *L. monocytogenes* isolates to determine if waterways could serve as a potential reservoir of this pathogen.

Methods: Water (n = 329) and point source (n = 30) samples from an urban and an agricultural source watershed in Nova Scotia (Canada) were collected over 18 months. After enrichment in LEB/Fraser broths, samples were plated on Palcam agar followed by speciation on RAPID'Lmono agar of

eight typical isolates from each positive sample. *L. monocytogenes* was also detected using Taqman quantitative PCR (qPCR). *L. monocytogenes* isolates were serogrouped, pulsotyped and compared with clinical isolates (n = 19) obtained from Nova Scotia during the study period.

Results: *Listeria* spp. was isolated from 72.1% of the rural water samples while 35.4% of the urban water samples tested positive for the genus. The *L. innocua* (56.3%) and *L. welshimeri* (43.1%) groups dominated in the rural and urban areas, respectively. Prevalence of *L. monocytogenes* was significantly ($P < 0.05$) higher when water samples were analyzed by qPCR with 67.8% positives vs. 31.6% by the culture method. *L. monocytogenes* co-occurred with other *Listeria* spp. in 45% of the culture-based positive samples, with serogroup IIa dominating (67.7%), followed by IVb (16.1%), IIb (15.8%) and IIc (0.4%). *L. monocytogenes* was detected by qPCR in 43, 100 and 0% of the cow feces (n = 24), raw sewage (n = 3) and septic tank (n = 3) samples, respectively. Absence of distinct clusters in a multidimensional scaling plot based on the AscI and ApaI pulsotypes of the environmental watershed and human *L. monocytogenes* isolates suggested the presence of shared genetic characteristics among the isolates.

Significance: *Listeria* spp. are common in rural and urban surface waters, which could potentially act as a reservoir for *L. monocytogenes* outbreak strains.

T11-12 Phenotypic and Genotypic Characterization of *Salmonella enterica* serovar Enteritidis Isolates Associated with a Mousse Cake-related Outbreak of Gastroenteritis in Ningbo, China

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Introduction: *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*Salmonella* Enteritidis) is a major pathogen responsible for causing the largest number of sporadic cases and outbreaks of human salmonellosis worldwide.

Purpose: In this study, an outbreak of *Salmonella* Enteritidis involving 112 cases in Ningbo, China was investigated with a combination of genotypic sub-typing methods and phenotypic analysis.

Methods: All isolates from this outbreak were sub-typed by Pulsed-Field Gel Electrophoresis (PFGE) and Multiple-Locus Variable number of tandem repeat Analysis (MLVA), to track and to confirm that this outbreak was associated with contaminated mousse cakes. Low temperature stress tolerance was determined and these isolates were tested for their antibiotic resistance, virulence genes and plasmid replicon types, to identify the pathogenic factors and potential hazards of this outbreak-associated *Salmonella* Enteritidis.

Results: The Pulsed-Field Gel Electrophoresis (PFGE) and Multiple Locus Variable number tandem repeat Analysis (MLVA) profiles showed that most of the outbreak clinical isolates (22/23) were indistinguishable from each other and were identical to the isolates obtained from implicated mousse cakes, demonstrating that this outbreak of gastroenteritis was caused by *Salmonella* Enteritidis-contaminated mousse cakes. Moreover, all isolates, irrespective of source, had an identical antibiotic susceptibility pattern. Five virulence-associated genes in *Salmonella* pathogenicity islands (SPI) and the plasmid-associated virulence genes *spvB/C* were present in both the food and clinical isolates.

Significance: All of these isolates can survive well under low temperature treatment, indicating that manufacturers of foodstuffs with raw ingredients (not subjected to thermal process) should use an effective approach to prevent or eliminate the microbial hazards to public health.

T12-01 Could the Chilling Process be Optimized to Decrease *Campylobacter* on Broiler Carcasses?

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Introduction: *Campylobacter* is one of the most common causes of diarrheal illness in many industrialized countries. Most cases of campylobacteriosis are associated with eating raw or undercooked poultry meat or from cross-contamination of other foods by these items. Risk assessment studies have indicated that campylobacteriosis associated with chicken products may be reduced 30 times by a 2-log reduction of *Campylobacter* concentration on carcasses.

Purpose: The objective of this work is to define chilling conditions allowing to reducing *Campylobacter* levels on poultry carcasses.

Methods: This study was set up to investigate four major parameters in the chilling process (temperature, duration, air velocity and initial concentration of *Campylobacter*) individually and in interaction on the behavior of *Campylobacter* using the Doehlert shell design. Three experimental designs were performed using a chilling prototype. Chicken legs were artificially contaminated before chilling. After chilling, *Campylobacter* counts were conducted in accordance with the ISO standard 10272-2.

Results: The maximum contamination reduction reached a rate of 63% corresponding to a reduction of 1.5 log CFU/g. Duration of chilling ($P = 0.04$) and initial concentration ($P = 0.03$) had significant effects: the reduction rate increased when the duration increased and the initial concentration decreased. An interaction between temperature and initial concentration had also a significant effect ($P = 0.01$) on *Campylobacter* contamination. If initial concentration was 10^3 CFU/g, temperature ($P = 0.0045$) had a significant effect: the reduction rate decreased when the temperature increased. Interaction between temperature and air velocity had also a significant effect ($P = 0.007$) on *Campylobacter* contamination.

Significance: The most important result is that carcasses presenting more than 10^3 CFU/g of *Campylobacter* would not be significantly decontaminated during the chilling process. Moreover this work shows that a chilling process with low temperature can significantly reduce the bacterial load on chicken carcasses presenting not more than 10^3 CFU/g.

T12-02 Survival of *Salmonella* on Raw Poultry Exposed to 10% Lemon Juice and Vinegar Washes

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Introduction: Formative research found a subset of consumers reported using acidic solutions (diluted lemon/lime juice or vinegar) to wash raw poultry. While studies have demonstrated the ineffectiveness of acidic marinades to eliminate pathogens from raw meat, the effect of acidic washes on raw poultry has not previously been examined.

Purpose: Determine the fate of *Salmonella enterica* 19214 inoculated onto raw poultry and subsequently exposed to acidic washes similar to those described by consumers. It was hypothesized that this would demonstrate the ineffectiveness of such washes to make raw poultry safe or pathogen free.

Methods: Chicken breasts were inoculated with approximately 5 x 10^8 CFU of *Salmonella enterica* 19214 (resistant to tetracycline, streptomycin and chloramphenicol). Inoculated breasts were then washed for 10 s, 30 s, 2 min or 5 min in control (tap water) or acidic (10% vinegar or 10% lemon juice) solutions. Following washing, *S. enterica* 19214 levels were determined both in the wash water and on the chicken using antibiotic media.

Results: Following washing with 10% vinegar (pH 3.1) for the 4 time periods, 1.7 x 10^7 - 3.3 x 10^7 CFU *S. enterica* were recovered from the chicken while 4.3 x 10^6 - 7.6 x 10^6 *S. enterica* were recovered from the vinegar wash solution. Following washing with 10% lemon juice (pH 2.6), 1.2 x 10^7 - 2.8 x 10^7 CFU were recovered from chicken, while 1.9 x 10^6 - 1.7 x 10^7 were recovered from the lemon juice wash itself. Results indicate that acid washes result in live *Salmonella* both in the wash as well as remaining on the chicken.

Significance: Washing raw poultry in a diluted lemon juice or vinegar solution is an inefficient method for removing *S. enterica* and results in live pathogens both in the wash water and on the chicken, increasing the risk for cross contamination.

T12-03 Preliminary Evaluation of Commercial Antimicrobials to Inhibit Growth of *Salmonella* on Chicken Liver

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❖ ***Developing Scientist Competitor***

Introduction: Antimicrobials like peracetic acid (PAA), chlorine (Cl), and Cetylpyridinium chloride (CPC) are commonly used in the poultry industry to inhibit growth of *Salmonella* on parts and carcasses but have not been tested on giblets.

Purpose: The objective of this study was to determine the effects of commercial antimicrobials on *Salmonella* inoculated chicken liver.

Methods: A cocktail (Kentucky, Typhimurium, and Enteritidis) of rifampicin resistant *Salmonella* at 10^6 CFU/ml was used to dip inoculate previously frozen chicken livers. Samples (n = 3; 1-lb samples) of inoculated livers were treated accordingly: 1) a negative control (NC), 2) positive control (CP), 3) immersion chilled positive control (CCP), 4) immersion chilled with 20 ppm CL, 5) immersion chilled and 10 s dip in 200 ppm PAA (PAA200), 6) immersion chiller with 24 ppm PAA (PAA24), 7) livers dipped in 0.3% CPC for 10 s, rinsed, and immersion chilled, 8) livers dipped in Citralow at 1.2 pH for 10 s then immersion chilled (LPH). Immersion-chilled livers were held in 4°C tap water for 45 min before collecting and rinsing with 100 ml buffer peptone water. The rinsate was serially diluted and plated on brilliant green agar with rifampicin. The twice replicated experiment was analyzed by ANOVA and Duncan's Multiple Range test.

Results: Results of CP indicated an initial load of 5.68 log CFU/ml which was significantly higher ($P < 0.05$) than other treatments (5.10 Cl , 5.09 PAA24, 4.98 CCP, and 4.80 PAA200). The LPH and the CPC were significantly lower (4.73 and 4.60; respectively) than the CP and other antimicrobials. In conclusion, all the antimicrobials tested reduced SC on liver tissue; however, CPC and Citrilow had the highest reduction.

Significance: These findings provide the industry information to select adequate antimicrobials to improve food safety. Further research is needed to understand the interactions of the antimicrobials with the liver tissue.

T12-04 Ability of Cecal Cultures to Inhibit Growth of *Salmonella* Typhimurium during Aerobic Incubation

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Introduction: Poultry can serve as reservoirs for *Salmonella*; however, chicks provided cultures of cecal bacteria develop resistance to colonization by *Salmonella*. Research has indicated that cecal bacteria metabolize organic acids to produce substances that inhibit *Salmonella* growth.

Purpose: The ability of cecal bacterial cultures to inhibit *Salmonella* growth when incubated aerobically in media supplemented with lactate and succinate was examined, and bacteria in the cultures were identified.

Methods: Cecal cultures were prepared by inoculating broth media with cecal contents of broilers and incubating media anaerobically at 35°C for 48 h. Media supplemented with 0, 50, 100, or 150 mM of lactate and succinate were inoculated with 0.1 ml of the cecal culture, 10^4 CFU/ml of *Salmonella* Typhimurium, or the cecal culture and *Salmonella*. Inoculated media were incubated aerobically at 35°C for 14 days, and aliquots of media were removed on Days 0, 7, and 14 to enumerate CFU/ml (n = 3).

Results: After 14 days of incubation of media inoculated with *Salmonella* only, there were significant ($P < 0.05$) increases in the number of *Salmonella* recovered from media supplemented with 0, 50, 100, or 150 mm of lactate and succinate. However, after incubation of media inoculated with *Salmonella* and cecal cultures, there was no significant increase in the number of *Salmonella* recovered from media that was not supplemented with lactate and succinate, while there were significant decreases in the number of *Salmonella* recovered from media supplemented with 50, 100, or 150 mm of lactate and succinate. *Enterococcus*, *Bacillus*, and *Proteus* were recovered from the media.

Significance: Findings indicate that cecal cultures incubated aerobically can metabolize lactate and succinate to produce substances that inhibit the growth of *Salmonella*. Identifying the bacteria that produce these substances will aid the formulation of defined probiotics that can reduce the colonization of broilers by *Salmonella*.

T12-05 Effect of Salt Concentrations on the High Pressure Inactivation of *Listeria monocytogenes*

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Introduction: National and international health agencies have recommended a significant reduction in daily intake of sodium by reducing the amount of NaCl in foods, specifically processed meats. One role of NaCl in processed meat is to decrease a_w, thereby inhibiting microbial growth. Thus, sodium reduction could increase the risk of survival and growth of spoilage and pathogenic microorganisms on these products. Therefore, alternate processing technologies to improve safety of sodium reduced foods are necessary.

Purpose: Examine the effect of salt types and concentrations on high pressure inactivation of *L. monocytogenes* in ground chicken.

Methods: Ground chicken formulated with three salt types (NaCl, KCl, $CaCl_2$), three salt concentrations (0, 1.5, 2.5%) and inoculated to 10^8 CFU/g with a four-strain cocktail of *L. monocytogenes* were subjected to four pressure treatments (0, 100, 300, 600 MPa) and two durations (60, 180 s) in an experiment with factorial design. Surviving cells were enumerated by plating on modified Oxford agar and analyzed by factorial ANOVA using mixed model.

Results: Pressure treatments at 100 or 300 MPa did not significantly ($P < 0.0001$) reduce *L. monocytogenes* populations. At 600 MPa, salt types, concentrations and duration of pressure treatment all had a significant effect on *L. monocytogenes* populations. Formulations with increasing concentrations of NaCl or KCl, showed significantly lower reduction in *L. monocytogenes*, while with $CaCl_2$ formulations, increase in concentration resulted in a significantly higher reduction. For instance, increase in NaCl concentration from 0 to 1.5 and 2.5% resulted in a reduction of 6.16, 2.52 and 1.34 log CFU/g, respectively, when exposed to 600 MPa for 60 s. In case of $CaCl_2$, increase from 0 to 1.5 and 2.5% resulted in a reduction of 6.16, 7.38 and 7.77 log CFU/g, respectively.

Significance: The results demonstrate that high pressure processing is a viable process to improve microbial safety of sodium reduced foods.

T12-06 Thermal Inactivation of *Salmonella* Species in Pork Burger Patties

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Introduction: While models have been developed to predict *E. coli* O157:H7 inactivation in beef patties at different internal endpoint cooking temperatures, analogous data and models for *Salmonella* inactivation in pork burger patties are not available. Such data would inform our understanding of the risk of salmonellosis from the consumption of pork burgers.

Purpose: Our aim was to quantify and model the inactivation of *Salmonella* in pork burger patties for different internal cooking temperatures and fat levels.

Methods: Pork mince with either a 'regular' or reduced fat content was inoculated with one of 3 *Salmonella* serovars (*Salmonella* 4,[5],12,i:-, *Salmonella* Senftenberg and *Salmonella* Typhimurium) and formed into 144 patties of ~100 g with ~8 cm diameter and ~2 cm thickness. Each patty was then cooked to one of 7 internal endpoint temperatures (45, 48, 51, 54, 57, 60, 63°C), determined by a thermocouple placed at the geometric center of the burger; uncooked patties were retained to estimate the initial concentration of *Salmonella*. *Salmonellae* were enumerated in cooked and raw burgers and a generalized linear model (log (*Salmonellae*) vs fat and endpoint internal temperature) was derived from these data.

Results: The concentration of *Salmonella* in the raw pork burger patties was 7.47 ± 0.29 log CFU/g. In pork mince with regular fat content (9.2%), the rate of *Salmonella* inactivation was estimated at -0.254 log CFU/g per 1°C increase above 40°C in end-point temperature, with a 'five-log' reduction in *Salmonella* estimated to occur at a geometric center temperature of 62.7°C. Higher fat content of the mince slightly enhanced *Salmonella* survival in less 'done' burgers although the significance of this observation is not strong ($P = 0.056$) and the difference is eliminated as the internal temperature approaches 63°C.

Significance: The data and model can be used to inform commercial pork burger cooking process validation and used to support quantitative risk assessments.

T12-07 Thermal Inactivation Kinetics of *Listeria monocytogenes* and *Vibrio parahaemolyticus* in Buffer and Mussels

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Introduction: *V. parahaemolyticus* is the leading shellfish-related pathogen in the U.S while *L. monocytogenes* is associated with cross-contamination of processed mussels. Mild thermal processes are a potential tool for pathogen reduction. To establish adequate and precise processing times and temperatures, thermal inactivation kinetics of target pathogens must be determined in actual foods.

Purpose: To establish and compare the thermal inactivation kinetics of *Listeria monocytogenes* and *Vibrio parahaemolyticus* in buffer and mussels.

Methods: Five-strain cocktails of each microorganism in phosphate buffered saline (PBS) (2 ml vials) or blended mussels in vacuum-sealed bags were treated at 56, 58 and 60°C (*Listeria*) or 46, 48 and 50°C (*Vibrio*) in a circulating water bath with temperatures monitored by thermocouples. At appropriate time intervals, samples were removed, placed in an ice bath, serially diluted and plated on tryptic soy yeast extract agar (*Listeria*) or marine agar (*Vibrio*). Colonies were enumerated after 24 h at 37°C (*Listeria*) or 48 h at 32°C (*Vibrio*). Each experiment was replicated thrice. D- and z-values were calculated using the first-order model.

Results: D-values for *L. monocytogenes* in PBS were 4.42 ± 0.16, 1.45 ± 0.22 and 0.58 ± 0.04 min at 56°C, 58°C and 60°C, respectively and for *V. parahaemolyticus* were 0.86 ± 0.05, 0.51 ± 0.06, 0.12 ± 0.02 min at 46°C, 48°C and 50°C, respectively. D-values were higher in mussels: 12.58 ± 2.80, 5.28 ± 0.65 and 1.46 ± 0.01 min at 56°C, 58°C and 60°C, respectively, for *L. monocytogenes* and 8.94 ± 0.68, 1.70 ± 0.08 and 0.88 ± 0.09 min at 46°C, 48°C and 50°C, respectively, for *V. parahaemolyticus*. No significant difference between z-values was observed in PBS, 4.52°C and 4.68°C, and mussels, 4.33°C and 3.98°C, for *L. monocytogenes* and *V. parahaemolyticus,* respectively.

Significance: *L. monocytogenes* had higher thermal resistance than *V. parahaemolyticus* in buffer and mussels at the tested temperatures. These data will aid the seafood industry in developing appropriate heating processes, such as steaming or microwaving, to eliminate foodborne pathogens.

T12-08 Thermal Inactivation of Hepatitis A Virus in Homogenized Clams (*Mercenaria mercenaria*)

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Introduction: Epidemiological evidence suggests that hepatitis A virus (HAV) is the most common pathogen transmitted to humans via bivalve mollusks such as clams, cockles, mussels, and oysters. Potential control of HAV-related foodborne illness outbreaks associated with clam consumption can be achieved by use of adequate thermal processes.

Purpose: The purpose of this study was (i) to characterize the thermal inactivation behavior of HAV in clams, (ii) to compare first-order and Weibull models in describing the data in terms of selected statistical parameters, and (iii) to calculate z-values and activation energy for each model.

Methods: Fresh clams were purchased from a local seafood market and homogenized using a Waring blender. Five milliliters of HAV stock (7.04 ± 1.34 log PFU/ml) were added to 25 g of homogenized clams and held at 4°C for 24 h. An aliquot (6 ml) of the homogenized inoculated clam sample was added to moisture barrier vacuum-sealed plastic bags. Thermal inactivation was performed for up to 6 min at 50, 56, 60, 65 and 72°C. Weibull and first-order models were compared to describe survivor curve kinetics and thermal death times. A comparison test (ANOVA, Post Hoc test) was used to analyze the effects of temperature and time on survival ratio.

Results: The D-values for HAV were in the range of 47.37 ± 1.23 to 1.55 ± 0.12 min for the first-order model and 64.43 ± 3.47 to 1.25 ± 0.45 min for the Weibull model at temperatures ranging from 50 to 72°C, respectively. The z-values determined for HAV in clams were 12.97 ± 0.59°C using the Weibull model and 14.83 ± 0.0.28°C using the first-order model. The calculated activation energies for the first-order model and the Weibull model were 145 and 170 kJ/mole, respectively.

Significance: The results of this study could be used to develop commercial or home thermal process recommendations for clams with negligible risk for the presence of infectious HAV.

T12-09 Reducing *Vibrio parahaemolyticus* in Oysters Using Natural and Environment Friendly Phytochemicals

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❖ ***Developing Scientist Competitor***

Introduction: *Vibrio parahaemolyticus* (Vph) is a halophilic bacterium implicated in numerous outbreaks of gastroenteritis involving raw or undercooked oysters, thereby underscoring the need for effective interventions for controlling the pathogen in oysters.

Purpose: This study investigated the efficacy of bactericidal concentrations of three GRAS-status phytochemicals, namely trans-cinnamaldehyde (5 mM), carvacrol (6 mM) and eugenol (18 mM) in decreasing Vph in oysters during depuration. Moreover, the efficacy of these phytochemicals in reducing Vph in shucked oysters was determined.

Methods: A two-isolate cocktail of Vph (~8 log CFU/ml) was added to artificial seawater tanks to facilitate pathogen accumulation in oysters. Infected oysters were transferred to depuration tanks containing water with or without the phytochemicals. Oysters collected at 0, 6, 12, 24 h of depuration were homogenized in neutralizing broth and enumerated on Thiosulfate Citrate Bile Salt Sucrose agar following serial dilutions. Additionally, shucked oysters were surface-inoculated with ~6 log CFU of Vph and subjected to air-drying for 30 min. The oyster meat was treated with the phytochemicals for 2 min, and surviving Vph was enumerated as before. Both experiments had duplicate samples and repeated three times.

Results: In the depuration experiment, the average accumulation of Vph was ~6 log CFU/oyster. Approximately 4.5 to 5.0 log CFU of Vph/oyster was recovered from control samples. However, the presence of phytochemicals in the depuration water decreased Vph by ~3.5 log CFU/oyster ($P < 0.05$). Additionally, trans-cinnamaldehyde and eugenol reduced Vph on shucked oyster meat to undetectable levels, whereas carvacrol decreased the pathogen by ~5.0 log CFU/oyster ($P < 0.05$).

Significance: The results suggest that trans-cinnamaldehyde, carvacrol and eugenol could potentially be used to reduce Vph in oysters. However, the effect of these phytochemicals on the sensory characteristics of oysters needs to determined. Additionally, follow up studies under commercial settings are necessary to validate these results.

T12-10 Microbiological Quality of Imported and Domestic Seafood

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Introduction: *Per capita* seafood consumption in the USA has increased during recent decades. Seafood importation and domestic aquaculture farming have also increased. Moreover, several recent outbreaks of human gastroenteritis have been linked to the consumption of contaminated seafood.

Purpose: The goal of this study was to investigate the microbiological quality of three seafood species obtained from four retail stores located on the Eastern Shore of Maryland.

Methods: A total of 468 frozen catfish, shrimp, and tilapia imported (60, 85, 84) and domestic (96, 71, 72) samples were analyzed for aerobic plate count (APC), total coliforms, *Escherichia coli* and three major foodborne pathogens (*Campylobacter jejuni, Salmonella,* and *Vibrio*) using standard methods. Presumptive isolates were confirmed using BAX polymerase Chain Reaction. Measurement outcomes were evaluated by one-way ANOVA or *t*-test when quantitative and by Fisher's exact test when qualitative.

Results: All samples were positive for APC and the average log CFU/g for APC ranged from 3.9 to 4.1 in the three types of seafood. Total coliforms were found in 41% of the samples and 8.8% were positive for *E. coli*. Approximately 3%, 27% and 8% of the samples were positive for *Campylobacter jejuni, Salmonella,* and *Vibrio*, respectively. With respect to comparisons between imported versus domestic, statistically significant differences in rate of detection of total coliforms were observed in shrimp but there were no statistically significant differences between average log CFU/g for APC or the rate of detection of *E. coli*. With respect to the major pathogens, there was a relatively large difference between *Salmonella* prevalence in imported versus domestic tilapia (33.3% of imported and 19.4% of domestic).

Significance: These findings suggest potential food safety hazards associated with imported and domestic seafood and warrant further large-scale studies. Attention should be paid to sanitary and spoilage condition of the seafood.

T12-11 *Vibrio parahaemolyticus* Levels in Atlantic Coast Shellfish

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Introduction: *Vibrio parahaemolyticus* (*Vp*) is the leading cause of bacterial infections from seafood consumption in the US. In 2012 and 2013, the number of *Vp* infections from consumption of Atlantic Coast shellfish more than doubled from the previous ten years' averages.

Purpose: This study determined the levels of total and pathogenic (*tdh*+ and/or *trh*+) *Vp* in shellfish harvested from Massachusetts, Connecticut, New York, and New Jersey and correlate those levels to water parameters.

Methods: Shellfish samples (oysters from all states and hard clams from CT and NY) were collected twice a month from May through September, 2014. Replicate shellfish samples were analyzed for each collection using a standard 3-tube MPN enrichment. Confirmation of total (*tlh*+) and pathogenic (*tdh*+ and/or *trh*+) *Vp*in enrichments was by real-time PCR.

Results: Of 112 shellfish samples, 109, 83, and 90 samples contained levels of total, *tdh*+, and *trh*+ Vp above the limit of detection (LOD; 0.03 MPN/g), respectively. In samples > LOD, mean levels were 1.5 log MPN/g (range: -1.44, 3.63), -0.46 log MPN/g (range: -1.82, 1.63), and -0.50 log MPN/g (range: -1.82, 1.63) for total, *tdh*+, and *trh*+ Vp, respectively. Mean water temperatures and salinities over the study were 20.4°C (range: 9.4 - 26.3) and 23.7 ppt (range: 10.1 - 30.0). Overall, there was a significant positive correlation between water temperature and total ($r = 0.72$, $P < 0.001$), *tdh*+ ($r = 0.25$, $P < 0.01$), and *trh*+ ($r = 0.21$, $P < 0.05$) Vp. A significant negative correlation was observed between salinity and total ($r = -0.29$, $P = < 0.01$) and *tdh*+ ($r = -0.30$, $P = < 0.01$) *Vp*, but not *trh*+ *Vp*.

Significance: As few illnesses were reported from these areas in 2014, these data provide valuable information on the baseline levels of total and pathogenic *Vp* that are generally not associated with illness. Additionally, the correlations with water parameters will allow refinement of the existing risk prediction model.

Poster Abstracts

P1-01 Broad Specific Antibodies to Norovirus

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Introduction: Notoriously difficult to contain, common, and associated with significant costs, norovirus outbreaks could be controlled, tracked, or even prevented by rapid, sensitive, and reliable diagnostic tests able to detect intact virus particles in clinical, food, or environmental samples.

Purpose: Our broadly specific, high affinity IgG anti-norovirus reagents will prove essential in the development of reliable and accurate immunoassays for norovirus diagnostics or detection.

Methods: For rapid and accurate diagnostics, the most useful antibodies would possess a high affinity for any human norovirus particle in its native state while not displaying crossreactivity to other closely related viruses. To this end, eight peptide antigens mimicking conserved GI and GII linear epitopes in the VP1 norovirus capsid protein were chosen based on their estimated accessibility to antibodies when displayed on the surface of intact icosahedral norovirus particles. Balb/c mouse polyclonal sera made in response to the peptides were tested for reactivity on GI.1, GI.2, GII.1, GII.2, GII.3, GII.4, GII.5, GII.6, GII.7 virus-like particles (VLP). Four out of the eight peptides generated serum antibodies with desirable reactivity patterns.

Results: Hyperimmune mice were sacrificed for hybridoma fusions, generating high affinity IgG anti-norovirus mAbs possessing the same reactivity patterns observed in serum assays. Extrapolating from the initial analysis of the reactivity patterns seen on the VLPs tested, we infer that certain key immunodominant epitopes within peptide antigens KLp5 and KLp6 generate antibodies which, when combined, appear capable of detecting potentially 100% of all GI norovirus strains. Similarly, key immunodominant epitopes within peptide antigens KLp7 and KLp8 generate antibodies with the potential to detect 80% of all GII noroviruses strains.

Significance: Such antibodies will be a welcome and useful reagent for the development of many different types of norovirus diagnostic immunoassays including dipstick, ELISA, IMS, and IMS-PCR.

P1-02 The Changing Face of Foodborne Illness Surveillance in the World of Culture-independent Diagnostic Testing

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Introduction: Culture-Independent Diagnostic Testing (CIDT) panels for gastrointestinal illnesses have changed the paradigm of foodborne illness surveillance. Since 1996, isolate-based pulsed-field gel electrophoresis reigned as the gold standard laboratory surveillance method for foodborne illness. In this new world of increasingly isolate-free diagnostic testing, public health surveillance must find a way to adapt.

Purpose: The purpose of this study was to evaluate the impact of culture-independent diagnostic testing in clinical and reference laboratories on public health laboratory surveillance.

Methods: One year of data submitted to the Tennessee Public Health Enteric Bacteriology Laboratory, comprised of 2,443 submissions of *Salmonella, Shigella*, Shiga Toxin-producing *E. coli* (STEC) and *Campylobacter*, was evaluated for submission type (isolate or specimen), organism requested, recovery rate and workload (picks or workups). Specifically, workload was evaluated by counting the number of isolates picked and worked up biochemically or with a molecular method to identify the organism.

Results: Culture-independent diagnostic testing comprised 25 - 45% of all *Salmonella, Shigella*, Shiga Toxin-producing *E. coli* and *Campylobacter* testing in all but the first month of the study. When comparing syndrome panels to EIA's, syndrome panel testing comprised over 80% of the initial clinical testing in the later nine of the twelve months. Recovery rates from CIDT submissions were 63% for *Salmonella*, 73% for *Shigella*, 54% for STEC and 40% for *Campylobacter*. There is a diminishing return on investment for continued workups, with a different level of effort required depending on the organism and recovery rate sought after.

Significance: These data suggest the wide variety of recovery rates and workups required to identify *Salmonella, Shigella*, Shiga Toxin-producing *E. coli* and *Campylobacter* from a CIDT specimen indicating a need for public health laboratory recommendations. This guidance will be valuable until public health can implement an isolate-free surveillance system for foodborne illness pathogens.

P1-03 Structure Sensitivity of Food Antimicrobials: Interactions between LAE and Methylparaben in Modified Model Systems

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Introduction: To date there is still a lack in suitable model systems that closely simulate food composition and account for the "structure sensitivity" of preservatives. Hence the efficacy of antimicrobial components such as lauric arginate (LAE), a cationic preservative which is approved for certain meat products, is often overestimated when evaluated in pure broth assays.

Purpose: We hypothesized that a suitable approach to improve the antimicrobial activity of LAE in complex food matrices is to combine this structure-sensitive component with a less structure-sensitive preservative that may attack different cellular levels and to examine the efficacy of this combination in a modified model system that contains bovine serum albumin (BSA) as an interacting agent.

Methods: Minimal lethal concentrations (MLC) of LAE (1 wt% LAE and 1 wt% Tween 80 in pure water; pH 6) against *Listeria innocua* LTH 3096 (inoculation level: 10^2 CFU/ml; nutrient broth) in the presence of 0 - 2 wt% BSA (pH 6) and/or 0 - 0.25 wt% methylparaben (pH 6) were determined using plate counts (detection limit: 10^2 CFU/ml; n = 4). Measurements were carried out over 12 days at 4°C to simulate typical storage conditions of meat products.

Results: While the presence of 2 wt% BSA significantly increased the MLC of LAE against *L. innocua* from 20 to 200/225 µg/ml, the antimicrobial activity of methylparaben was not strongly influenced by the protein content. In both assays (± BSA) 1000 µg/ml methylparaben caused a reduction in viable cell concentration of ca. 6 log. However, a slightly lower MLC of ~2500 µg/ml was detected in systems without BSA addition. Combinations were found to be synergistic and the presence of only 1000 µg/ml methylparaben remarkably reduced the concentration of LAE needed to cause total growth inhibition in model assays (± BSA).

Significance: The combination has great potential for meat applications and may thus be of interest to the meat industry.

P1-04 Report on the Field Testing and Evaluation of Air Liquide's CO2+ Process and Pathogen Reduction during a Meat Mixing Process

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Introduction: "CO2+" is a proprietary process developed by Air Liquide that allows for the introduction of a biocide (or other liquid food processing aid) directly into the stream of liquid CO_2 prior to bottom injection into a meat mixer.

Purpose: The CO2+ process delivers accurate doses of prescribed biocide such that a small volume of biocide (less than one liter per 1,000 pound batch size) can be evenly distributed throughout the entire mass of meat during the standard liquid CO_2 injection cycle. Air Liquide and the USDA-ARS Wyndmoor, PA, have collaborated in a Cooperative Research and Development Agreement to investigate the effectiveness of antimicrobial agents in the reduction of pathogenic bacteria such as *Salmonella, E. coli, Listeria*, and *Campylobacter* both in lab and full-scale process.

Methods: Antimicrobial agents were identified and tested in the lab for their effectiveness in reducing bacterial load. Six of these antimicrobials were selected for field testing in full-sized meat batches utilizing bottom injection liquid CO_2 chilling technology. Antimicrobial agents were introduced throughout the meat mix via Air Liquide's CO2+ process. Samples were collected and sent to an independent laboratory for processing.

Results: Testing showed that all of these antimicrobials were effective against *Salmonella* on poultry trim products. Results showed a pH reduction ranging from 5.8 to less than 4.0, and a reduction of up to 2 logs in APC and Enterobacteriaceae, depending on dosage and antimicrobial product.

Significance: New technology for pathogen reduction in ground and trim meat products.

P1-05 Evaluation of the 3M™ Molecular Detection System for the Detection of *Salmonella* in Feeds and Pet Foods

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Poster

Introduction: *Salmonella* is one of the most important foodborne zoonotic pathogens. Feedstuffs contaminated with *Salmonella* may cause a transient animal infection, and may also cause animal infections for an extended period. This can result in significant health and economic impact to both humans and animals.

Purpose: To evaluate the performance of the 3M Molecular Detection Assay *Salmonella* through comparison with a cultural method for artificially and naturally contaminated feed and pet food matrices, including: (1) Raw material (Soybean, Fish, Rice Bran Oil and Premix); (2) Canned; (3) Feed with supplements and vitamins.

Methods: All samples were weighed into 25-g portions and enriched in 225 ml 3M Buffered Peptone Water ISO. Sample enrichments were incubated at 37 ± 1°C for 18 - 24 h and then tested by two methods: (a) 3M Molecular Detection Assay *Salmonella* (including Matrix Control); and (b) cultural method ISO 6579. Presumptive positive molecular results were confirmed onto XLD and HE agars and typical colonies subjected to biochemical confirmation following ISO 6579 procedures.

Results: One hundred ninety-five (195) samples were tested in total; 58 samples were spiked with a low level (3 - 18 CFU) of *Salmonella* Typhimurium; 137 samples were tested with natural contamination. Overall, 3M Molecular Detection System showed 98.25% sensitivity, 98.28% specificity, 98.97% accuracy and no significant difference from the reference method using the Chi-square statistic.

Significance: For all feed and pet food matrices evaluated, the 3M™ Molecular Detection Assay *Salmonella* demonstrated comparable results to the reference methods for the rapid, automated detection of *Salmonella*.

P1-06 Rapid, Multiplexed Detection of Shiga Toxin-Producing *E. coli* Using a Liquid Crystal-based Immunoassay

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Introduction: Shiga Toxin-producing *E. coli* (STEC) are primary pathogens of concern in food products. Once ingested, STEC colonization may result in hemorrhagic colitis and hemolytic uremic syndrome, leading to severe illness and death. A rapid test is critical for both food producers and consumers for infection prevention.

Purpose: The study evaluated a rapid, multiplexed liquid crystal-based immunoassay as compared to the USDA and FDA reference methods for detection of the 7 USDA mandated STEC serotypes (O26, O45, O103, O111, O121, O145 and O157) in various food matrices.

Methods: The assay involved screening different antibody coated microsphere panels against pathogens and examining their detection in a liquid crystal media. Selectivity was validated using 54 pure culture STEC strains and 52 pure culture non-STEC strains. Finally, the assay was optimized for the raw ground beef, beef trim and spinach food matrices and tested in parallel against the USDA/FSIS-MLG 5B.05 and FDA BAM4A reference methods, using multiple STEC strains.

Results: Two assay panels of antibody were designed to detect the 7 STEC serotypes. The liquid crystal-based assay selectively detected 98% (53 of 54) of the STEC while excluding 96% (50 of 52) of the non-STEC strains. Assay detection of 1 - 4 CFU of an individual STEC strain in 325 g of raw ground beef or trim, or 200 g of spinach, was statistically equivalent (95% CI) to the respective USDA and FDA reference methods, after a pre-enrichment for as short as 9.5 h (N = 30 for each matrix, dPOD of -0.10 with 95% CI (-0.33, 0.14) for ground beef and trim, and dPOD of 0.13 (-0.11, 0.36) for spinach).

Significance: This liquid crystal-based immunoassay provides a major time reduction from sample to detection compared to the reference methods, and appreciably reduces the risk for exposure to STEC contaminated food.

P1-07 Performance of the 3M™ Molecular Detection Assay *Listeria monocytogenes* as Compared to the Canadian Reference Method MFHPB-30

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Introduction: In recent years, 132 cases of Listeriosis on average were reported annually in Canada. *Listeria monocytogenes* is usually associated with environmental contamination within food production facilities and is a significant food safety concern and cost. The 3M™ Molecular Detection Assay *Listeria monocytogenes* is used with the 3M™ Molecular Detection System for the rapid and specific detection of *Listeria monocytogenes* in food and environmental samples. The 3M™ Molecular Detection Assays employs isothermal amplification of nucleic acid sequences to achieve specificity, efficiency and rapidity, utilizing bioluminescence to detect the amplification of the target.

Purpose: The objective of this study was to evaluate the performance of the 3M™ Molecular Detection Assay *Listeria monocytogenes* against the Compendium of Analytical Methods MFHPB-30 in a variety of food matrices for the inclusion in the Compendium of Analytical Methods.

Methods: The 3M™ method and comparative reference method (MFHPB-30) were analyzed by testing 5 food categories with 3 matrices per category (RTE Meat and Poultry, Fish and Seafood, Dairy, Fruit and Vegetable Products, Raw Poultry). Three separate inoculum levels were used; 20 samples at 1 - 5 CFU/25 g, 20 samples at approximately 1 log CFU/25 g higher, and 5 negative controls.

Results: Statistical analysis was conducted using the Probability of Detection statistical mode. This showed a relative sensitivity of 99.5%, relative specificity of 98.2%, false positive rate of 1.8%, and false negative rate of 0.5% for a total test efficacy of 99.0%. This exceeded the criteria outlined by the Health Canada MMC. Level of Detection results showed a range of 0.5 - 6.375 MPN/25 g.

Significance: The 3M™ Molecular Detection Assay *Listeria monocytogenes* showed excellent performance and exceeded the MMC requirements. This new method offers the capability of detecting *Listeria monocytogenes* in foods after only 24 - 48 hours of incubation, thereby significantly reducing presumptive reporting times over the reference method.

P1-08 Relative Limit of Detection Comparison for Two Rapid Detection Methods to Accurately Detect *Listeria monocytogenes* Analyte in Hot Dog and Deli Turkey Matrices

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Introduction: The presence of *Listeria monocytogenes* in Ready-to-Eat (RTE) foods is a significant public health risk. Detection methods with enhanced sensitivity may reduce false negatives and aid in preventing contaminated product entering the food supply.

Purpose: To compare the Atlas® *Listeria monocytogenes* LmG2 Detection Assay to the Dupont™ BAX® System PCR Assay for detection of *Listeria monocytogenes* in RTE meat matrices using a relative Limit of Detection (rLOD) approach.

Methods: Hot dog and deli turkey were bulk batch inoculated targeting 4 - 8 CFU/sample, held at 4°C for 48 - 72 h and then portioned into twenty replicate samples per matrix and method. Five un-inoculated samples were included for each method. For the Atlas®, 1:9 and 1:4 enrichment ratios were prepared in PALCAM broth + 0.02 g/l nalidixic acid and incubated at 35 ± 2°C for 24 h (25 g hot dogs) or 48 h (125 g deli turkey), respectively. For the BAX, a 1:9 dilution was prepared in UVM broth for both matrices, and incubated at 30 ± 2°C for 24 h (25 g hot dogs) or 26 h (125 g deli turkey), respectively, prior to secondary enrichment in MOPS-BLEB for 24 h prior to analysis. End-point enriched samples were 10-fold serially diluted and subjected to testing by each respective method. Culture based MPN was utilized to estimate starting concentrations.

Results: For hot dogs, the Atlas detected 20/20 positives for the 1:1000 dilution, whereas BAX detected 7/20. The rLOD value was 14.383, indicating significantly different method sensitivities ($P < 0.001$). Similarly, for deli turkey at the 1:10,000 dilution, Atlas detected 14/20 positives and BAX 0/20. The rLOD value was 112.441, indicating significantly different method sensitivities ($P < 0.001$). Un-inoculated replicates were negative for both methods. Between test Chi-square analyses at each dilution further supported rLOD analyses.

Significance: Rapid methods with enhanced sensitivity may provide more accurate test results for products with extremely low level contamination.

P1-09 Evaluation of New Molecular Methods for the Detection of *Listeria,* Including *L. monocytogenes* and Newly Recognized *Listeria* Species

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Introduction: Improved rapid methods for foodborne pathogen detection are an ongoing need. Two new molecular methods for the detection of *Listeria monocytogenes* and *Listeria* spp. have been developed based on modification of existing products. The new methods yield faster sample-to-answer times and improved ease-of-use compared to the current methods.

Purpose: The aim of this study was to evaluate performance of the new methods, specifically for inclusivity, exclusivity and for the detection of *Listeria monocytogenes* and *Listeria* spp. in environmental samples.

Methods: Pure cultures of known identity, both inclusive and exclusive strains from diverse sources were tested using the new molecular methods after growth in demi-Fraser broth or non-selective broth. Paired food-plant environmental sponge samples were collected using a sampling device wetted with Dey-Engley Neutralizing Broth then tested using the traditional FDA-BAM method and the new molecular methods. Presumptive results from the new methods were confirmed using microscopic, biochemical and molecular characterization according to the FDA-BAM method, followed by 16S sequencing.

Results: Of the forty-four pure cultures, all were correctly identified using the new methods. Among the ninety environmental sample locations analyzed, three positive samples were identified by both the new molecular method and the FDA-BAM method. Using 16S sequencing, two of these isolates were identified as either *Listeria riparia* or *Listeria booriae*, two newly recognized *Listeria* species that cannot be differentiated based on the partial 16S rDNA sequence amplified.

Significance: This study demonstrates that recently reported and newly recognized *Listeria* species can be found in food processing environments with advanced detection and characterization methods. In addition, this study shows that the new detection method is reliable, accurate and offers advantages to the end user, including a faster time-to-result compared to the cultural method.

P1-12 Comparison of 3M™ Molecular Detection Assay (MDA) *Listeria monocytogenes* with ISO Standard Method for Rapid Detection of *L. monocytogenes* Artificially Inoculated on Food Contact Surfaces with or without Organic Load

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Introduction: Food contact surfaces contaminated with *L. monocytogenes* is an important issue for Ready-to-Eat food manufacturers, since surfaces could be a major source of reoccurrence of Listeriosis. It is crucial to implement rapid detection methods at the factory to control such Listeriosis. A commercial rapid detection system based on loop-mediated isothermal amplification coupled with bioluminescence has been developed, and requires broader validation with food contact surfaces in detecting *L. monocytogenes*.

Purpose: This study aims to evaluate the performance of 3M Molecular Detection System (MDS) in comparison with ISO method for detection of *L. monocytogenes* on stainless steel and polyethylene surfaces artificially inoculated at low levels with or without organic load.

Methods: The 3-strain cocktail of *L. monocytogenes* was inoculated on stainless steel and polyethylene surfaces (10 x 10 cm) to achieve 10^0, 10^1 and 10^2 CFU/100 cm^2. To prepare the surface with organic load, fresh raw salmon was homogenized with peptone water and the suspension was spread evenly across the surface prior to inoculation. A total of 120 samples were subjected to both MDS and ISO methods.

Results: The MDS method performed equally effectively compared with ISO method at inoculum levels of 10^0 and 10^2/100 cm^2, on both surfaces. Reported sensitivity and specificity for inoculum at 10^1 CFU/100 cm^2 were more than 80%, except for stainless steel surfaces without organic load that had a reported sensitivity and specificity of 75% and 92%, respectively.

Significance: This study demonstrates that MDS can provide cost effective, rapid and accurate detection of *L. monocytogenes* at low inoculum levels on stainless steel and polyethylene surfaces in 26 - 30 hours with a single enrichment.

P1-13 Volatiles Compounds Suitable of Rapid Detection as Quality Indicators of Aerobically Stored Fresh Salmon

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Introduction: Spoilage is a process, in which food deteriorates to the point, when the quality of the product is unacceptable for consumers due to the microbiological, chemical or physical changes. The activity of microorganisms leads to the synthesis of volatile organic compounds (VOCs), which might be considered as the indicators of spoilage and causing off-odors in highly perishable foods, like raw fish.

Purpose: The objectives of this study were to select specific spoilage organism (SSO) and to identify VOCs as possible indicators of freshness of raw salmon fillets stored aerobically at different temperatures.

Methods: The growth of total viable count (TVC), psychrotrophs, *Pseudomonas* spp., coliforms, lactic acid bacteria (LAB), *Brochothrix thermosphacta*, H_2S producing bacteria, yeasts and molds were investigated. The VOCs profiles were determined using HS-SPME-GC-MS. Weighted samples of salmon fillets were stored at 4 and 10°C for 14 days and 21°C for 3 days, respectively. SSO and volatile indicators were identified with use of modeling, statistical and multivariate analysis.

Results: *Shewanella putrefaciens*-like bacteria (H_2S producing bacteria) can be considered as SSO for aerobically stored salmon fillets, as it was characterized by the highest maximum growth rate at 4°C. However, spoiled salmon samples were dominated by: psychrotrophs, probably with *Pseudomonas* spp., as major representative, followed by *B. thermospacta* and H_2S producing bacteria. The predicted shelf lives of salmon fillets, at TVC = 7.0 CFU/g, were 3.02, 1.82 and 0.60 days for storage at 4, 10 and 21°C, respectively. While, the sensory rejection point was observed at TVC = 7.5 CFU/g (on 4^{th}day at 4°C). Regardless of storage temperature, significant ($P < 0.05$) and high correlations between microbial counts and selected VOCs were observed for trimethylamine, ethanol, 3-methyl-1-butanol, acetoin, acetic acid and 2,3-butanediol.

Significance: This study showed that the identified VOCs might be suitable as indicators of rapid spoilage detection for aerobically stored fresh salmon.

P1-14 Cooked Beef Food Matrix Validation Study Using Real-time PCR BAX Method for Testing *E. coli* O157:H7

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❖ ***Developing Scientist Competitor***

Introduction: Humans can become infected with *E. coli* O157:H7 through the consumption of contaminated food and develop hemolytic uremic syndrome (HUS) which is a serious illness that can result in renal failure, seizures, paralysis and death. In 1994 FSIS declared *E. coli* O157:H7 an adulterant if found in raw beef food products and since then the BAX® System Real-Time PCR Assay has been validated for the detection of *E. coli* O157:H7 in raw beef products.

Purpose: The purpose of this study was to discover if the PCR Assay validated for *E. coli* O157:H7 could be used to detect *E. coli* O157:H7 cells at a concentration of 2 CFU/25g within cooked ground beef patties also subjected to freezing temperatures.

Methods: This study inoculated cooked ground beef patties with an estimated 2 CFU of *E. coli* O157:H7 per sample and then froze the samples to -15°C for a minimum of 24 h. The samples were then enriched with pre-warmed MP Media and incubated at 42°C. After the desired incubation time points of 8 h, 10 h and 24 h were met, 5ml aliquots were removed from the samples and tested per the PCR Assay method.

Results: This study found that the PCR Assay for *E. coli* O157:H7 can be used to detect *E. coli* O157:H7 in cooked ground beef. The results from this study also indicate the matrix required incubation for a time period of greater than 8 h or for a minimum of 10 h to obtain a positive result in 100% of the inoculated samples using the PCR Assay.

Significance: Cooked ground beef was previously thought to be protected from *E. coli* O157:H7 adulteration but could cause future outbreaks especially if the ground beef product is undercooked or if cross contamination occurs.

P1-15 Sensitivity, Specificity, and Robustness of a Monoclonal Antibody-based Direct Sandwich Enzyme-linked Immunosorbent Assay for Cashew Detection

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❖ ***Developing Scientist Competitor***

Introduction: Globally, cashew nut is the second largest produced tree nut following coconut. Although safely enjoyed by most, upon exposure, sensitive individuals experience adverse reactions to cashew.

Purpose: The objective of this study was to evaluate a new commercially available monoclonal antibody-based direct sandwich enzyme-linked immunosorbent assay (ELISA) kit for cashew detection.

Methods: Cashew nut seeds and food ingredients were purchased from local grocery stores and the ELISA kits were purchased from BioFront Technologies (Tallahassee, FL). Whole cashew nut seeds were subjected to autoclaving (121°C, 15 psi, 15, 30 min), blanching (100°C, 5, 10 min), frying (191°C, 1 min), microwaving (500, 1000 W, 3 min), and roasting (140°C, 30 min; 168, 177°C, 12 min). Soluble proteins were extracted in the provided extraction buffer (flour-to-buffer ratio 1:10 w/v) at 60°C for 10 min and quantified by the Bradford method. Samples were subsequently analyzed by ELISA.

Results: The ELISA was sensitive (limit of detection: 0.07 ± 0.02 ppm, linear detection range: 0.2 - 20.0 ppm), reproducible (intra- and inter-assay variability < 15% CV), and rapid (post-extraction testing time: ~40 min). The target antigen was detected in the processed samples. Compared to the unprocessed control, percentage cashew recovery was 89.0 - 99.2% for all processed cashew nuts except those autoclaved for 30 min (44.2 ± 0.9% recovery, $P \leq 0.05$). At 10,000 ppm, no cross-reactivity was observed in 120 tested food matrices except pistachio seeds (signal equivalent to 0.7 ± 0.1 ppm cashew). The signal registered by pistachio was eliminated at 1000 ppm.

Significance: The results suggest that under the test conditions, the ELISA was specific, sensitive, and robust for cashew detection.

P1-16 Study to Demonstrate the Detection of Cross Contamination of Surfaces with Raw Chicken Juice Using Simple Acid Phosphatase Detection Device

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Introduction: The use of a rapid detection method for cross contamination has been highlighted as a possible method for the reduction of *Campylobacter* infections from store purchased chickens. These potential sources of *Campylobacter* require an easy method to quickly decide if a surface has been contaminated or requires better sanitation.

Purpose: This study highlights a simple detection device (Hygiena CrossCheck) that will detect raw chicken juice dried onto a surface and that has been transferred multiple times through contact and still remains detectable. This viability of contaminant through a hand contact environment highlights the need for rapid detection in these situations.

Methods: The juice from grocery store packaged raw chicken was decanted. The chicken juice was then inoculated onto a surface and allowed to dry overnight at ambient temperature. The chicken juice was also rubbed onto gloved hands which then touched 20 sterile squares of stainless steel in sequence. Using the CrossCheck device each square was tested in turn. The assay consists of swabbing the surface and then activating the device. After 5 min incubation at 37°C the level of acid phosphatase is indicated by an RLU level in the luminometer. The assay is extremely sensitive and will detect acid phosphatase down to nanogram levels.

Results: The acid phosphatase in the raw chicken from the initial drying was 34,000,000 RLUs; the background level of acid phosphatase was 24,000. This initial signal dropped with sequential transfers until ACP through the gloved hands became insignificant, i.e., less than the background after 7 transfers on to sterile squares.

Significance: The use of rapid acid phosphatase detection is a useful tool in the detection of cross contamination and is suggested as a proxy measurement to aid reduction of *Campylobacter* contamination.

P1-17 Rapid Detection of Enterobacteriaceae from Food Preparation Surfaces Using Simple Bioluminogenic Device

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Introduction: The rapid detection of Enterobacteriaceae from food preparation surfaces is particularly important to the cleanliness of food manufacturing facilities.

Purpose: The Hygiena MicroSnap Surface Express system allows collection, detection and enumeration of Enterobacteriaceae from surfaces in the same working shift.

Methods: MicroSnap Surface Express is an all-in-one device containing a surface swab and ready-to-use patented media. Upon sample collection, the entire device is incubated from 2 to 6 h to detect as low as < 10 CFU/swab. The device is then activated. The reagent bathes the growing sample and light is produced proportionally to the level of bacteria present (CFU). The system is self-sterilizing; after activation the device and bacteria collected are rendered non-viable. In this study, *Salmonella enterica* ATCC 13076 and *Escherichia coli* ATCC 8739 were grown overnight and diluted in diluent, inoculated onto the swab and incubated at 37°C for 1, 2, 3, 4, 5, 6, 7 and 8 hours. The devices were activated at each time point and measured in Hygiena SystemSURE Plus and EnSURE luminometers. Reference counts were run on traditional pour plates and read at 24 h.

Results: *S. enterica* and *E. coli* started to be detected in 2 h with confirmed detection in both luminometers in 4 h. The RLUs S/N which uses the non-inoculated background as baseline were as follows: for *E. coli* and *S. enterica* the S/N at each time were as follows 1 hour (1 and 1), 2 hours (2 and 4) and 3 hours (9 and 30).

Significance: This rapid method will allow microbiological cleanliness to be run by more food manufacturers in-house allowing better surveillance and control of surface borne cross-contaminating pathogenic Enterobacteriaceae.

P1-18 Rapid and Simple Molecular Workflow for the Detection of *Listeria* in Food and Environmental Samples

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Introduction: Thermo Scientific™ SureTect™ assays are PCR based tests for the detection of pathogens in food, animal feed and environmental samples. SureTect *Listeria* assays and Applied Biosystems™ 7500 Fast Real-Time PCR instrument were combined to bring the SureTect system´s streamlined workflow on to a high throughput 96-well PCR platform.

Purpose: The purpose of the study was to verify the performance of the SureTect *Listeria* assays on the Applied Biosystems 7500 Fast PCR platform with pasteurized whole milk, cold smoked salmon, roast beef, and stainless steel.

Methods: Hybrid *Listeria monocytogenes* and *Listeria* species assays were compared to a slightly amended version of the ISO 11290-1:1996 including Amendment 1:2004 reference method. The reference method's primary enrichment was conducted in 24LEB followed by secondary enrichment in Fraser Broth. Food matrices were spiked with *L. monocytogenes* or *L. innocua* isolates. Isolates for roast beef and whole milk were heat stressed prior to spiking. Ten replicate bags were set up for each matrix. All matrices were held at +4°C for 4 days prior to the enrichment. The spiking level was between 0.5 - 0.83 CFU/25 g food. The stainless steel was spiked with 24 CFU/plate to account for the *Listeria* die off on the surface. Samples were tested after a 22-hour enrichment step. PCR results were confirmed by plating on Thermo Scientific™ *Brilliance*™ *Listeria* chromogenic plates followed by identification of *Listeria* species using Thermo Scientific™ Microbact™ *Listeria* 12L kit.

Results: The hybrid *Listeria* methods were comparable to the amended reference method for the four matrices following an enrichment time of 22 h.

Significance: The study provided the evidence that the high throughput SureTect methods offer rapid, easy to use and reliable workflow for the detection of *Listeria* in food and environmental samples.

P1-19 Performances Assessment of the 3M^{TM} Molecular Detection Assay *Salmonella* Kit According to the ISO 16140 Standard for *Salmonella* Species Detection in Spices, Aromatic Herbs, Concentrates, Culinary Products, Cocoa and Milk Products

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Introduction: The 3M Molecular Detection Assay *Salmonella* kit uses isothermal amplification of specific DNA target sequences. The amplification is detected by bioluminescence.

Purpose: An independent study was conducted at ADRIA as part of the NF VALIDATION approval process, in order to extend the scope of the ISO 16140 validation study to spices and aromatic herbs, concentrates and culinary products, cocoa and cocoa based products, and milk powders. The ISO 6579 standard was used as reference method.

Methods: The 3M Molecular Detection Assay *Salmonella* test protocol includes a single enrichment step in Buffered Peptone Water (ISO), and specific enrichment procedures are dedicated to tested matrices. Two incubation temperatures are validated depending on the tested food categories (37°C and 41.5°C). After lysis, DNA amplification is performed in the 3M Molecular Detection Instrument.

Results: Two hundred eighty-nine samples were analyzed for relative accuracy, sensitivity and specificity study. The results demonstrate equivalent performances between the 3M Molecular Detection Assay *Salmonella* method and the ISO 6579 methods. Depending on the tested (matrix/strain) pairs, the relative detection limits of the 3M Molecular Detection Assay *Salmonella* method vary from 0.3 to 3.6 CFU/25 g for the alternative method, those of the ISO standard vary from 0.4 to 2.8 CFU/25 g.

Significance: The alternative method is a reliable method for *Salmonella* spp. detection in spices and aromatic herbs, concentrates and culinary products, cocoa and cocoa based products, and milk powders. The 3M Molecular Detection Assay *Salmonella* kit offers important economic savings by reducing time to result and handling time.

P1-20 Comparison of Two Methods for the Isolation of Shiga Toxin-Producing *Escherichia coli* O157 from Cattle Feces at Slaughter

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Introduction: Accurately determining the prevalence of *E. coli* O157 in beef cattle is largely dependent on the performance of detection and isolation procedures.

Purpose: In this study, we compared two methods of isolating *E. coli* O157 to determine which was the most suitable for isolating *E. coli* O157 from cattle fecal enrichments.

Methods: A total of 1500 cattle fecal samples were tested. For Method A, a 25-g portion of cattle feces was enriched in BAX MP media (DuPont) for 18 - 20 h. Samples were then screened for *E. coli* O157 using the BAX real-time PCR assay (DuPont) and confirmation was performed on PCR positive broths using immunomagentic separation (IMS). Method B involved enriching a 25-g portion of feces in Buffered Peptone Water for 6 h prior to performing IMS. For each method, cefixime-tellurite sorbitol MacConkey agar was used as the primary culture media, though IMS beads from Method A enrichments were also plated onto three additional media.

Results: *E. coli* O157 was isolated from 96 of 1500 samples, with 37 (2.5%) samples positive using Method A and 90 (6.0%) samples positive using Method B. Overall, 6 (6.3%) samples were exclusively positive using Method A, 31 (32.3%) samples were positive by both methods and 59 (61.5%) samples were solely positive by Method B. Many of the samples that yielded an isolate using Method B did not proceed to confirmation in Method A, as the PCR screen returned a negative result for O157.

Significance: Method B proved superior to Method A at isolating *E. coli* O157 from cattle feces. However, as the PCR screen test used in Method A has been validated for beef trim but not fecal enrichments, further work is required to understand the factors which lead to screen test negative results in complex fecal enrichments.

P1-21 Comparative Evaluation of the bioMérieux VIDAS® UP *Listeria* LPT™ and Romer Labs® SDIX RapidChek® *Listeria* F.A.S.T.™ Assays with Naturally-contaminated Environmental Sponge Samples

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Introduction: The bioMérieux VIDAS® UP *Listeria* LPT™ assay (LPT) is a rapid phage-ligand based automated immunoassay. The Romer Labs® SDIX RapidChek® *Listeria* F.A.S.T. assay (SDIX) is a rapid antibody-antigen based lateral flow immunoassay. Both assays are validated for the detection of *Listeria* spp. in environmental sponge samples.

Purpose: The purpose of this study was to compare the performance of LPT and SDIX by testing both naturally-contaminated environmental sponge samples collected from food processing facilities and a *Listeria* spp. pure culture inclusivity panel.

Methods: Five food processing facilities were enrolled for the study. Ten Zone 3 sites (e.g., drains, floors, walls) were identified in each facility. Two environmental sponge samples were collected from adjacent areas at each site, and were randomly assigned to either LPT or SDIX. Samples were enriched for 24 h, then screened, and culturally confirmed after an additional 24-h incubation period. False positive and false negative rates were calculated. The inclusivity panel consisted of 50 *Listeria* spp. pure cultures. Cultures were screened with each assay, and percentages of positives were calculated.

Results: In testing the naturally-contaminated environmental sponge samples, SDIX had a higher false positive rate (3.70%) and false negative rate (21.74%) than LPT (0.00% and 4.35%, respectively). LPT also identified 100.00% of the inclusivity panel cultures as positive, while SDIX only identified 96.00% as positive. The two cultures producing negative results were *Listeria grayi* cultures.

Significance: In this comparative study the LPT assay outperformed the SDIX assay when testing both a set of naturally-contaminated environmental sponge samples and an inclusivity panel of *Listeria* spp. pure cultures.

P1-22 Validation of RapidChek® NextDay™ *Listeria* Species Test System for the Detection of *Listeria* spp. in Ready-to-Eat (RTE) Foods

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Introduction: The USDA-FSIS *Listeria* Control Program requires that some RTE food processing facilities withhold the release of product pending *Listeria* test results. In order to reduce the cost of holding product, we developed a rapid, 27 - 48 h, lateral flow test strip-based method for the detection of *Listeria* spp. in several RTE food types.

Purpose: The purpose of this study was to validate under AOAC-Research Institute guidelines, a new lateral flow test strip-based method for the detection of *Listeria* spp. in RTE foods.

Methods: For each food type, a set of 5 non-spiked, 5 high-level spiked, and 20 low-level spiked samples were tested by the new lateral flow test strip method at 27 and 48 h and the respective cultural reference method. Low level spike levels ranged from 0.5 to 1.575 CFU per analytical unit (25 or 125 g).

Results: A total of 260 low-level spiked RTE food samples were tested by both methods. The number of confirmed positives for the RapidChek method tested at 27 h and 48 h, was 160 and 169, respectively, and 158 for the cultural reference method. All non-spiked samples were negative for *Listeria* spp. by all methods. The overall Chi-square was 0.032 (P = 0.858) and 0.995 (P = 0.316) after a 27 h and 48 h enrichment, respectively. Probability of Detection (POD) analysis showed no significant differences between the test method tested at either 27 or 48 h and the respective cultural reference method. These results indicated that the test method was equivalent in performance to the cultural reference method at both enrichment times.

Significance: The new test method should provide the end user with a rapid and reliable tool for monitoring and controlling *Listeria* species in Ready-to-Eat foods and minimize the introduction of *Listeria monocytogenes*-contaminated food products into commerce.

P1-23 A New Immunoassay for the Detection of Staphylococcal Enterotoxins G (SEG), H (SEH) and I (SEI)

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Introduction: Staphylococcal food poisoning is one of the most common foodborne diseases resulting from the ingestion of staphylococcal enterotoxins (SEs) preformed in foods by enterotoxigenic strains of coagulase-positive staphylococci, mainly *Staphylococcus aureus*. Immunological methods are considered as the most practical and powerful methods for the analysis of SEs in foods because they give sensitive and reliable results. To date, the commercially available test kits are able to detect SEA to SEE, but not SEG, SEH and SEI.

Purpose: The goal of this study was to develop and briefly evaluate an immunoassay for the screening of SEG, SEH and SEI in food products.

Methods: Using recombinant SEG, SEH and SEI as immunogens, polyclonal antibodies were raised in rabbit and then immunopurified and conjugated to alkaline phosphatase. The purified antibodies and the conjugates were used to develop a two-step sandwich ELISA assay adapted to the VIDAS automated system.

Results: When tested individually, the antibodies showed no significant cross-reactivity with the other non-related toxins (for example, SEA, SEB, SEC, SED, SEE, SEH, SEI did not cross-react with anti-SEG antibodies) tested at a concentration of 100 ng/ml. In a multiplex assay, the limit of detection (LOD) using pure toxins was < 0.1 ng/ml. The ELISA detected SEG, SEH and SEI in two artificially contaminated food products (raw milk cheese and meat product) in a dose-dependent manner, with an LOD <0.4 ng of toxin per g of products when the food extracts were pre-concentrated with polyethylene glycol 20,000.

Significance: The new ELISA in this study showed high sensitivity and specificity for the detection of SEG, SEH and SEI in food samples, providing a promising tool to ensure food safety.

P1-24 Evaluation of BAM with VIDAS Immunoassay Method for Identifying *Salmonella* on Tomatoes Surface

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Introduction: *Salmonella* has been implicated as a major cause of human foodborne illness worldwide, and several outbreaks of salmonellosis associated with tomatoes have recently been reported. There is an increase demand for effective detection methods which are rapid, accurate and easy to apply.

Purpose: This study evaluated the Mini Vidas *Salmonella* (SLM) (bioMérieux) method in relation to its ability to detect *Salmonella* cells artificially inoculated on tomato surfaces.

Methods: Tomatoes without any defects (peel ruptures, bruised areas) were used in the experiment. A total of 168 tomatoes were artificially inoculated on the surface at levels of 10, 10^2 and 10^3 CFU/tomato with *Salmonella* Brazil and left to dry inside the air cabinet. These contaminated tomatoes were analyzed simultaneously by Mini Vidas (SLM) and BAM methods. Test for significant difference (x^2) between the methods was evaluated as described by McNemar´s test.

Results: The proportion of samples confirmed positive by the alternative method is not statistically different from the proportion confirmed positive by the reference method (BAM) in all inoculated levels tested. Overall, the Mini Vidas (SLM), method recovered *Salmonella* in 106 test samples compared to 87 for the cultural method, resulting in an 82% overall agreement between the two methods.

Significance: The results indicated that the rapid method Mini Vidas *Salmonella* (SLM) may be effective for the analysis of this microorganism on tomato surface.

P1-25 Prevalence of *Salmonella* spp. in Açai Pulp

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Introduction: One of the most common bacterial pathogens involved in foodborne outbreaks is *Salmonella* spp. Açaí pulp is commercialized pasteurized and frozen or it is used as basis for diverse products. In Brazil, this fruit is widely consumed and recently, it has conquered interest in the United States market.

Purpose: The objective of this study was to investigate the prevalence of *Salmonella* spp. in açai (Euterpe oleracea Mart.) pulp.

Methods: A total of thirty-six (36) açai pulp were collected from industries and one (1) obtained in "natura." For the isolation of *Salmonella* spp samples were analyzed using the Food and Drug Administration's Bacteriological Analytical Manual (FDA- BAM). Serotyping was carried out in the Enterobacteriaceae Reference Laboratory of Instituto Oswaldo Cruz (FIOCRUZ), RJ, Brazil.

Results: *Salmonella* Brazil was confirmed in one sample of açai pulp "in natura." All pulps from the industries were negative for *Salmonella* spp.

Significance: These results indicate that *Salmonella* spp can be found in açai pulp ("in natura") and practices/treatments should be followed to eliminate them.

P1-26 Prevalence of *Salmonella* on Tomatoes from Organic and Conventional Production in Rio de Janeiro (Brazil)

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Introduction: Outbreaks of salmonellosis associated with tomatoes have been related over the past fifteen years. Tomatoes may become surface contaminated with *Salmonella* spp. from production to consumption.

Purpose: The purpose of this study was to evaluate the prevalence of *Salmonella* spp. on tomatoes surface from organic and conventional production. Samples were collected in the street vendors and supermarkets, and analyzed by two methods; the mini-VIDAS SLM assay a specific enzyme-linked fluorescent immunoassay (ELFA) rapid method and the conventional FDA BAM.

Methods: A total of 263 tomatoes were collected during a two-year (2010-2012) survey. A hundred twenty-seven were from organic production, 61 from supermarkets and 66 from street vendors. From conventional production 136 samples were collected, being 84 from supermarkets and 52 from street vendors. All samples were simultaneously surface analyzed by mini-VIDAS SLM and FDA BAM.

Results: *Salmonella* spp. was not detected by the conventional method and two false positives were found by mini-VIDAS SLM.

Significance: Surface tomatoes from organic and conventional production analyzed in the city of Rio de Janeiro showed no potential *Salmonella* risk to their consumption.

P1-27 Modifications to the ANSR® *Salmonella* Method for Improved Ease of Use

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Introduction: ANSR® *Salmonella* is a molecular diagnostic assay that allows for rapid detection of *Salmonella* spp. in foods and environmental samples. Following single-step enrichment, the assay is completed within 40 minutes, via an isothermal nucleic acid amplification reaction based on the NEAR™ technology.

Purpose: To evaluate performance of the molecular method after improvements were made. Molecular method performance was compared to that of either the USDA-FSIS or FDA/BAM reference culture methods for ice cream, peanut butter, dry dog food, raw ground turkey, raw ground beef, and sponge samples from a stainless steel surface.

Methods: For both the molecular and reference methods, 40 test portions of each food product were inoculated with *Salmonella* spp. at a level to produce fractional positive results, along with 5 high level samples and 5 uninoculated controls. Half of the test portions were analyzed by the molecular method after 16 h and 24 h enrichment and half by the reference procedure.

Results: All high level samples tested positive and all negative controls tested negative for each food product by both molecular and reference methods. At the fractional positive level, a total of 120 test portions were analyzed by each method. There were a total of 75 positive results by the molecular assay and 80 by the reference procedures. There were no statistically significant differences in the number of positive results obtained by the alternative and reference methods for any of the products tested as determined by probability of detection analysis.

Significance: A modification to the reagent formulation and a minor procedural change to the molecular method did not affect performance of the assay. Enhanced reagent formulation provided increased pellet solubility, thus eliminating any need for pipette mixing of the lysed sample and assay reagents. This improved operator ease of use, while minimizing the risk of contamination.

P1-28 Rapid Detection of *Listeria monocytogenes* in Milk Using Confocal Micro-raman Spectroscopy and Chemometric Analysis

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Introduction: *Listeria monocytogenes* is a facultatively anaerobic, Gram-positive, rod-shape foodborne bacterium causing invasive infection, Listeriosis, in susceptible populations. Rapid and high-throughput detection of this pathogen in dairy products is critical as milk and other dairy products have been implicated as food vehicles in several outbreaks.

Purpose: Here we evaluated confocal micro-Raman spectroscopy (785 nm laser) coupled with chemometric analysis to distinguish six closely related *Listeria* species, including *L. monocytogenes*, in both liquid media and milk.

Methods: Raman spectra of different *Listeria* species and other bacteria (i.e., *Staphylococcus aureus*, *Salmonella enterica* and *Escherichia coli*) were collected to create two independent databases for detection in media and milk, respectively. Unsupervised chemometric models including principal component analysis and hierarchical cluster analysis were applied to differentiate *L. monocytogenes* from *Listeria* and other bacteria. To further evaluate the performance and reliability of unsupervised chemometric analyses, supervised chemometrics were performed, including two discriminant analyses (DA) and soft independent modeling of class analogies (SIMCA).

Results: By analyzing Raman spectra via two DA-based chemometric models, average identification accuracies of 97.78% and 98.33% for *L. monocytogenes* in media, and 95.28% and 96.11% in milk were obtained, respectively. SIMCA analysis resulted in a satisfied but relatively lower average classification accuracy (93.06%) due to the potential identification of samples as belonging to multiple classes.

Significance: This Raman spectroscopic-based detection of *L. monocytogenes* in media and milk can be finished within a few hours and requires no extensive sample preparation.

P1-29 Multiplex Microbead Immunoassay for Detection of the Ten Most Common Shiga Toxin-Producing Escherichia coli Pathogen Serogroups in Food

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Introduction: Non-O157 Shiga Toxin-producing *Escherichia coli* (STEC) are a growing concern, as they have been linked to many recent outbreaks. Although six STEC serogroups are now regulated as adulterants in the US, important emerging threats include highly virulent serogroups (e.g., O104) that are not included in the "Top Six."

Purpose: We have generated a 10-plex immunoassay for rapid detection and identification of the 10 serogroups most commonly involved in United States outbreaks of STEC: O26, O45, O91, O103, O104, O111, O113, O121, O145 and O157.

Methods: We used antibodies (Ab) specific for the LPS O-antigens of STEC, in a sandwich immunoassay format, using the same polyclonal Ab for both capture and detection. We used the Luminex MAGPIX® fluorescent magnetic microsphere platform, which facilitates assay multiplexing. After developing the assay with pure cultures grown overnight in BHI, we validated it in milk, lettuce, and ground beef spiked with 3 strains of each serogroup.

Results: Our assay yielded 100% sensitivity and specificity for the 30 strains tested, for all 3 foods with all 10 individual assays comprising the 10-plex. Background was low, and S/N ranged from 62 to 251. We observed limited cross-contamination of samples, which was verified via multiplex PCR STEC assay.

Significance: This assay may be used for detection and identification of the 10 most common STEC serogroups in contaminated/adulterated milk, lettuce, and ground beef. It can be used to accelerate outbreak identification and trace-back. Importantly, the Luminex format provides immunomagnetic bead isolation of live bacteria, which may be used for subsequent confirmation and additional characterization.

P1-30 Detection of *Cyclospora cayetanensis* on Fresh Produce: Assessment of Improved Produce Wash, DNA Extraction, and Molecular Detection Procedures

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Introduction: *Cyclospora cayetanensis* is a coccidian parasite causing foodborne diarrheal disease linked to consumption of contaminated fresh produce such as leafy greens and raspberries. During the U.S. outbreaks of 2013 and 2014 a total of 935 cases of cyclosporiasis were confirmed in several states. In both outbreaks, some illnesses were linked to the consumption of imported fresh cilantro.

Purpose: Detection of *Cyclospora cayetanensis* on produce relies heavily on efficient oocyst recovery and sensitive molecular methods since this organism cannot be enriched or cultured. The objective of this study was to evaluate new methods of detection for *Cyclospora cayetanensis* on produce for future implementation into FDA regulatory analyses.

Methods: Samples of cilantro (25 g) and raspberries (50 g) were seeded with oocysts. Six or more replicates (n) at each seeding level were tested. Oocysts were recovered from seeded samples using a detergent wash solution and DNA was extracted using a commercial kit. Molecular detection was performed using a conventional nested PCR assay currently used in FDA labs and a new optimized qPCR assay.

Results: Nested PCR detected *C. cayetanensis* DNA in 100%, 94%, 44%, and 38% of cilantro replicates seeded with 200 (n=16), 10 (n=16), 5 (n=16), and 2 (n=8) oocysts, respectively. Using qPCR, detection rates were 100%, 94%, 69%, and 63%, respectively. Detection rates using nested PCR for raspberries seeded with 200 (n=18), 10 (n=16), 5 (n=16), and 2 (n=6) oocysts were 100%, 63%, 19%, and 33%, respectively, and 100%, 75%, 31%, and 33%, respectively, using qPCR.

Significance: The low detection limit for *C. cayetanensis* was 10 or fewer oocysts on two commodities, which is the presumed infective dose for this organism. In addition, qPCR provides a robust, streamlined, and faster alternative to nested PCR. These results support public health and the FDA mission by providing improved detection methods for the foodborne parasite, *Cyclospora cayetanensis*.

P1-31 Using Denaturing Gradient Gel Electrophoresis Method to Rapidly Evaluate Virulence Genes Diversity of Infectious *Salmonella* Strains

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Introduction: *Salmonella* spp. is a major foodborne pathogen infecting human beings at present across the world. Some studies suggest that mutations in virulence genes of infectious *Salmonella* spp. are a result of host-adaptation convergent evolution. Sequencing methods were used to study virulence-related gene diversity present in different *Salmonella*strains. But those methods are laborious, time-consuming and need a lot of funding.

Purpose: This study aimed at using the DGGE method to rapidly evaluate virulence-related gene diversity present in different *Salmonella*strains.

Methods: In this study, thirty-one *S. choleraesuis* (n = 31), ten *Salmonella* Typhimurium (n = 10) and eight *Salmonella* Enteritidis (n = 8) strains were used to detect virulence genes by PCR. Fourteen virulence genes were selected, which have different functions and locations. High carried rate genes in different serovar *Salmonella*spp. were evaluated using DGGE assay.

Results: The results showed that most *S. choleraesuis* strains (93.75%) did not carry *sopE1* gene, and most *Salmonella* Typhimurium strains did not have *sopE1*, *siiD*, *spvC* and *gipA* genes. All *Salmonella* Enteritidis strains (100%) did not carry *gipA* gene, and most of them did not have *fljB*, *siiD*, *spvC* and *flgK* genes. Different serovar have different virulence gene profiles. In this study, high carried rate genes in different serovar *Salmonella spp*., such as *sopB*, *spvC*, *mgtC* and *ssaQ* genes, were evaluated using DGGE assay. The results showed that *sopB*, *spvC* and *mgtC* genes have no different from three serovar *Salmonella* strains by DGGE, except *ssaQ*gene.

Significance: In this study, DGGE method was used as a rapidly screening method to search for mutations in virulence genes of infectious *Salmonella* spp. It may facilitate epidemiologic studies with other *Salmonella* or related foodborne bacteria and could serve as an appropriate alternative sub-typing method.

P1-32 Rapid Detection of *Escherichia coli* O157:H7 in Alfalfa Sprouts Using Liquid Crystal-based Immunoassay

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Introduction: *E. coli* O157:H7 a major foodborne pathogen that has posed serious problems for food safety and public health. Although *E. coli* O157:H7 has been associated with various types of food, alfalfa sprouts are considered one of main food vehicles for *E. coli* O157:H7. A rapid and sensitive detection assay for this pathogen is critical to control outbreaks and ensure food safety.

Purpose: The aim of this study was to evaluate a novel liquid crystal-based system as a rapid and sensitive assay for detection *E. coli* O157:H7 in alfalfa sprouts.

Methods: The liquid crystal-based immunoassay for *E. coli* O157:H7 was developed using immunomagnetic beads (IMB) and liquid crystal. When *E. coli* O157:H7 is present, formation of *E. coli* O157:H7-IMB aggregates distorts liquid crystal matrix and causes the bending of light, which is then detected by Crystal Diagnostics Xpress system. The assay was tested for its sensitivity using artificially inoculated alfalfa sprouts, and its specificity was tested with various non-O157 Shiga Toxin-producing *E. coli* (STEC) strains and common foodborne pathogens.

Results: The developed immunoassay was able to detect *E. coli* O157:H7 with detection limits of 10^5 CFU/ml without any enrichment. When 6 h sample enrichment step was added, the assay could detect *E. coli* O157:H7 as low as 1 CFU in 100 g of alfalfa sprouts. The total assay was completed

within 30 min. The developed assay was highly specific to *E. coli* O157:H7, and did not show any cross-reactivity with non-O157 STEC or with other common foodborne pathogens.

Significance: The novel immunoassay based on liquid crystal technology shows a great potential as a rapid and sensitive detection method for *E. coli* O157:H7 in food.

P1-33 Use of an Alternative Non-proprietary Enrichment Media for the Detection of *Escherichia coli* O157:H7 with the Atlas® System in 375 Gram Beef Samples with High Bacterial Background Flora

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Introduction: Detection of *Escherichia coli* O157:H7 using pathogen detection methods can be challenging in food matrices with high bacterial background flora such as beef products. High levels of bacterial background flora in beef products can inhibit *E. coli* O157:H7 growth during enrichment. Selective media promoting *E. coli* O157:H7 growth can enhance detection of the target organism in beef products using molecular pathogen detection methods.

Purpose: To validate use of an alternative non-proprietary enrichment media to detect *E.coli* O157:H7 including non-motile strains (NM) with the Atlas EG2 assay in artificially inoculated raw ground beef and raw beef trim samples containing high bacterial background flora.

Methods: Three hundred seventy-five gram test portions of raw ground beef and raw beef trim with > 10^5 CFU/g bacterial background were inoculated with high and low levels of *E. coli* O157:H7 and *E. coli* O157:NM and enriched in Tryptic Soy Broth + 8 mg/l novobiocin and 1.5 g/l dipotassium phosphate (TSBn+dpp). All samples were enriched for 8 - 18 h at 42°C, transferred into Roka G2 Sample Tubes and loaded onto the instrument. The instrument combines target capture, transcription-mediated amplification, and hybridization protection assay. The results of the new method were compared to the FSIS MLG 5.08 reference method.

Results: *E. coli* O157:H7 and *E. coli* O157:NM was specifically detected in artificially inoculated 375 g raw ground beef and raw beef trim samples as early as 8 h enrichment in TSBn+dpp media. The new method performed equally compared to the reference method MLG 5.08 determined by POD analysis.

Significance: The Atlas method provides fast and highly accurate detection of pathogenic *E. coli* O157:H7 and *E. coli* O157:NM in 375 g raw ground beef and raw beef trim samples with high bacterial background flora enriched in a non-proprietary commercially available media and results were comparable to the FSIS reference method.

P1-34 Development of a Reliable ELISA Kit for the Determination of Soya Protein

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Introduction: Soya is widely used in processed foods and it is one of the more common allergic ingredients. For some ELISA kits, it is difficult to evaluate the contamination of soya protein in processed foods.

Purpose: To accurately detect soya protein in processed foods, we developed a reliable ELISA kit and evaluated the performance of this kit.

Methods: A soya protein-specific sandwich ELISA kit was developed using a special extraction solution (patent pending) and polyclonal antibodies against beta-conglycinin. For standard, soya protein was extracted from defatted soya (Enrei) powder with the extraction solution and the protein content was determined by a 2D Quant kit (GE healthcare).

Results: Our kit was able to detect both raw and heat treated soya protein and the recovery was more than 80%. The kit showed no cross-reactivity to other food ingredients such as peanut, wheat, egg, milk, buckwheat and many kinds of nuts (e.g., almond, cashew, pistachio, walnut, pecan, hazelnut), and low cross-reactivity to some kinds of beans. The limit of detection (LOD) and the limit of quantification (LOQ) were 0.156 microgram/g food and 0.31 microgram/g food, respectively. The recovery of soya protein from model processed foods ranged from 77% to 122% and was higher than other commercial kits. The average intra-assay variation (repeatability) was C.V. 1.0% calculated by measuring in three assay on the same day. The inter-assay variation (reproducibility) was C.V. 2.6% calculated by measuring for three days.

Significance: Our ELISA kit is useful and valuable for detecting soya protein in both non-processed and highly processed foods.

P1-35 Simultaneous Detection of Major Shiga Toxin-Producing *E. coli* (STEC) Serotypes in Ground Beef by Immunomagnetic Bead-based Fluorescent Assay

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✦ ***Undergraduate Student Award Competitor***

Introduction: The USDA Food Safety and Inspection Service (FSIS) considers raw beef products contaminated with *E. coli* O157:H7 and Big 6 Shiga Toxin-producing *Escherichia coli* (STEC) serotypes including O26, O45, O103, O111, O121 and O145 to be adulterated, and mandates testing of domestic and imported beef for these STEC serotypes in order to mitigate food safety risks associated with these pathogens. It is estimated that STEC causes 176,000 infections each year in the US, and therefore a rapid and simple detection assay that can simultaneously detect the presence of these STEC serotypes is highly desirable.

Purpose: The purpose of this study was to develop an immunomagnetic bead-based assay for the simultaneous detection of *E. coli* O157:H7 and Big 6 STEC serotypes in ground beef.

Methods: Immunomagnetic beads functionalized with antibodies to target STEC serotypes were loaded into a microplate and used as a bead-based array platform for STEC identification, and the presence of target STEC was determined by measuring fluorescence signal from detection antibodies. Twenty-five grams of each ground beef sample was inoculated individually with target STEC serotypes at concentrations from 10^0 to 10^5 CFU/g. The samples were enriched in 225 milliliters of tryptic soy broth and incubated at 37°C. Each sample was collected at different times (6, 9, 12, and 18 h) and tested with the developed assay.

Results: The developed immunoassay could detect target STEC serotypes as low as 2,000 CFU/ml without any enrichment. The totally assay can be completed within 4 h, and no cross-reactivity was observed. When ground beef samples contaminated with STEC were tested, the assay was able to detect 1 CFU/g of STEC with 9 h enrichment.

Significance: This study demonstrated the developed immunomagnetic bead-based immunofluorescent assay can have great potential to simultaneously detect multiple STEC serotypes in ground beef present at low concentrations.

P1-36 A Novel Membrane-based Electro-separation Method for Sample Clean-up and Norovirus Concentration

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❖ ***Developing Scientist Competitor***

Introduction: Norovirus elution and concentration from complex food matrices is difficult, often resulting in inefficient recovery and inhibition in downstream molecular detection. Membrane-based electro-separation is a technique to exchange charged particles through membrane from one solution to another using electric current as the driving force. Norovirus has a net negative surface charge in neutral buffer environment and when placed in an electric field, it can move towards the anode.

Purpose: Membrane-based electro-separation method was designed, developed and evaluated for its efficacy in concentrating and recovering noroviruses from phosphate buffer in a proof-of-concept study.

Methods: ElectroPrep Electrodialysis system (Harvard Apparatus) was fitted with a 1 µm or 10 µm polycarbonate membrane (for pore-size selection) and two flanking 300 kDa MWCO cellular acetate restricting membranes as to create two chambers (one for sample addition (3.5 ml); one for sample collection (1.5 ml)). 10^6 genome copies per ml of murine norovirus (MNV-1) was seeded into sample chamber and the linked chambers were submerged in sodium phosphate buffer (20 mM, 50 mM, 100 mM, 150 mM) containing 0.01% SDS (pH 7.5) as the electrolyte. Constant electric potential 20 V was provided for 30 min (0 V applied for 30 min as control). 500 µl was collected from each chamber for RNA extraction and quantification by RT-qPCR. All the experiments were performed in duplication.

Results: %Recovery of MNV-1 from the collection chamber were 14.2%, 15.0%, 8.8%, and 16.0% with a 1 µm separation membrane and 27.6%, 24.3%, 21.6%, and 22.6% with a 10 µm separation membrane when 20 mM, 50 mM, 100 mM, and 150 mM electrolyte concentration were used. In control where 0 V were applied, 1.2% and 3.3% recoveries were obtained with 1 µm and 10 µm separation membranes, respectively, in 20 mM electrolyte.

Significance: Norovirus recovery in the collection chamber is higher when electric potential is applied. With further optimization, membrane-based electro-separation method may provide a quick, easy, and cost-effective sample clean-up technique for norovirus detection.

P1-37 Validation of 12 Additional Food Matrices with the Atlas *Salmonella* SG2 Detection Assay

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Introduction: *Salmonella* has been implicated as a major cause of human foodborne illness worldwide. The renewed focus on *Salmonella* prevention created the need for a faster time to result without compromising quality.

Purpose: To conduct a matrix extension validation for the Atlas *Salmonella* SG2 Detection Assay for the detection of *Salmonella enterica* spp. in 12 additional food matrices.

Methods: After enriching for 10 to 24 h for tomatoes, cookie dough and chicken carcass rinses, 16 to 24 h for string cheese, soy flour and pasteurized dried egg powder and 24 to 28 h for peanut butter, raw almonds, milk chocolate, cocoa powder, whey powder and non-fat dried milk, an aliquot of the enrichment is transferred and processed by a fully automated platform for bacterial lysis, template specific sample extraction, amplification, and probe detection. A total of 12 foods were compared to the FSIS MLG 4.07 or the FDA BAM-5 reference method in an internal study and three foods in an external study.

Results: The internal matrix studies demonstrated the Atlas method was equivalent to the FSIS MLG 4.07 and FDA BAM-5 reference methods for raw tomatoes, chicken carcass rinse, string cheese, pasteurized dried whole egg, soy flour, cocoa powder, milk chocolate, non-organic creamy peanut butter, raw almonds, non-fat dried milk, cookies dough and whey powder. The independent laboratory study demonstrated similar results to the internal study for pasteurized dried whole egg, non-organic creamy peanut butter and milk chocolate.

Significance: The Atlas method detected *Salmonella enterica* spp. in a wide variety of food and offers the benefits of high sensitivity and high specificity for the detection of *Salmonella enterica* while reducing the overall time to results compared to the US reference methods.

P1-38 Low Level Detection of *E. coli* based on Electrochemical Biosensor

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Introduction: Even with overall improvements in safety and regulation of food, the US Food and Drug Administration (FDA) is still announcing considerable numbers of recalls and safety alerts due to foodborne pathogens.

Purpose: Since an outbreak of foodborne pathogens may have a severe impact on society resulting in illness, fatalities and financial losses, a rapid, cost-effective, and early detection biosensor would be a valuable tool not only for the food industry but also for regulatory bodies. In this work, the aim is to fabricate a flexible, label-free electrochemical biosensor based on chemiresistive and voltammetric techniques for the detection of *Escherichia coli* (e.g., *E. coli* O157), responsible for numerous foodborne and water-borne infections worldwide.

Methods: The biosensor is constructed on electro-spun membrane by synthesizing chemically sensitive conductive functionalized polymeric thin film to immobilize the analyte detecting molecules. The functional conductive thin films are obtained by co-polymerization of 3, 4-ethylenedioxythiphene and 3-thiopheneethanol and are produced via oxidative chemical vapor deposition (oCVD). oCVD can be used to deposit films with thicknesses ranging from Angstroms to microns onto many kinds of substrates such as paper, fabric, plastic, and glass.

Results: Real time detection and monitoring of *E. coli* with a lower than 10 CFU/ml level has been measured by chemiresistive and voltammetric techniques in this biosensor configuration.

Significance: Besides its sensitivity, ease of use makes it deployable for in-field detection of food pathogens. The high surface area of the biosensor detector membrane allows processing of very large sample sizes, a character which many potential biosensor platforms lack.

P1-39 TEMPO® BC Method for the Next-day Enumeration of *Bacillus cereus* Group in Food and Environmental Samples: AOAC® Research Institute Validation Study

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Introduction: Growth of species of *Bacillus cereus* group in food products is a public risk. TEMPO BC is an automated test for the enumeration of the *Bacillus cereus* group in food products within 22 - 27 hours.

Purpose: As part of the AOAC® Research Institute validation process, the alternative method was compared to the FDA Bacteriological Analytical Manual (BAM) for the food and environmental samples during the internal and independent laboratory method comparison studies.

Methods: Twelve naturally and artificially contaminated matrices were tested including meat, poultry, dairy, seafood, oatmeal, rice and stainless steel surface. Five replicates of three lots for each food and environmental matrix were tested for a total of 180 samples. Inoculated cards were incubated for 22 - 27 h at 30 ± 1°C and then read using the automated reader. Standard method testing was performed as detailed in the FDA-BAM.

Results: The enumeration values obtained from both methods were converted into log and an overall bias was determined. The overall calculated bias was -0.043 with a 95% confidence (-0.119; 0.034) interval that contains zero, indicating that there was no significant difference between the TEMPO BC and the reference method.

Significance: The alternate method provides an automated method for the enumeration of *Bacillus cereus* group species in food and environmental samples with next day results. The automated method eliminates the subjectivity and potential for plate count variability along with additional advantages of increased efficiency, better traceability and significant labor savings.

P1-40 Comparative Study on the Detection of *Salmonella*, *Listeria* Species and *Listeria monocytogenes* with the 3M™ Molecular Detection System in a Variety of Dairy Food Matrices

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Introduction: *Salmonella* and *Listeria* are pathogens of worldwide public health significance frequently associated with foodborne disease. Detection via traditional methods require up to 4 - 5 days to results. The 3M™ Molecular Detection System (MDS) is an alternative method based on bioluminescence coupled with isothermal amplification of DNA that provides results in real time.

Purpose: The purpose of the study was to compare the performance of the 3M MDS against the ISO 6579:2002 e ISO 11290-1 reference methods for detection of *Salmonella*, *Listeria* spp. and *Listeria monocytogenes* in a variety of food matrices typical of the dairy industry within the Santa Fe region in Argentina.

Methods: Food samples from a variety of dairy matrices were artificially inoculated with overnight cultures of *Salmonella* Typhimurium ATCC 14028 or *Listeria monocytogenes* ATCC 19114. A total of 47 samples were analyzed for *Salmonella* spp., 27 for *Listeria* spp. and 32 for *Listeria monocytogenes*. Samples were enriched by combining 25 g with 225 ml of media and incubating for 18 - 24 h at 37°C for *Salmonella* spp., or for 24 - 28 h at 37°C for *Listeria* spp. and *Listeria monocytogenes*. Samples were analyzed by either the 3M MDS and ISO 6579:2002 or ISO 11290-1 reference protocols.

Results: All artificially inoculated samples were detected as positive by the 3M MDS after 15 min. The non-inoculated samples were detected as negative by the 3M MDS and confirmed to be free of the pathogen via culture method. The results reflect 100% accuracy, inclusivity and exclusivity, with no false positives or false negatives observed and excellent correlation with the corresponding ISO methodologies.

Significance: Results demonstrate the suitability of the 3M MDS for rapid analysis of dairy food matrices, allowing full confidence in the release of ingredients and finished products based on timely and highly accurate results.

P1-41 MALDI-TOF MS Biotyping for Characterization of Antimicrobial-resistant *Escherichia coli* from Concentrated Animal Feeding Operations and Associated Wildlife

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❖ ***Developing Scientist Competitor***

Introduction: Antimicrobial resistant (AMR) bacteria represent one of the most significant challenges to food safety and security. Wildlife serve as one potential vector in the spread of AMR bacteria, thus interventions which limit wildlife contact with livestock or produce can control the dissemination of AMR bacteria. Accordingly, to pinpoint the problem wildlife vectors, methods are needed to rapidly characterize the AMR phenotypes of associated bacterial isolates.

Purpose: Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) Biotyping and bioinformatics were used to identify bacteria and correlate mass spectra to AMR phenotypes. AMR *Escherichia coli* collected from wildlife and their associated concentrated animal feeding operations (CAFOs) were used as the test bed.

Methods: MALDI Biotyping of 237 presumptive *E. coli* isolates grown on selective media was conducted using the Bruker Ultraflex II TOF/TOF configured in positive ion reflector mode with an accelerating voltage of 20 kV, following an ethanol/formic acid extraction of the bacteria. Antimicrobial susceptibility testing with 17 relevant antibiotics was performed on isolates confirmed as *E. coli* using the disk diffusion method. Correlations of mass spectra with the determined AMR phenotypes were accomplished using the principal component analysis (PCA) clustering feature of the MALDI Biotyper RTC software (Ver. 3.1.) and with novel algorithms developed by our laboratories.

Results: MALDI-TOF MS confidently identified (Biotyping score ≥ 1.8) 156 of the 237 isolates as *E.coli*, 35 isolates as beta- or gamma- proteobacteria sp., and 46 isolates could not be identified. Detailed integrations of mass spectral PCA clustering patterns proved useful for differentiation of isolates with specific AMR phenotypes. Further, within this dataset, multiple ions were identified that uniquely correlated with a specific antimicrobial resistance.

Significance: This study demonstrates that MALDI-TOF MS is a viable strategy for the identification of *E. coli* associated with wildlife/CAFOs and for discriminating between their AMR phenotypes.

P1-42 Evaluation of the DuPont™ BAX® System Real-Time *Salmonella* Assay for Detecting *Salmonella* on Stainless Steel and Epoxy-coated Concrete Environmental Surfaces Using Two Sponge Types in Two Different Enrichment Media

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Introduction: In a commercial environment, contact surfaces can be a source of foodborne contamination. Environmental monitoring programs are generally used to measure the effectiveness of sanitation procedures by sampling and detecting pathogens or indicators on these surfaces.

Purpose: This study evaluated the performance of a real-time PCR assay and the FDA-BAM cultural method for detecting *Salmonella* on stainless steel and epoxy-coated concrete surfaces. Surface samples were inoculated with *Salmonella* and a competitor*, Citrobacter braakii*, then evaluated using two different sponge types enriched in either Buffered Peptone Water (BPW) or Lactose Broth (LB).

Methods: Stainless steel (n = 80) and epoxy-coated concrete (n = 80) surfaces were artificially co-inoculated with either a *Salmonella* cocktail or *Salmonella* Newport, respectively, and with a background organism, *Citrobacter braakii,* to obtain fractional recovery. Each surface was swabbed with one of two sponge types then added to 225 ml of either pre-warmed (35°C) LB or BPW. Samples were incubated at 35 ± 2°C for 22 h before proceeding with BAX® System method testing and reference culture confirmation.

Results: Of the 80 fractionally inoculated stainless steel surfaces, PCR detected 2/20 presumptive positive samples after 22 h of enrichment in BPW using sponge #1 and 7/20 using sponge #2. In LB, 6/20 using sponge #1 and 10/20 using sponge #2 were detected. Of the 80 fractionally inoculated epoxy-coated concrete surfaces, PCR detected 3/20 presumptive positive samples after 22 h of enrichment in BPW using sponge #1 and 7/20 using sponge #2. In LB, 9/20 using sponge #1 and 6/20 using sponge #2 were detected. All positive results were confirmed following the FDA-BAM reference culture method.

Significance: Regardless of sponge type, the results for epoxy-coated concrete and stainless steel environmental samples enriched in either BPW or LB, demonstrate no significant statistical difference as indicated by POD analysis. Overall, the DuPont™ BAX® System Real-Time PCR assay for *Salmonella* demonstrates equivalence to the reference culture method.

P1-43 Evaluation of the DuPont™ BAX® System Real-time Genus *Listeria, Listeria monocytogenes* and *Escherichia coli* O157:H7 Assays for Detecting *Listeria monocytogenes* and *Escherichia coli* O157:H7, Respectively, in Whey Protein Concentrate 34

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Introduction: Whey is a natural dairy product that is a constituent of numerous high-value finished products. To assure safe, high-quality dairy products, reliable rapid methods are needed to dependably screen this commodity for foodborne pathogens before it is converted into value-added products.

Purpose: To evaluate the performance of real-time PCR assays, the FDA-BAM reference method, and traditional culture method to detect two separate significant human pathogens, *Escherichia coli* O157:H7 and *Listeria monocytogenes,* in artificially inoculated samples of Whey Protein Concentrate 34 (WPC34).

Methods: WPC34 powder was artificially inoculated with either a *L. monocytogenes* or *E. coli* O157:H7 strain to obtain fractional recovery, after being held at room temperature for 2 weeks to stress the organisms. *L. monocytogenes*-spiked 125 g portions (n = 20) were enriched in 1125 ml pre-warmed (30°C) Demi-Fraser (DF) Broth, homogenized and incubated (30°C) for 22 - 26 h. A secondary enrichment in pre-warmed (35°C) MOPS-BLEB was incubated (35°C) for 18 - 24 h. *E. coli* O157:H7-spiked portions of 375 g (n = 20) were enriched in 1500 ml pre-warmed (42°C) modified Tryptic Soy Broth (mTSB), homogenized and incubated (42°C) for 18 - 24 h. A set of reference samples (n = 20) were also enriched according to the FDA-BAM method. After incubation, all samples were processed with real-time PCR testing and the appropriate culture method.

Results: Of the *L. monocytogenes*-spiked samples, the real-time PCR assay for Genus *Listeria* and *L. monocytogenes* returned 5/20 presumptive positive results from MOPS-BLEB. Of the *E. coli* O157:H7-spiked samples, the real-time PCR assay returned 5/20 presumptive positive results after a BHI regrowth. Reference method samples returned 13/20 presumptive positive results after a BHI regrowth. All culture results matched PCR results.

Significance: The results of this study demonstrate the DuPont™ BAX® System can rapidly, accurately and reliability detect *E. coli* O157:H7 in 375 g and *Listeria monocytogenes* in 125 g samples of WPC34.

P1-44 Evaluation of the DuPont™ BAX® System PCR Assays for Genus *Listeria* and *Listeria monocytogenes* for Testing Stainless Steel and Concrete Environmental Surfaces Using 90 Milliliters of Primary Enrichment Media

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Introduction: Due to increases in industry concern and USDA regulatory policies for environmental *Listeria* testing in facilities producing Ready-to-Eat meat products, a new importance has been placed on developing rapid *Listeria* spp. and *L. monocytogenes* testing methods that are industry-validated, easy to use and cost effective. One way to help support these goals is to develop enrichment protocols that use less media per sample in conjunction with automated PCR testing methods.

Purpose: The purpose of this study was to assess the ability of two commercial PCR assays for Genus *Listeria* and *L. monocytogenes* to detect *Listeria* from stainless steel and concrete environmental surfaces after a modified primary enrichment conducted in 90 ml of Demi-Fraser broth.

Methods: Stainless steel and concrete surfaces (n = 20 each) were inoculated with *L. monocytogenes* and *L. welshimeri*, respectively, at levels likely to give fractional recovery. Each surface was also inoculated with *Enterococcus faecalis* as background flora. For the alternative method, sample sponges were added to 90 ml of Demi-Fraser broth and incubated for 22 - 26 h at 30 ± 2°C, then 100 µL of primary enrichment was added to 9.9 ml MOPS-BLEB and incubated for an additional 18 - 24 hours at 35 ± 2°C. For the reference method, protocols were followed according to the USDA-FSIS MLG method. All samples were processed in the BAX® System instrument and confirmed according to the reference culture method.

Results: For the alternative enrichment, PCR and culture results were identical with 13 positive samples from stainless steel and 6 from concrete. The reference method enrichment produced 10 positive samples on stainless steel and 6 on concrete also with identical results from culture and PCR.

Significance: The alternative and reference methods generated results that were statistically indistinguishable. These data suggest that the method as modified is an acceptable alternative to the reference method.

P1-45 Assessment of a Monoclonal Antibody-based Direct Sandwich Enzyme-linked Immunosorbent Assay for Cashew (*Anacardium occidentale*) Detection in Food Matrices

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❖ ***Developing Scientist Competitor***

Introduction: Cashew is the second most globally produced tree nut and accounts for significant portion (14%) of tree nut-induced allergies in the U.S. The ability to rapidly detect cashew traces in complex food matrices is therefore important.

Purpose: The objective of this study was to assess the applicability of a commercially available monoclonal antibody-based direct sandwich enzyme-linked immunosorbent assay (ELISA) kit to detect cashew in selected foods.

Methods: ELISA kits were purchased from BioFront Technologies (Tallahassee, FL). Cashew nut seeds, food ingredients, and commercial samples were purchased from local grocery stores. Full fat flours were extracted in kit-provided extraction buffer for 10 min at 60°C (flour-to-buffer ratio of 1:10 w/v) and soluble protein content was determined using the Bradford method. Immunoreactivity of the samples was assessed using the ELISA kit procedure.

Results: Among the 25 tested commercial samples, with and without declared cashew, no false positive or false negative results were obtained by the assay. The percentage recovery for 10 ppm cashew spiked ice cream, dark chocolate, milk chocolate, white chocolate, cereal, corn flake, sponge cake, and sugar cookie was 92.8 ± 3.6%, 66.1 ± 7.5%, 83.7 ± 4.4%, 87.7 ± 3.4%, 93.1 ± 1.5%, 89.5 ± 2.2%, 88.4 ± 4.4%, and 98.7 ± 1.3%, respectively. In case of dark chocolate, addition of 5% non-fat dry milk in the extraction buffer significantly ($P \leq 0.05$) improved cashew recovery (66.1 ± 7.5% to 86.0 ± 7.8%). The recovery range for incurred cashew (0.5, 1, 2, and 5%) in the laboratory prepared sponge cake, sugar cookie, and corn flake was 87-118%.

Significance: The tested ELISA kit was able to rapidly detect cashew in the tested foods.

P1-46 Recovery of Aflatoxin B_1 in a Range of Food Commodities Utilizing a Matrix Resistant ELISA

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Poster

Introduction: Aflatoxin B_1 is a well-known potent human carcinogen produced by toxigenic fungi. Given its ubiquitous presence in a wide variety of foods and beverages, aflatoxin B_1 levels must be measured and monitored to prevent contaminated food from reaching the consumer. Enzyme linked immunosorbent assays (ELISAs) are frequently employed as a rapid and inexpensive method to screen samples that may contain aflatoxin B_1 concentrations above the legal permissible limit. To date, many available ELISAs are limited in the range of commodities that can be tested because they are subject to matrix interferences and require an additional clean-up step.

Purpose: The aim of this study was to evaluate if a single ELISA kit can accurately detect aflatoxin B_1 in commodities that typically pose matrix interferences, including nuts, spices, and other common cooking ingredients.

Methods: The various comestibles (almonds, macadamia, peanuts, chili, cinnamon, coriander, ginger, and coconut) were purchased from local markets and evaluated for matrix interferences. A Student's *t*-test was performed to determine any significant differences between the extracted commodity and extraction solvent alone. Samples were then fortified with aflatoxin B_1 at various concentrations ranging from 2.5 – 20 ppb (n = 3 for each spike level) and measured in three independent experiments by a previously developed competitive inhibition ELISA. The % recoveries and %CVs were calculated.

Results: All food items exhibited minimal matrix interferences. The mean signal of the commodities did not demonstrate a statistically significant difference compared to the mean signal of the extraction solvent alone ($P < 0.05$). All commodities showed excellent recoveries of 82 - 111% with %CVs of less than 13%. The only exception was cinnamon, which had a recovery of less than 30%.

Significance: The data demonstrates that a single ELISA kit can be used to successfully quantify aflatoxin B_1 in most commodities without the need for special extraction methods or clean-up procedures.

P1-47 Implementation of a Streamlined Testing Algorithm to Decrease Turnaround Time for *Listeria monocytogenes* Foodborne Outbreak Investigations

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Introduction: During a multi-state investigation of a *Listeria monocytogenes* illness cluster in 2014, the Virginia Division of Consolidated Laboratory Services (DCLS) isolated *L. monocytogenes* from the implicated food which possessed an indistinguishable Pulsed Field Gel Electrophoresis (PFGE) pattern to five previously identified clinical cases. Utilization of a rapid food sample testing algorithm enabled DCLS to more promptly identify contaminated food products to include in the public recall notification, enabling regulatory authorities to remove the product from the market.

Purpose: The algorithm for isolation of *L. monocytogenes*, biochemical confirmation, and molecular subtyping by PFGE prior to the outbreak required approximately ten days from sample receipt. Given the critical need for expedited results, algorithm modifications were proposed and evaluated

Methods: The existing DCLS Food Microbiology and PFGE methods were evaluated using a risk-based approach to maximize efficiency while maintaining quality laboratory practices. The testing algorithms were compared, redundancies identified, and a harmonized *L. monocytogenes* algorithm developed.

Results: Four process redundancies in the *Listeria* testing algorithms were identified: (*i*) transfer of suspicious colonies to blood agar plates (BAP) on Day 5, (*ii*) confirming *L. monocytogenes* isolates prior to submission to PFGE on Day 7, (*iii*) subculture of isolates to blood heart infusion agar (BHI) from BAP in PFGE, and (*iv*) overnight incubation of PFGE plugs for cell lysis. The response time for *L. monocytogenes* was reduced from 10 days to 6 days by (i) streaking for isolation on BHI and BAP on Day 4, (*ii*) submitting BHI plates to PFGE on Day 5, (*iii*) performing biochemical confirmation concurrently with PFGE analysis, and (*iv*) PFGE processing using a "1 day" method.

Significance: Use of an improved laboratory testing algorithm allowed for rapid notification to regulatory agencies, and consumers, thereby, reducing or eliminating potential illnesses associated with contaminated product.

P1-48 Pathatrix Auto™: The Development and Validation of a Method for Detecting *Listeria* Species in Pooled Food and Environmental Samples

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Introduction: The Pathatrix Auto™ pathogen isolation platform provides a workflow that is able to process sample volumes up to 25 ml containing as many as ten individual food or environmental sample enrichments in the same sample pool.

Purpose: Development and validation have been performed for this workflow, which is the first of its kind in the market, and allows food safety professionals to utilize this pooling approach prior to detecting *Listeria* by Real-time PCR.

Methods: The core of this isolation technology is the automated purification of all *Listeria* species of significance by antibody-conjugated magnetic beads specific for *Listeria*. The captured bead-bound bacteria are then lysed, and the supernatant is added to an AOAC- and AFNOR-validated lyophilized MicroSEQ® *Listeria* spp. (or *Listeria monocytogenes*) Real-time PCR assay. By combining the specificity of antibody-based capture and the sensitivity of Real-time PCR, we endeavored to reliably detect 1 CFU in 25-g food samples, as well as in swab- and sponge-based environmental samples.

Results: A wide variety of over 30 different sample types (n > 300 total samples) were tested during internal validation. This workflow correctly identified 96% of all samples against the Reference Method by Real-time PCR, and 98% by selective agar plating.

Significance: The ability to pool individual samples, in addition to the ease of use of this workflow, enables the processing of hundreds of samples per hour at a fraction of the cost of platforms that do not accommodate a pooled sample format, thus creating an economic benefit to food producers by providing a workflow that is able to rapidly and inexpensively screen for rare contamination events in both the environment and food products. This work demonstrates that one can use PCR technology to detect pathogens without the costs and labor associated with the traditional one-sample-per-assay relationship.

P1-49 *E. coli* Detection in Food: Development of a Fully Automated Pathogen Purification and Detection System

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Introduction: There is an increasing call by authorities for regulation of pathogenic *E. coli* O157:H7 and non-O157 "Big 6" *E. coli* in foods, particularly beef products. Real-time PCR represents a powerful tool for specific and rapid detection of foodborne pathogens with time to results of less than 24 hours, particularly desirable for products with short time-to-market requirements.

Purpose: The purpose of the current study was to develop a fully automated method for the purification and real-time PCR detection 1) of *E. coli* virulence factors O157, *stx1/stx2,* and *eae* as a rapid screening assay for the virulence factors and 2) *E. coli* O157:H7 and non-O157 O-serotypes in a further screen for pathogenic *E. coli* O-serotypes.

Methods: *E. coli* DNA was purified from enrichment cultures of raw beef trim, ground beef and spinach. Cultures were performed for 6 - 18 hours in mTSB at 41°C. A one-for-all fully automated purification method developed for *Salmonella* spp. and *Listeria* spp. was extended to *E. coli* using *mericon E. coli* O157 Screen Plus and *mericon E. coli* STEC O-type pathogen detection assays.

Results: Time course studies were performed for each matrix type to determine the enrichment time required. *E. coli* was detected for all 3 matrices at low level within 8 h. Limit of detection studies demonstrated that these new assays are equivalent to the standard reference methods in their ability to detect *E. coli* virulence factors and STEC O-types. The inclusivity and exclusivity of the two assays are as expected. A scheme for *E. coli* virulence factor screening with subsequent STEC O-type screen using these assays is presented.

Significance: The fully automated QIAsymphony Rotor-Gene Q provides a powerful, rapid, reproducible, and sensitive method for the detection of *E. coli* virulence factors and STEC O-type in beef products and spinach.

P1-50 The Use of the RapidChek *Listeria* NextDay™ Test System to Detect Low Levels of *Listeria* Species in Brine Samples

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Introduction: Different types of brine solutions are used to improve both flavor and the moisture content of different food products. These solutions are also used for food preservation, where they are often used by Ready-to-Eat meat producers to cure meat. The brine solutions are tested to make sure they are free of food pathogens.

Purpose: The aim of the study is to evaluate the performance of the RapidChek *Listeria* NextDay test system for the detection of low levels of various *Listeria* spp. in brine samples.

Methods: Two types of brine solutions were tested with the *Listeria* test method, chlorine based and citric acid brine. Hot dogs were cured in both brine solutions for 18 h at 4°C. Each brine solution was spiked with either *L. monocytogenes* ATCC 19115 (chlorine-based) or *L. innocua* ATCC 33090 (citric acid brine solution). Thirty brine samples, 20 low-level and 5 high-level spiked samples, and 5 non-spiked samples, of each variety were analyzed by the test method. Brine samples (50 ml) were enriched in 450 ml of the *Listeria* media and incubated at 30°C for 27 - 48 h. All samples were evaluated with *Listeria* test strips, and streaked to MOX plates for confirmation.

Results: The *Listeria* test method detected twenty out of twenty inoculated samples from both types of brine samples. There was no evidence of a matrix or a pH effect on the 27 - 48 h test method.

Significance: The *Listeria* test system detected low levels of *Listeria* spp. in brine samples within the given timeframe of 27 - 48 h with no matrix effect evident on the test. This should enable food producers to quickly and efficiently identify *Listeria* spp. in brine samples.

P1-51 Real-time, Specific and Sensitive Detection of *Salmonella* Using a Hand-held Immuno Field Effect Transistor (ImmunoFET)

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Introduction: Today, most foodborne pathogen testing requires dedicated, off-site labs, specialized equipment, trained personnel and can often takes days to complete. With the goal of shifting the paradigm to on-site, real-time results, we have developed a high sensitivity, incubation-free detection system for pathogen contaminants, in a portable, user-friendly format. Our platform technology utilizes a solid-state field effect transistor

functionalized with an antibody (an immunoFET) to detect specific analytes of interest in small volume liquid samples, with a turnaround time of minutes.

Purpose: The purpose of this study is to demonstrate the sensitivity and specificity of an immunoFET sensor for the detection of *Salmonella enterica* bacteria.

Methods: ImmunoFETs functionalized with an anti-*Salmonella* antibody were used to detect the presence of *S. enterica* at different concentrations in blinded, artificially contaminated saline solutions. Cross-reactivity of the immunoFET to other bacteria was evaluated using spiked solutions of *Escherichia coli* and *Listeria monocytogenes*.

Results: Preliminary data shows that the lowest concentration of *S. enterica* detectable with our immunoFET sensor is 100 colony forming units/ml. Further, the immunoFET accurately identified the presence or absence of *S. enterica* in spiked samples in our trials. We did not detect cross-reactivity of our sensor with samples spiked with *E. coli* or *L. monocytogenes*. Total time from sampling to result output is less than 10 min.

Significance: We have demonstrated that our immunoFET sensor can directly measure the presence of even low concentrations of *S. enterica* in liquid samples within minutes, eliminating the need for sample incubation. Our handheld sensor can be readily used with little training, at the point of testing, to accurately identify and quantify contamination.

P1-52 Rapid Detection of Yeast and Mold in Plant-extracted Health Foods Using DuPont™ BAX® System Y&M PCR Assay

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Introduction: Fungal spoilage in foods is not only a quality issue but also food safety concern since many common mold species are toxigenic. It is therefore imperative that manufacturers closely monitor their processes to prevent such contamination. While traditional yeast and mold culture method requires 5 - 7 days to achieve a result, the DuPont™ BAX® System Yeast and Mold PCR Assay provides accurate results within 2 days. This AOAC RI-Performance Tested Method (#010902) was validated in yogurt, corn starch and milk-based infant formula.

Purpose: The objectives of this study were to validate this PCR-based test method in plant extracts to detect yeast and mold at as low as 25 CFU/g and to compare the effectiveness of sample processing through different preparation procedures against growth/PCR inhibition issues coming from the complexity of food matrices.

Methods: Twenty naturally contaminated plant extracts were tested by the test method and China GB reference method. For the test method, samples were incubated at 25°C for 44 - 52hrs, followed by lysis and PCR processes according to the test kit User Guide. To avoid growth/PCR inhibition from the samples, various incubation time, different pooled sample procedures and lysate dilutions were investigated.

Results: For the twenty tested samples, the preferred sample processing was worked out for the test method, which gave identical results to the reference method. Chi-square analysis indicates the test method and the reference method returned statistically equivalent results.

Significance: The results demonstrate this PCR-based assay can be used to reliably and accurately detect yeast and mold in plant extracts at low levels to meet manufacturers' product specifications. Furthermore, the faster time to result allows the analysis lead time for Quality Control procedures to be reduced to 2 days, compared to 5 days required with the reference method, resulting in faster release of product, reductions in inventory and gains in working capital.

P1-53 Evaluation of the VITEK® MS MALDI-TOF Method for the Rapid Identification of Microorganisms

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Introduction: The bioMérieux VITEK MS®is a microbial identification system utilizing MALDI-TOF Mass Spec (MS) to identify bacteria, yeasts and molds from culture.

Purpose: The MS was validated according to AOAC guidelines for *Performance Tested Methods* and *Official Methods* precollaborative studies for the identification of microorganisms.

Methods: In the combined internal and independent laboratory studies 359 different claimed organisms (representing 208 Gram negative, 123 Gram positive and 28 yeast) were evaluated by the method. All test strains were cultured on appropriate agar medium and growth conditions before analyzing on the MS instrument.

Results: Overall percent correct identification in the combined Internal and Independent studies (6111/6401 MS results from the 359 different organisms) was 95.5%. For each specific group of microorganisms tested the percent correct identification was 94.4% for Gram-negative organisms, 96.1% for Gram-positive organisms and 97.5% for yeast. Assay ruggedness was demonstrated for three critical parameters, including the use of 1-µl loops from two different suppliers, the age of the culture on an agar plate before analysis and various suppliers of the agar media.

Significance: Overall results indicate that the VITEK MS method is an acceptable automated method for the identification of a wide variety of yeasts, Gram-negative and Gram-positive bacteria.

P1-54 Milk Protein and Ingredient Analysis by Commercial Milk-specific LFD Kits

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❖ ***Developing Scientist Competitor***

Introduction: Commercial lateral flow devices (LFDs) that target allergens are used by the food industry as a quick and rugged measure of equipment cleanliness or food product contamination. Milk LFDs that target total milk, casein or β-lactoglobulin are available, but little is known about their milk protein specificity and testing ability at high allergen levels which may produce false-negatives as a result of the hook effect.

Purpose: To determine the specificity of milk LFDs to milk proteins and milk-derived ingredients and to determine the overload level.

Methods: Casein (α-, β- and κ- casein) and whey proteins (α-lactalbumin and β-lactoglobulin) of milk were analyzed by eight milk (total milk, casein or BLG) LFDs. Milk-derived ingredients (MDI) (whey protein concentrate (WPC), sweet whey (SW), acid whey (AW), sodium caseinate (NaCas), non-fat dry milk (NFDM) and milk protein concentrate (MPC)) were analyzed with the eight milk LFDs and a total-protein kit. NFDM was tested with all of the kits to determine the overload level/hook effect.

Results: Some total milk LFDs only detected casein and not whey proteins. Similarly, some total milk kits did not detect MDIs containing only whey proteins (WPC, SW, AW). All LFD kits were able to detect NFDM. The LFDs became overloaded between 250 ppm -10,000 ppm NFDM protein; testing

dilutions of a sample may be important to ensure it is within the dynamic range of the kit. Variable intensities of LFD positive test lines were found throughout the allergen concentrations tested; LFDs are a qualitative test and corrective action should be taken whenever a positive result is obtained.

Significance: These results will help the food industry to effectively select a LFD for their purposes and interpret the test results.

P1-55 Validation of the Atlas EG2 Combo STEC Detection Assay for Big Six Non-O157 Shiga Toxin-Producing *Escherichia coli* (STEC) in Ground Beef, Beef Trim and Finely Textured Beef

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Introduction: Validated methods for detection of non-O157 STEC are needed to advance industry testing practices and protect public health.

Purpose: These studies independently validated AOAC-RI approved Roka Atlas EG2 Combo STEC Detection assay for detection of Big Six STEC in trim, ground and finely textured beef matrices.

Methods: Eight studies were conducted across 3 matrices. For beef trim, O26, O103, O111 and O121 were utilized. Raw trim was portioned into 30, 375-g samples, inoculated with a subfractional or high level 3-strain cold-stressed cocktail of the serogroup and enriched for 8 - 24 h at 1:4 in modified tryptic soy broth with casamino acids (mTSB+CAA) at 42°C. At 8 and 10 h, individual samples were taken for assay and 1 ml combined with 4 ml of non-inoculated enrichment to form 20 low and 5 high level pooled samples. Pooled samples were assayed at 8 and 10 h. For ground beef, O26 and O145 were similarly processed at 1:5 and assayed at 8, 10 and 12 h including pooling with 1 ml to 2 ml as above. Finely textured beef was evaluated with O26 and O45 at 1:5 and assayed at 10 and 12 h without pooling. Aerobic plate counts were conducted by pour plate method. A heat step was utilized in processing for Atlas EG2 Combo 8 hour samples. All samples were confirmed with USDA MLG 5B.04.

Results: Of 890 samples, the Atlas EG2 Combo reported 585 positive and 305 negative results. Two false negative results were associated with serogroup O103 in an individual and paired, pooled beef trim sample and one O26 in ground beef at 8 hours from low level replicates. Overall, the assay demonstrated 99.66% sensitivity and 99.34% specificity.

Significance: The Atlas EG2 Combo STEC Detection Assay is a sensitive and specific method for detection of Big Six STEC in trim, ground and finely textured beef matrices.

P1-56 Development of a Limits-based Pathogen Test Application for Detecting Specified Concentrations of *Salmonella enterica* in Ground Poultry

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Introduction: Positive qualitative testing results for prevalence yield little or no insight regarding pre-testing contamination levels in sampled product. Methods to rapidly discern highly contaminated product may be useful as a process control or intervention.

Purpose: These studies were conducted to develop a semi-quantitative, threshold-based testing application for a qualitative molecular detection technology.

Methods: Three phases of study were conducted to optimize detection of 0.5 and 1 CFU/g *Salmonella* contamination levels in raw ground turkey within the time frame of one production shift utilizing the Atlas Salmonella SEN Detection Assay. Studies consisted of replicate (n = 10 - 15) 375 g ground turkey samples inoculated with one of 5 levels of a 5-serovar *Salmonella enterica* cocktail (Newport, Hadar, Heidelberg, Typhimurium and St. Paul) for each experimental combination of contamination level, enrichment ratio, temperature and sampling time point. Phase 1 evaluated enrichment of 375 g samples in BPW at 1:1, 1:2 and 1:3 ratios at 35° and 42°C for 3 and 4 hours. Phase 2 optimized enrichment time and temperature to within half hour intervals and guard-banded conditions selected in phase 1. Phase 3 served to validate the application sampling parameters demonstrating repeatability. Ten replicate samples were evaluated for each experimental combination in triplicate studies. In all studies, mean relative light units were used as the semi-quantitative measurable response variable.

Results: Sampling parameters were established for a threshold based testing application to accurately detect 1 CFU/g contamination levels of *Salmonella* in 375 g ground turkey samples within one working shift and with 100% confidence.

Significance: This threshold based testing application provides an additional tool for process control and intervention, in combination with appropriate diversion, to reduce *Salmonella enterica* burden entering commerce.

P1-57 Development of a Liquid Crystal-based *Salmonella* Detection Assay

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Introduction: *Salmonella* is the most common causative agent of foodborne illness in meat. The CDC estimates that 1.2 million cases of salmonellosis occur annually in the United States, resulting in 400 deaths per year. Because elimination of *Salmonella* from food is nearly impossible, detection of the organism before it reaches the consumer is essential. Crystal Diagnostics (CDx) has developed a liquid crystal technology that detects microsphere aggregates formed when specific antibody coated microspheres interact with their target pathogen.

Purpose: This study evaluates anti-*Salmonella* magnetic microspheres in a rapid pathogen detection system.

Methods: Anti-*Salmonella* magnetic microspheres were assayed against 32 *Salmonella enterica* subsp. *enterica* serovars representative of serogroups A-F. *Salmonella* serovars were grown overnight in TSB; 6.75 x 10^6 CFU were assayed using the anti-*Salmonella* magnetic microspheres via immunomagnetic separation and detected using the liquid crystal technology. In addition, 21 non-*Salmonella* species were grown overnight in TSB or BHI and assayed undiluted. Finally, 50 ground beef samples from an institutional processing plant, along with appropriate controls, were enriched overnight in mTSB + novobiocin and assayed for *Salmonella*.

Results: When *Salmonella* serovars were grown in non-selective media, and processed through the existing CDx methodology, 30 of 32 serovars (94%) were detected. When 21 non-*Salmonella* species were assayed after overnight growth in non-selective media, two species (9.5%) were identified as *Salmonella*. After comparing CDx methods with culture-based methods, all 50 samples from the beef processing plant were correctly identified.

Significance: These preliminary data suggest that integration of commercially available anti-*Salmonella* antibody-coated microspheres into the liquid crystal based immunoassay will permit broad-based detection of *Salmonella*. Such an assay would permit rapid detection of this important foodborne pathogen.

P1-58 Evaluation of Different Methods to Detect Microbial Hygiene Indicators Relevant in the Dairy Industry

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❖ ***Developing Scientist Competitor***

Introduction: Bacterial groups including coliforms, Enterobacteriaceae (EB), and Gram-negatives represent indicators of poor sanitation or post-pasteurization contamination in dairy products worldwide. While Petrifilms and traditional selective media are commonly used for the testing of these indicator organism groups throughout the dairy industry, new rapid methods are also being developed.

Purpose: This project was designed to evaluate the ability of different methods to detect coliforms, Enterobacteriaceae and other Gram-negatives isolated from various dairy products and dairy processing environments.

Methods: Using the Food Microbe Tracker© database, a collection of 211 coliform, Enterobacteriaceae, and Gram-negative isolates from 25 dairy-related genera were assembled for the purpose of this study. The selected isolates were tested in pure culture (at levels of 15 - 300 bacteria/test) to evaluate their detection on 3M™ Coliform Petrifilm, 3M™ Enterobacteriaceae Petrifilm, Violet Red Bile Glucose Agar (VRBGA), Crystal Violet Tetrazolium Agar (CVTA), Brain-Heart Infusion Agar (BHI), and a novel flow cytometry based method.

Results: Of the 211 Gram-negative isolates, 82.5% (174/211) had characteristic growth on CVTA media. Within this set of Gram-negative organisms, 175 isolates representing 19 genera in the Enterobacteriaceae group and were screened using the Enterobacteriaceae selective/differential detection methods. Positive results were observed for 96.0% (168/175), 90.3% (158/175), and 86.3% (151/175) of isolates when tested on Enterobacteriaceae Petrifilm, VRBGA, and the flow cytometry instrument, respectively; optimization of the cut-off thresholds for the flow cytometry method may further improve the sensitivity and specificity of this method, but will require additional data. Additionally, 73.7% (129/175) of the Enterobacteriaceae isolates tested positive as coliforms.

Significance: The data obtained from this study indicate that detection of microbial hygiene indicator groups differs by testing methods and suggests that testing for Enterobacteriaceae or total Gram-negatives may represent more comprehensive hygiene indicators than traditionally used coliforms tests.

Poster

P1-59 Single Shift Detection and Real-time Enumeration of *Salmonella* in Poultry Rinsate Samples with the Use of a Ferrofluidic-based Platform

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Introduction: Foodborne illnesses remain a serious public health issue in the U.S, causing more than 48 million cases, 3,000 deaths, and 128,000 hospitalizations each year – an annual economic burden of $77.7 billion. Current detection methods are slow, labor-intensive, and expensive. We have developed a novel ferrofluidic technology platform (PIPER) to offer a rapid, cost-effective alternative for the detection and enumeration of foodborne pathogens in complicated food matrices.

Purpose: (1) To demonstrate PIPER's ability to successfully detect *Salmonella* Typhimurium in commercial poultry rinsate samples with only a 6-hour enrichment. (2) To enumerate spiked concentrations of the same pathogen in these samples with no enrichment.

Methods: The poultry sample (300 g) was rinsed with buffered peptone water (BPW) media (60 ml). For enumeration experiments, rinsate aliquots were spiked with serial dilutions of viable *Salmonella* Typhimurium (n = 6 for each concentration) and analyzed on PIPER (75 mins for 11 simultaneous tests per instrument). For enrichment experiments, poultry rinsate (20 ml) was mixed with BPW (20 ml) and spiked with 0 and 3 CFU of *Salmonella* Typhimurium. After 6 hours of enrichment, aliquots of each culture (n = 5 each) were analyzed on PIPER and compared to selective media plating.

Results: Both enriched and non-enriched samples achieved 100% relative correlation to selective plating. Following the enrichment period, positive samples were distinguished from negative samples with ~30 ppm false positive and false negative error rates (0.9999 tolerance intervals, alpha = 0.05). Without enrichment, PIPER successfully detected *Salmonella* Typhimurium and showed good linearity over a dynamic range of 5 orders of magnitude (R^2 = 0.93). All *Salmonella* Typhimurium concentrations were discriminated with a confidence of 0.995 (alpha = 0.005).

Significance: The PIPER platform could have significant impact in food safety industry with the demonstrated ability to successfully enumerate a common foodborne pathogen in a complex matrix within 75 minutes, and detect its presence within a single work shift.

P1-60 Production of Monoclonal Antibodies against *Vibrio parahaemolyticus* and Their Application to Immunoassay Using Magnetic Nanoparticle

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Introduction: *Vibrio parahaemolyticus* are Gram negative halophilic marine bacteria occurring naturally in the estuarine environment and are one of the major reasons developing acute gastroenteritis.

Purpose: The objectives of this study are to produce monoclonal antibodies (MAbs) against *V. parahaemolyticus* and to develop immuno-selective filtration for the rapid detection of *V. parahaemolyticus*.

Methods: To produce monoclonal antibodies against *V. parahaemolyticus*, heat killed *V. parahaemolyticus* (HKVP) and formalin killed *V. parahaemolyticus* (FKVP) were prepared. The MAb for *V. parahaemolyticus* was produced from cell fusion and cloning. MAb was conjugated to 74 nm magnetic nanoparticles to develop the immuno-selective filtration.

Results: A MAb was produced from HKVP 4H9-9, 16 hybridoma cell. HKVP 4H9-9 MAb was conjugated to 74 nm magnetic nanoparticles to develop the immuno-selective filtration. The detection limit of immuno-selective filtration for *V. parahaemolyticus* was 10^1 cell/ml. The immuno-selective filtration was specific to *V. parahaemolyticus.* Cross-reactivity to *V. alginolyticus* and *S. aureus was.*

Significance: These results strongly support that the application to immuno-selective filtration could be used as rapid, simple and effective methods.

P1-61 *Listeria* Detection after 24 Hours Using a Combined Sampler/Enrichment System and Romer Labs' RapidChek® *Listeria* NextDay™

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Introduction: Environmental sampling for *Listeria* presents challenges in large and complex facilities, such as aseptic handling of sponges and sample enrichment bags on the manufacturing floor, and assembling sample sets for laboratory processing. Sample integrity and sampling workflow may be improved by using a sampler consisting of a pre-wetted, polyurethane foam swab incorporated into the screw cap of a 10 ml enrichment tube. After swabbing, the sample and 1 ml neutralizing broth are delivered back to the tube, to which 9 ml enrichment broth is added.

Purpose: This study evaluated detection of low levels of *Listeria monocytogenes* by RapidChek® *Listeria* NextDay™ after 24 h incubation in a combined sampler/enrichment tube system with 9ml broth, and the traditional sampling sponge and enrichment bag with 60 ml broth.

Methods: For each method, 3 CFU/sample (n = 20) and 10 CFU/sample (n = 5) *L. monocytogenes* were spiked along with 1 x 10^5 CFU/sample competitor background. Samples were cold-stressed 24 h, and then incubated at 30 ± 2°C in the aforementioned volumes of enrichment broth for 24 h before boiling 0.4 ml from each enriched sample and testing by lateral flow device. All samples were confirmed per USDA MLG 8.09 guidelines by streaking to MOX agar, followed by subsequent examination for colonies with *Listeria* spp. morphology and esculin hydrolysis.

Results: At 3 CFU/ml, both sampler/enrichment methods had 19 confirmed positive results. At 10 CFU/ml, both sampler/enrichment methods had five confirmed positive results. With a Mantel-Haenzel $\chi^2$of 0, both sampler/enrichment methods had no false positive or negative results, and indicated 100% sensitivity, specificity and accuracy. The results indicate that both environmental sampling methods evaluated are equivalent.

Significance: The combined swab sampler/enrichment tube method can facilitate improved aseptic sampling, sample management, and laboratory workflow, while reliably detecting low levels of *Listeria* in environmental samples after 24 h incubation using this lateral flow test system.

P1-62 Water Activity Variation at Elevated Temperatures and Thermal Resistance of *Salmonella* in Selected Low-moisture Foods

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❖ ***Developing Scientist Competitor***

Introduction: Water activity (a_w) is a major factor affecting pathogen heat resistance in low-moisture foods. However, there is a lack of data for a_w at elevated temperatures that occur during actual thermal processing conditions, and therefore its influence on thermal tolerance of pathogens.

Purpose: The purpose of this study was to gain in-depth understanding of the relationship between temperature-induced changes in a_w and thermal resistance of *Salmonella* in wheat flour and peanut butter at elevated temperatures.

Methods: Equilibrium isotherms (moisture content vs. water activity) for wheat flour and peanut butter at 20 to 80°C were generated using a vapor sorption analyzer and a newly developed thermal cell with relative humidity sensor, and compared using ANOVA. The thermal resistance (D_{80C}-values) of *Salmonella* in wheat flour and peanut butter with initial a_w of 0.4 (measured at room temperature) were determined via isothermal treatment of small (< 1 g) samples in aluminum test cells in a temperature-controlled water bath.

Results: When increasing sample temperature from 20 to 80°C (at constant moisture content), the a_w of wheat flour increased significantly from 0.4 to 0.7 ($P < 0.05$), but the a_w of peanut butter decreased from 0.4 to 0.01 ($P < 0.05$). The corresponding estimated D_{80C}-values of *Salmonella* in wheat flour and peanut butter with room temperature a_w of 0.4 were 5.9 min and 14.9 min, respectively. The significantly higher D_{80C}-value of *Salmonella* in peanut butter than in wheat flour may be partially attributed to the reduced a_w in peanut butter in comparison to the increased a_w in wheat flour at 80°C, given equivalent a_w values measured at room temperature.

Significance: Improved understanding of the temperature-induced changes in a_w of low-moisture products is critically important in designing and validating thermal processing methods to control *Salmonella* in low-moisture foods.

P1-63 Validation of *Enterococcus faecium* NRRL B2354 as a Surrogate for *Salmonella* in Thermal Treatment of Wheat Flour at Different Water Activities

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Introduction: Non-pathogenic surrogates are important tools for evaluating the efficacy of pathogen-reduction processes for low-moisture food products. *Enterococcus faecium* NRRL B2354 (EFB2354) has been identified as a surrogate for *Salmonella* in validating almond pasteurization processes; however, very limited information is available to support its use in other low-moisture products.

Purpose: The objectives were: (1) To quantify thermal resistance of EFB2354 in wheat flour and compare it to the thermal resistance of *Salmonella* Enteritidis PT30 (SPT30) under the same conditions, and (2) To evaluate the stability of EFB2354 thermal resistance in wheat flour during storage (60 days) at 4°C.

Methods: Wheat flour was inoculated with EFB2354 and SPT30, respectively, and equilibrated to different a_w (0.25, 0.45 and 0.65 for EFB2354; 0.31, 0.43 and 0.60 for SPT30). The samples were sealed in small metallic cells, heated isothermally (70 - 85°C), cooled after treatment, plated, and enumerated to obtain thermal inactivation parameters (D- and z-values). For the storage test, thermal inactivation parameters were obtained every 14 days.

Results: Results indicated log-linear kinetics for both organisms at all a_w levels (R^2=0.93 - 0.99). The D-values of EFB2354 were higher than those of SPT30 at all a_w levels (e.g., at a_w 0.44 ± 0.01 and 75, 80, and 85°C, D-values of EFB2354 were 22.02 ± 2.20, 7.29 ± 2.12 and 2.26 ± 0.04 min, respectively; the corresponding D-values of SPT30 were 9.97 ± 0.46, 5.51 ± 0.22 and 2.11 ± 0.09 min). The z-values of both varied from 10.1 to 21.8°C, depending on a_w. The lowest were at a_w 0.44 ± 0.01, with $z_{EFB2354}$=10.12°C, z_{SPT30}=14.83°C. No significant changes in population or thermal resistance were observed during 60 days at 4°C storage.

Significance: EFB2354 appears to be a valid surrogate for *Salmonella* in thermal treatment of wheat flour, and exhibits stable thermal resistance after 60 days storage at 4°C.

Poster

P1-64 Influence of Biofilm Formation in the Resistance of *Salmonella* to Desiccation and Thermal Treatment in Wheat Flour

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❖ ***Developing Scientist Competitor***

Introduction: *Salmonella* has several defense mechanisms to cope with stresses such as desiccation; biofilm formation has been reported as one. But most defense mechanisms tend to help cope with more than one type of stress; for example *Salmonella* Enteritidis pSB311 forms a biofilm that reportedly may make it resistant to disinfectants and high temperatures.

Purpose: Assess the influence of biofilm formation in resistance to desiccation and thermal treatments of *Salmonella*in wheat flour.

Methods: Seven strains of Salmonella Enteritidis were used, three strong biofilm formers (G3, MD4, and UK1), three weak formers (G2, MD9, P97) and a mutation of G1 (silenced CsgB gene involved in curly fimbriae formation). A lawn or biofilm of *Salmonella*, depending on the strain, was harvested with maximum recovery diluent (MRD), then pelletized, re-suspended in 3 mL of MRD and used to inoculate organic wheat flour (10^8 CFU/g). Inoculated samples were equilibrated to a_w0.45 in a controlled humidity chamber. Aluminum cells were filled with 0.7g of flour, isothermally treated at 80°C in an oil bath, sampled every 5 min up to 20 min (triplicates), cooled, serially diluted and plated on Tryptic soy agar with 0.6% (w/v) yeast extract, 0.05% (w/v) ferric ammonium citrate, and 0.03% (w/v) sodium thiosulfate. Thermal resistance (D-values) was calculated using the first order kinetics model. Significant differences were analyzed with Kruskal-Wallis and Dunn' tests (α=0.05).

Results: D-value for strong biofilm formers (10.2-27.8 min, R^2=0.78-0.89) was significantly higher ($p<0.05$) than weaker formers (3.1-8.0 min, R^2=0.86-0.91), with a strong linear correlation (spearman ρ=1) between biofilm capacity and D value. Population was significantly reduced (-0.28 to -1.49 Log reduction) for all strains ($p<0.05$) after equilibration; with no significant influence of biofilm formation capacity.

Significance: This study found evidence suggesting the influence of biofilm formation on thermal resistance of *Salmonella*, but not on desiccation survival.

Poster

P1-65 Survival and Heat Resistance of Freeze-dried Pathogens on Various Carriers

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Introduction: Dry inocula are often used in challenge or validation studies on low water activity foods. Ingredients like wheat flour, wheat starch, or whey can be used to create dry inocula that mimic a product's formula. The survival of *Salmonella* at low water activities has been well documented for specific species and environments using dry inoculation techniques. However, there are gaps in the literature concerning other foodborne pathogens' survival using dry inocula. In addition, the heat resistance of foodborne pathogens in dry inoculation studies is not characterized.

Purpose: The objectives of this investigation were to evaluate the stability (population and heat resistance changes during storage) of dry inocula by monitoring: (1) survival of *Escherichia coli O*157:H7, *Listeria monocytogenes, Salmonella mons,* and *Staphylococcus aureus* in calcium carbonate, wheat starch, wheat flour, and whey, (2) the change in heat resistance with respect to storage time.

Methods: Dry inocula were prepared by harvesting lawns of *E. coli* O157:H7, *L. monocytogenes* Scott A*, S. mons,* and *S. aureus* grown on tryptic soy agar with 0.1% peptone. Pathogen slurries were frozen overnight at -70°C with one gram of each carrier in separate vials and transferred to a freeze dryer. Inocula were diluted with uninoculated carrier and then stored at 3°C for the duration of this study. Survival and heat resistance were assayed every ten days for 100 days.

Results: Not all carriers are suitable for the four pathogens. For example, the population of *E. coli* O157:H7 freeze-dried on whey powder decreased by three orders of magnitude within the first week of the study. Trends in heat resistance of the pathogens were similar throughout storage. The influence of the carrier on stability and heat resistance was varied.

Significance: Use of carriers for dry inocula in challenge studies must be validated as part of the experimental design.

P1-66 Factors Affecting X-ray Inactivation of *Salmonella* in Low-moisture Foods

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✦ ***Undergraduate Student Award Competitor***

Introduction: Microbial safety of low-moisture foods is an important goal in the food processing industry. Ionizing irradiation, like x-rays, electron beams, or gamma-rays, can inactivate bacteria within the product without significant impact on the quality of the food. However, it is necessary to identify the factors affecting inactivation of pathogens in low-moisture food products, for improved process modeling, design, and validation.

Purpose: The objective was to quantify the effect of X-ray dose, dose rate, and food structure on *Salmonella* inactivation in wheat products.

Methods: Wheat kernels, meal, and flour were inoculated with *Salmonella* Enteritidis PT30, conditioned to 0.4 a_w, and irradiated at three dose levels with high (6.17 Gy/s) or low (0.617 Gy/s, only for flour sample) dose rate using a 70 kV X-ray food irradiator. Treated samples (~1 g) were plated on modified tryptic soy agar, incubated, and enumerated.

Results: Dose rate (high/low) did not affect *Salmonella* inactivation in wheat flour at 0.4 a_w ($P > 0.05$) However, D_{10}-values (up to 2 log reduction) were 0.51, 0.60, and 0.40 kGy for wheat kernel, meal, and flour, respectively, which implies that structural differences can affect X-ray inactivation of *Salmonella* in low-moisture products.

Significance: Considering the fact that low-moisture raw materials are processed to various particle sizes, the parameters of X-ray irradiation should be validated for each product type, even if the chemical compositions are identical.

P1-67 Ionizing Irradiation Inactivated *Bacillus cereus* in Naturally Contaminated Mesquite Pod Flour

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Introduction: Mesquite pod flour has unique cinnamon/chocolate flavor with high sugar content (~40%), and is becoming increasingly employed in conventional and gluten-free applications. As the flour is made from the pods of wild trees (*Prosopis* species), it is prone to contamination with human pathogenic bacteria such as *Bacillus cereus.* Thermal treatment, though effective in killing bacteria, changes its unique flavor. Therefore, non-thermal processing technologies are needed to reduce the population of microorganisms in the flour.

Purpose: The objective of the study was to investigate the efficacy of ionizing radiation in inactivating *B. cereus* and mesophilic bacteria in naturally contaminated mesquite pod flour.

Methods: Two types of mesquite pod flours (*P. alba* and *P. pallida*) naturally contaminated with *B. cereus* were irradiated with gamma rays at doses up to 10 kGy. The survival population of *B. cereus* spores and vegetative cells along with native microflora were determined using selective chromogenic media (after heat activation for spores).

Results: Results showed that the total plate counts were 4.5 and 5.7 log CFU/g and the populations of *B. cereus* were 3.8 and 5.4 log CFU/g in the non-irradiated *P. alba* and *P. pallida* flours, respectively. The populations of *B. cereus* spores were 3.4 and 4.9 log CFU/g in *P. alba* and *P. pallida* flours, respectively, which accounted for at least 80 % of mesophilic spores. The D_{10}-values (radiation dose required to achieve 90% reduction) of *B. cereus* and spores were in the range of 0.94 - 1.55 kGy. Populations of microflora, mesophilic spores, *B. cereus* and *B. cereus* spores decreased with increasing radiation doses. At 6 kGy, the populations fell below 1 log CFU/g.

Significance: Our results demonstrated that irradiation at 6 kGy was sufficient to reduce *B. cereus* population to undetectable level, and the technology may be used to enhance the microbial safety of the low moisture food.

P1-68 Development of Inoculation Methods for *Enterococcus faecium*, a Potential Surrogate Bacteria for *Salmonella,* on Whole Black Peppercorns and Cumin

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Introduction: Increasing illnesses associated with *Salmonella*-contaminated spices has highlighted need for surrogate organisms to validate inactivation methods.

Purpose: To develop inoculation methods for *Enterococcus faecium* on whole black peppercorn and cumin seeds that achieve a minimum of 6 log CFU/g on the surface and are stable for a period of 28 d.

Methods: Whole black peppercorns and cumin seeds were inoculated with *Enterococcus faecium* NRRL B-2354 cells using three inoculation methods: liquid-grown, agar-grown, and biofilm-incorporated cells. Liquid-grown *E. faecium* were incubated 24 h in tryptic soy broth (TSB) at 37°C, washed and applied to spices. Tryptic soy agar (TSA) grown cells were scraped from TSA plates grown for 24 h at 37°C, and applied directly to spices. *E. faecium* was incorporated within a native microbiota biofilm on spices over a 24 h period during static incubation in TSB at 37°C. Inoculated spices were dried to a_w 0.5, and held for 28 days. Spices (n = 3) were sampled after drying (time 0), and after 1, 7, 14, 21, and 28 days of storage. Cells were enumerated following serial dilution and plating onto bile esculin agar (BEA) and TSA.

Results: *Enterococcus faecium* recovery was high after 28 d storage, with greater than 6 log CFU/g recovered from peppercorns and cumin seeds. Significant reductions ($P < 0.04$) in recoverable *E. faecium* from peppercorns occurred only for the TSB grown cells (0.58-log CFU/g reduction) after 28 d. On cumin seeds, larger reductions after 28 days occurred for TSB grown, TSA grown and biofilm incorporated methods (1.2, 0.5 and 0.6-log CFU/g reductions, respectively).

Significance: The inoculation method influenced the recoverability of *Enterococcus faecium* from whole peppercorns and cumin seeds after drying. Developing stable inoculation methods is a crucial step toward determining if *E. faecium* NRRL B-2354 is an appropriate surrogate organism for commercial validation of *Salmonella* inactivation methods for spices.

P1-69 In-package Decontamination of *Salmonella* Montevideo on Black Pepper Using Pulsed Light

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Introduction: Spices are often contaminated with pathogens and typically used as seasonings without undergoing further cooking. Pulsed light system is a rapid non-thermal inactivation technology for controlling foodborne pathogens such as *Salmonella*.

Purpose: In this study, efficacy of pulsed light treatment for inactivation of *Salmonella* Montevideo on black pepper was investigated.

Methods: Black peppercorns (20 g) were dip-inoculated with 2 ml of *Salmonella* Montevideo and air dried for 24 h in a sterile environment. One-gram samples of peppercorns were vacuum packaged in polyethylene bags and then pulsed light treated for 10 to 40 s at a distance of 11.5 to 16.6 cm from the lamp at voltages of 3000 to 3800 V. Reduction in microbial population was enumerated by spread plating onto tropic soy agar with yeast extract.

Results: Increased treatment time increased microbial reduction. Reductions of 2.48, 3.79 and 5.37 log CFU/g were obtained at 20, 30 and 40 s, respectively, at a voltage of 3800 V and distance of 11.5 cm. Increased voltage also resulted in increased inactivation. Reductions of 0.57, 1.13 and 2.48 log CFU/g were obtained at 3000, 3400 and 3800 V, respectively, at 11.5 cm distance after 20 s treatment. Samples treated closer to the lamp were more readily inactivated. Reductions of 3.95, 2.55 and 0.63 log CFU/g were obtained at 11.5, 14.1 and 16.6 cm, respectively, after a 40 s treatment at 3400 V. The effect of treatment time, voltage, and distance were statistically different ($P < 0.05$).

Significance: These results suggest that pulsed light has potential for use for inactivating *Salmonella* on black pepper. Reductions up to 5.60 ± 0.46 log CFU/g were obtained within 40 s indicating that pulsed light can provide rapid decontamination of black peppercorns packaged in the final package.

P1-70 Ethanol Vapor to Inactivate *Salmonella* on Peppercorns

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Introduction: Outbreaks of foodborne illness associated with consumption of contaminated spices have highlighted a need for improved methods of pathogen control in spice products.

Purpose: The purpose of this study was to evaluate the efficacy of ethanol vapor produced by atmospheric and vacuum-assisted boiling to inactivate *Salmonella* on artificially-contaminated peppercorns.

Methods: A mixed biofilm containing four *Salmonella* serovars was established on the surface of black peppercorns. The contaminated peppercorns (5 g) were placed onto loosely packed glass wool in a modified glassware distillation apparatus. Ethanol (95%) was boiled at atmospheric pressure (AB) and vacuum-assisted boiling (VB; - 20 in Hg) conditions, and vapor was in contact with the peppercorns for 1, 5, or 10 min. During treatment, ethanol vapor temperature was measured in the distillation head downstream from the peppercorns. Following treatment, peppercorns

were serially diluted in peptone-Tween-cellulase diluent, surface-plated onto XLT4 and TSA agars, and incubated at 37°C for 24 h prior to colony enumeration.

Results: Ethanol vapor temperature was 72 ± 2°C for AB treatments and 50 ± 2°C for VB treatments. Ethanol vapor treatment at AB conditions resulted in *Salmonella* population reductions of 3.90 and 5.95 log CFU/g on peppercorns after 10 min as determined by plating onto TSA and XLT4, respectively. Under VB conditions for 10 min, *Salmonella* populations decreased by 2.69 and 4.55 log CFU/g as determined on TSA and XLT4, respectively. Native microbiota of uncontaminated peppercorns decreased by only 1.15 log CFU/g (TSA only) after 10 min treatment under AB conditions.

Significance: Atmospheric ethanol vapor treatment produced greater than a 5-log reduction in *Salmonella* populations on contaminated peppercorns.

P1-71 Survival of Five Serotypes of *Salmonella enterica* in Dried Carrots, Dried Minced Onions, White Flour, and Black Pepper Held at 25, 35, and 42°C

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Introduction: Low moisture foods and spices have been associated with 14 different reported outbreaks in the United States from 2007 to 2012. Low moisture foods generally do not undergo thermal processing due possible effects on sensory qualities and functionality of the product. For this reason, low moisture foods pose a potential risk of salmonellosis.

Purpose: Research was performed to test the survival of several serotypes of *Salmonella* in low moisture foods at various temperatures.

Methods: The serotypes used (*Salmonella* Agona*, Salmonella* Montevideo*, Salmonella* Tennessee*, Salmonella* Typhimurium phage type 42*,* and *Salmonella* Weltevreden) were associated with outbreaks in low moisture foods. Dried carrots, dried onions, black pepper, and flour samples were inoculated and stored at 25°C, 35°C, and 42°C. For ten weeks, samples were plated weekly on XLD agar.

Results: *Salmonella* in low moisture foods generally demonstrates biphasic death curve; an initial exponential reduction which tapers off over time. This trend was observed with samples stored at 25°C. The survival of samples stored at 35°C varied depending on the product. Onion and flour samples showed the expected biphasic trend, while the pepper and carrot samples did not. Carrot and pepper samples stored at 35°C showed a greater reduction of 6 logs in the thirty days compared to the onion and flour samples, which showed a 4-log reduction in thirty days. This more rapid decrease continued until no growth occurred at the limit of detection (< 5 CFU/g). All products held at 42°C fell below the limit of detection before samples at other temperatures. A 7-log reduction for samples held at 35°C and 42°C was observed. Once a sample fell below the limit of detection, a qualitative ELISA test showed the presence of *Salmonella* in the samples.

Significance: These results question the biphasic model of survival of *Salmonella* in some low moisture foods at varying temperatures.

Poster

P1-72 Thermal Resistance and Stability of *E. faecium* Using Lyophilized Cells for the Inoculation of Low-Water Activity Foods

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Introduction: Preparation of homogeneous, stable, and quantifiable inoculums is essential in process validation. Low-water activity matrices present an additional challenge as methods must not change product parameters. Using lyophilized cells may be a viable method for inoculating low-water activity foods.

Purpose: This study assessed the homogeneity, stability, and thermal resistance of lyophilized *Enterococcus faecium* during storage after inoculation into low-water activity foods.

Methods: *E. faecium* was grown on tryptic soy agar with 0.6% yeast extract (TSAYE) at 37°C for 24 hours. Cells were harvested and added to 1 liter of tryptic soy broth with 20% polyethylene glycol. The solution was lyophilized, yielding ~8 log CFU/g, and added to ground black pepper and oat flour, separately. Inoculated matrices were mixed for 30 mins then stored at 32% relative humidity at ambient temperature (~25°C). Ten 1-g samples were enumerated to assess homogeneity. Stability and thermal resistance were assessed in triplicate at periodic intervals over 42 days. Thermal resistance was determined by using aluminum test cells in an oil bath and enumerating on TSAYE.

Results: Maximum acceptable variability for homogeneity was defined as < ± 0.3 log CFU/g. Day 42 populations of the black pepper and oat flour were 7.84 ± .16 and 7.86 ± 0.03 log CFU/g, respectively. Initial populations of *E. faecium* in flour and pepper were 8.38 ± 0.11 and 8.38 ± 0.09 log CFU/g, respectively. Although population decreases were only about 0.5 log CFU/g over 42 days, these changes were significant ($P < 0.05$). The $D_{80°C}$-values at days zero and 42, respectively, for *E. faecium* were 2.65 min and 3.52 min in black pepper and 6.04 min and 8.47 min in flour.

Significance: Addition of lyophilized cells appears to provide a homogeneous, fairly stable inoculum in black pepper and oat flour. Care must be taken assessing thermal resistance of the *E. faecium* during a validation process as it may change over the time period examined.

P1-73 Differential Persistence and Survival of *Salmonella* Serovars in Spices with Low-water Activity

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Introduction: Although *Salmonella* outbreaks from low-moisture products are relatively rare, they often impact large numbers of people. Spices represent one such vehicle for transmission to humans. *Salmonella* contamination of spices poses great risks to U.S. consumers since spices are often added just prior to consumption or are ingredients in RTE products.

Purpose: The purpose of this study was to determine the persistence and survival of *Salmonella* serovars in different varieties of spices at low water activity level.

Methods: *Salmonella* serovars (Rissen, Anatum and Montevideo) were inoculated into black pepper, white pepper, ground cumin, and ground celery seed, respectively, at a starting level of 5 log CFU/g and incubated at ambient temperature (25°C) in a desiccator. The water activity in these spices remained low in the desiccator ($a_w < 0.8$). Culture populations were determined at day 0, 1, 2, 3, 4, 5, 6, 7, 14, 28, and 56 post inoculation (dpi).

Results: Although no growth of *Salmonella* was documented in spices at low water activity, *Salmonella* serovars can survive in certain spices for an extended period of time. The survival of *Salmonella* in spices is both serovar dependent and spice dependent. For example, in white pepper, all serovars showed a 2-log decrease after 24 h inoculation. While serovar Rissen was undetectable after 7 days, serovar Montevideo was able to be

recovered continuously through 28 dpi. Among all the varieties of spices investigated, all *Salmonella* serovars survived better in ground cumin than other spice vehicles.

Significance: Certain *Salmonella* serovars can survive in spices even at low water activity level and may persist for an extended period of time. Pinpointing specific growth dynamics of *Salmonella* adapted to persist in various spice substrates will aid in assigning more accurate microbiological risk assessments to this important and widespread food group.

P1-74 Standardizing an Oregano Inoculation Procedure for Use in Challenge Studies on Reduction of *Salmonella* in Dry Spices

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Introduction: Spices have attracted interest among food safety professionals after being found to be the vehicle of *Salmonella* causing foodborne outbreaks. As for other low moisture foods, the FDA has recommended validating lethality treatments to ensure achievement of target log reductions in *Salmonella.* However, standardization of methods to conduct such validation studies is needed and there is little information about the best methods.

Purpose: To standardize a procedure for inoculating oregano as part of challenge studies on decontamination of dry spices.

Methods: Oregano was inoculated with a cocktail of *Salmonella* Rissen and *Salmonella* Montevideo using liquid and dry inoculation procedures. Liquid inoculation (LI) involved two inoculation steps, each mixing 10 ml of the *Salmonella* cocktail (10.2 log CFU/ml) with 50 g of oregano. After each inoculation, oregano was dried at 35°C until it reached its original a_w (0.45). Dry inoculation (DI) was conducted by mixing the oregano with silica or talc powder (TP) containing (8.4 and 6.3 log CFU *Salmonella*/g, respectively). *Salmonella* was then recovered by plating on TSA, incubating at 35°C for 3 h and then overlaying with XLT4 before continuing incubation up to 24 h.

Results: For LI, counts of *Salmonella* in the oregano were 3.8 log CFU/g after the first step and 6.6 log CFU/g after the second step, whereas for DI the counts were 6.7 log CFU/g and 6.0 log CFU/g when using silica and TP, respectively. The mean concentration of *Salmonella* in oregano when using only the first LI was significantly different from the second LI and the DI procedures ($P < 0.05$).

Significance: These methodologies can potentially be extended to other spices for evaluating decontamination treatments in the laboratory, or conducting in-house validation studies using surrogate organisms instead of *Salmonella*.

P1-75 Environmental Adaptation and Stress Response Mechanisms of *Salmonella enterica* in Peanut Oil, Peanuts and Chia Seeds

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Introduction: Outbreaks of *Salmonella* in recent years have been linked to an assortment of low-water activity (a_w) foods, such as nuts, peanut butter and chia seed powder. The unusual emergence in environments that should otherwise promote death highlights the need for elucidation of response networks that enhance survival.

Purpose: The purpose of this study is to evaluate the response of *Salmonella enterica* to two stressors commonly encountered in low-a_w food processing, desiccation and heat.

Methods: Five serotypes of *S. enterica* were desiccated in three food matrices with varying a_w: peanut oil (a_w = 0.560), peanuts (a_w = 0.330) and chia seeds (a_w = 0.585) to identify survival characteristics in low-a_w environments. Oil-desiccated cells were subsequently used in heat experiments to identify cross-protective mechanisms. In these assays, tolerance to lethal heat at 70°C following six-day oil desiccation was evaluated. Survival at 70°C following exposure to a sub-lethal heat of 45°C was also assessed.

Results: *S. enterica* exhibited serotype-specific differences in survival capabilities in the low-a_w foods. Serotypes Hartford and Tennessee demonstrated an aggressive phenotype across all three low-a_w foods, while Typhimurium was identified as the least aggressive serotype. Interestingly, persistent serotypes survived to upwards of 90 days in peanut oil. Exposure to 70°C following six-day oil desiccation revealed significantly ($P < 0.05$) higher concentrations in all serotypes compared to non-desiccated controls. Moreover, significantly ($P < 0.05$) higher concentrations were observed following pre-adaptation to 45°C.

Significance: These results indicate that *S. enterica* is capable of long-term desiccation in low-a_w foods and can lead to enhanced resistance to heat treatments. These are problematic to the food industry, which often use these two hurdles in tandem to reduce *Salmonella* contamination.

P1-76 Influence of Monostearin on the Survival of *Salmonella* in a Low-water Activity Peanut Protein Model Food System at 37°C and 70°C

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Introduction: Several multi-state outbreaks of *Salmonella* in low-water activity (a_w) foods have raised concern of its survival in peanut-based products. *Salmonella* has demonstrated the ability to survive in low a_w foods for long periods of time. Peanut butters may be formulated with emulsifiers containing monoglycerides, some of which are reported to have antimicrobial effects.

Purpose: The purpose of this study was to investigate the role of monostearin on the survival of *Salmonella* in a peanut flour model food system at a_w of 0.46 at 70°C and 37°C.

Methods: Monostearin was homogenized with 50% (w/w) defatted peanut flour and 55% (w/w) peanut oil to obtain 0% and 1.625% (w/w) emulsifier concentrations and was equilibrated to a_w = 0.46 (± 0.04). A dried cocktail of *Salmonella* Agona*, Salmonella* Montevideo*, Salmonella* Tennessee*,* and *Salmonella* Typhimurium was added and the samples were treated at 70°C for 48 h and 37°C for 28 d. Surviving *Salmonella* were recovered at various times using supplemented tryptic soy agar and log CFU values were compared at each time point using two-way ANOVA.

Results: At 70°C, survival of *Salmonella* was not affected by monostearin ($P = 0.28$, $\alpha = 0.05$). The average log reduction was 3.02 with monostearin and 3.59 without monostearin after 48 h. At 37°C, survival was not affected by monostearin ($P = 0.80$, $\alpha = 0.05$). The average log reduction was 1.94 with monostearin and 1.78 without monostearin after 28 d.

Poster

Significance: This data indicates that monostearin does not affect the survival of *Salmonella* in peanut-based products at a_w = 0.46 at 70°C for 48 h and at 37°C for 28 d. These findings will be useful for predictive modeling of *Salmonella* survival in a low-water activity (a_w = 0.46) model food system.

P1-77 Re-Interpretation of Water Activity Effects on Temperature Dependency of *Salmonella* Inactivation in Low-moisture Foods

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Introduction: Understanding *Salmonella* thermal resistance in low a_w products is critical for process design and validation, as well as for developing models that are more mechanistic, robust, and reliable. Although several studies have reported secondary models for a_w and temperature effects on thermal resistance, none has linked the differences in these effects across products to underlying differences in the moisture isotherms of those products.

Purpose: The objective was to quantify how a_w affects the temperature dependency of *Salmonella* inactivation kinetics in low a_w products, and to evaluate commonalities and differences within and across product categories with differing equilibrium isotherm characteristics.

Methods: We recovered decimal reduction time values (D-values) reported for multiple low a_w products (including wheat flour, whey protein, and multiple peanut butter products). Those D-values were plotted and analyzed as functions of temperature and a_w. Then the temperature changes required to reduce the D-value by 90% (z_T) were calculated and analyzed in relation to a_w.

Results: The observed D-values showed a reasonable log-linear decrease with temperature, but the relationship with a_w varied among products. In peanut butter, a positive correlation ($P < 0.05$) was observed between z_T and a_w (i.e., z_T increasing > 60% with room-temperature a_w increasing from 0.2 to 0.8), while for low-fat peanut butter, whey protein, and wheat flour, a negative correlation was observed ($P < 0.3$, < 0.15, and < 0.05, respectively), with a very similar pattern across these three products.

Significance: These results reinforce the need for a better understanding of the role of a_w in inactivation processes. The results are consistent with previously reported tendencies for low a_w products and support the possibility that a significant part of the differences observed across products might be related to physicochemical aspects of moisture equilibrium, parallel to the biological explanations that have been proposed.

P1-78 *Salmonella* and Other Enterobacteriaceae at Peanut Processing Stages

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Introduction: Due to recent outbreaks, peanuts have been considered a product of potential risk for *Salmonella*. In spite of this, little is known about the occurrence of *Salmonella* in peanut processing. Due to recent outbreaks, peanuts have been considered a product of potential risk for *Salmonella*. In spite of this, little is known about the occurrence of *Salmonella* in peanut processing.

Purpose: The aim of this study was to investigate the presence of *Enterobacteriaceae*, coliforms, *Escherichia coli* and *Salmonella* in peanuts during processing stages (drying, shelling, sorting, blanching, roasting) in Brazil.

Methods: One hundred samples from six processing plants were analyzed. *Salmonella* detection was carried out in 250 g of each sample by an immunoassay method - Mini-Vidas System. *Enterobacteriaceae* was enumerated in Violet Red Bile Agar with Glucose (VRBG), and Petrifilm 6404 was used for total coliforms and *E. coli*. The pH value and the water activity (a_w) of the samples were also determined.

Results: The a_w of the media was reduced to < 0.56 after drying and to < 0.35 after roasting step. The pH values ranged from 5.91 to 7.13. Samples showed *Enterobacteriaceae* counts between < 1 and 7.98 log CFU/g. The highest total coliform counts (7.32 log CFU/g) were observed before the drying step. *E. coli* was not detected in any analyzed sample, whereas *Salmonella* was isolated in two samples (0.004 MPN/g) collected during drying in the same processing plant.

Significance: Therefore, the results show that peanuts could be contaminated by *Salmonella* during post-harvest stages. This reinforces the importance of a well-designed roasting step to obtain a safe final product.

P1-79 Factors Affecting Dry Cross-contamination of *Salmonella* during Almond Processing

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Introduction: Outbreaks of *Salmonella* associated with low-moisture foods, such as almonds, are an important concern in food processing, which necessitates an improved understanding of the mechanism of bacterial transfer and physical/environmental factors affecting the process.

Purpose: The goal of this study was to quantify the effect of physical and environmental factors on the transfer of *Salmonella* within bulk almonds.

Methods: Un-inoculated almond kernels (200 g) and inoculated almonds (5 g, 7.85 log CFU/g) were conditioned at 0.2 and 0.4 water activity (a_w), placed in a stainless-steel drum (140 mm diameter and 64 mm depth), and rotated for four durations (60 - 600 s) with three rotational speeds (8, 16, and 24 rpm) in an environment chamber (in triplicate). At each condition, a four almond sample (~4 g) was retrieved from the drum, plated on modified tryptic soy agar, incubated, and enumerated.

Results: Bacterial transfer rate was compared based on physical and environmental factors. Bacterial transfer rates were higher ($P < 0.05$) at the higher a_w (0.0009 ± 0.0006% and 0.006 2 ± 0.0050% at 0.2 and 0.4 a_w, respectively). As the total number of revolution increased, the total bacterial transfer reached statistically different (95% CI) asymptotic values, 3.02 ± 0.13 and 3.80 ± 0.20 log CFU/g. However, the rotational speed did not affect ($P > 0.05$) transfer rates.

Significance: Environmental factors, such as a_w, appear to be critical factors affecting bacterial transfer in low-moisture products. A subsequent secondary model as a function of a_w will significantly increase the accuracy of an existing first-principle-based discrete element model, which will ultimately contribute to more accurate risk modeling and assessment for low-moisture food safety.

P1-80 Modeling of Cross-contamination of *Salmonella* during Almond Processing

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Introduction: Although low-moisture foods have been believed safe, recent outbreaks have brought safety concerns over how low-moisture food contamination occurs and how the contaminated foods travels through a system and are affected by the environment. To understand the nature of cross-contamination in low-moisture food products, first-principle models can be used to understand the interactions between dry particles and bacterial pathogens.

Purpose: The objective was to develop a first-principle based discrete element model of bacterial transfer and to assess the model performance.

Methods: Utilizing a Discrete Element Modeling software (LIGGGHTS: LAMMPS Improved for General Granular and Granular Heat Transfer Simulations), 200 almonds in a rotating drum being mixed with 5 inoculated almonds in a low-water activity environment at a rate of 8 rpms was simulated. The first model uses an analogy of heat transfer mechanics to bacterial transfer. Using a more fundamental approach, the second model depicts the electrostatic force between a simulated bacterial particle attached to a simulated almond particle. The Hamaker Constant of the Van der Waals equation for electrostatics was optimized to simulate realistic *Salmonella* adhesion properties. Both simulations were validated against experimental results of the same environment.

Results: Comparison between experimental results and simulation results using heat transfer as an analogy demonstrated an accurate representation of the system, with RMSE = 0.070 log(CFU/g). Comparison of results for electrostatic bacterial modeling and experimental results yielded RMSE = 0.108 log(CFU/g). Despite being less accurate of a fit, the electrostatic model provides fundamental insights into the mechanics of cross contamination and direct representation of the phenomena.

Significance: A first-principle based discrete element model can be used as a useful tool to elucidate the mechanism of bacterial transfer in low-moisture environment. Ultimately, the model will be used for scale-up validation and risk modeling to design safe process.

P1-81 Evaluation of the Thermal Resistance of *Enterococcus faecium* NRRL B-2354 and *Salmonella* Enteritidis PT30 on Four Different Almond Products

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Introduction: Almond Board of California (ABC) published protocols for the heat resistance verification of *Enterococcus faecium* NRRL B-2354 on inoculated almonds as a surrogate for *Salmonella* Enteritidis PT30. Since the heat resistance of *Enterococcus* and its correlation to *Salmonella* was completed using whole intact almonds to develop the guidelines, ABC was interested in evaluating the heat resistance of *Enterococcus* and *Salmonella* on other types of almond products during 28 days of storage.

Purpose: Evaluate the heat resistance of *Enterococcus* and *Salmonella* on Nonpareil whole, Monterey whole/broken, Natural splits, and Natural dice in an oven at 280°F/15 min after 0, 14, and 28 days of storage at 4°C.

Methods: Almond products were inoculated with *Enterococcus* or *Salmonella* and stored at 4°C for 28 days. The inoculated almonds were heat-treated after 0, 14, and 28 days of storage. Log reductions for almond products following heat treatment at each sampling time were compared using ANOVA.

Results: Log reductions of *Enterococcus* or *Salmonella* for each type of almond (except *Enterococcus* on Monterey whole/broken) following heat treatment over 28 days of storage were not significantly different ($P > 0.05$) and ranged between 0.29 ± 0.20 and 2.37 ± 1.09 logs (n = 9), or 0.91 ± 0.33 and 2.26 ± 0.59 logs (n = 9) for *Enterococcus* or *Salmonella*, respectively. No significant differences ($P > 0.05$) were observed between log reduction of *Enterococcus* and *Salmonella* for each type of almonds at each sampling time. Heat resistance of *Enterococcus* or *Salmonella* at each sampling time was significantly different ($P < 0.05$) based upon almond type; however, the average log reduction for all cases was less than 2.5 logs.

Significance: The shelf life of Nonpareil whole, Natural splits, and Natural dice almonds inoculated with *Enterococcus* can be extended to 28 days at 4°C without significant impact to the heat resistance of this organism. *Enterococcus* is an appropriate surrogate for *Salmonella* on all four almond types.

P1-82 Comparing the Effect of Product Structure on Thermal Resistance of *Salmonella* Enteritidis PT30 on/in Almond and Wheat Products

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❖ ***Developing Scientist Competitor***

Introduction: Low-moisture pasteurization processes must be validated to ensure efficacy. Although product characteristics are known to affect *Salmonella* thermal resistance, the impact of product structure is not well known or reported.

Purpose: The objective was to quantify the effect of structure on thermal resistance of *Salmonella* Enteritidis PT30 on/in multiple almond and wheat products.

Methods: Raw almonds, surface-damaged almonds, blanched almonds, and whole wheat kernels were surface-inoculated with *Salmonella* Enteritidis PT30 (~10^8 CFU/g). Almond meal, almond butter, wheat meal, and wheat flour were fabricated by grinding the same raw almonds or wheat kernels in a food processor, and inoculated with the same *Salmonella* (~10^8 CFU/g). All inoculated products were equilibrated to ~0.4 water activity (a_w) in controlled-humidity chambers. The inoculated whole almond and wheat kernels (vacuum-packaged in thin layer plastic bags) and fabricated samples (in aluminum test cells, sample thickness < 1 mm) were heated (in triplicate) in an isothermal water bath (80°C), pulled at multiple intervals, cooled in an ice bath, diluted in peptone water, and plated on modified tryptic soy agar to enumerate survivors.

Results: *Salmonella* thermal resistance ($D_{80°C}$) was greater ($P < 0.05$) on/in all the almond products than on/in the wheat products. The $D_{80°C}$ values on raw, damaged, and blanched almonds were statistically equivalent, but significantly less ($P < 0.05$) than in almond meal and butter of equivalent composition. Moreover, the $D_{80°C}$ value in wheat meal was significantly higher ($P < 0.05$) than on/in whole wheat surface and flour.

Significance: Product structure can affect *Salmonella* thermal resistance; however, that effect differs by product. The high oil content in the almond products may have caused the difference, as compared to the wheat products. In either case, knowledge of structure effects on thermal resistance is important to ensure accurate validation of pathogen intervention processes.

P1-83 Reduction of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on Inoculated Almonds Exposed to Different Heat Treatments

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Introduction: Outbreaks of salmonellosis and *Escherichia coli* O157:H7 gastroenteritis have been associated with consumption of tree nuts, and isolation of *Listeria monocytogenes* has led to recalls of these products. Thermal treatments used by the almond industry have been validated to inactivate *Salmonella* but little is known about reductions achieved for other pathogens.

Purpose: To determine the heat resistance of *E. coli* O157:H7 and *L. monocytogenes* on inoculated almonds.

Methods: Almond kernels were inoculated with *Salmonella* Enteritidis PT 30 (control), *E. coli* O157:H7 (five isolates from produce- or low-moisture-food–associated outbreaks), or *L. monocytogenes* (five isolates from produce-associated outbreaks) at 9 log CFU/g, and dried for 72 h and equilibrated (48 - 72 h) to a moisture content of 4.5 to 5%. Almonds were exposed to hot oil (121°C for 30 and 60 s), hot water (80°C for 30 and 60 s), or a dry oven (138°C for 15 min). Survivors were enumerated by plating on tryptic soy agar and appropriate selective media.

Results: During desiccation, average population decreases of 0.6, 1.8 (range: 1.1 - 2.7 log CFU/g), and 1.3 log CFU/g (range: 1.2 - 1.4 log CFU/g) were observed for *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes*, respectively. Reductions of *E. coli* O157:H7 (3.1 - 5.8 and 1.1 - 4.8 log CFU/g) and *L. monocytogenes* (4.5 - 5.0 and 2.3 - 2.7 log CFU/g) were similar to or greater than those of *Salmonella* (3.1 and 1.4 log CFU/g) after the hot oil (60 s) and dry oven treatments, respectively. Among the *E. coli* O157:H7 isolates, NML#11-1865, isolated from walnuts, was consistently more resistant to desiccation and to hot oil and dry oven treatments. Reductions in hot water (60 s) were similar among all strains evaluated (2.6–3.9 log CFU/g).

Significance: Among the isolates evaluated, *E. coli* O157:H7 and *L. monocytogenes* display similar or lesser thermal tolerance than *Salmonella* Enteritidis PT 30 on inoculated almonds.

P1-84 Presence and Levels of *Salmonella* on Raw California Pistachios

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Poster

Introduction: Little is known about the prevalence, levels, and distribution of *Salmonella* in most tree nuts. Such data are important in developing quantitative microbial risk assessments and scientifically-sound product sampling schemes for verification of food safety plans.

Purpose: The objective of this study was to determine the prevalence, levels, and distribution of *Salmonella* in raw California pistachios.

Methods: Pistachio samples were collected over 3 years from storage silos located at seven pistachio handlers representing ~98% of California production. Samples were divided between nuts from the sinker and floater streams (typically representing 85% and 15%, respectively, of the total harvest). Samples were stored at 4°C prior to enriching 100-g subsamples for the presence of *Salmonella*. Levels of *Salmonella* in positive samples were determined using a most-probable-number (MPN) method. Isolates were serotyped, phage typed when appropriate, and PFGE patterns were determined.

Results: Thirty-two of 3,968 100-g samples of pistachios (0.81%) were positive for *Salmonella*: 11 of 1,152 (1.0%), 6 of 1,380 (0.43%) and 15 of 1,436 (1.0%) in 2010, 2011, and 2012, respectively. The average prevalence in sinkers and floaters was 0.37% (11/2,936; range 0.10–0.71%) and 2.0% (21/1,032; range 0.63–3.7%), respectively. In every sinker sample levels of *Salmonella* were 0.0046 MPN/g [95% Confidence Limits (CL) 0.00064, 0.034 MPN/g]; the geometric mean level in floater pistachios was 0.012 MPN/g (range 0.0046 [95% CL 0.00064, 0.034 MPN/g] to 0.43 [95% CL 0.10, 1.8 MPN/g]). The 63 *Salmonella* isolates were serotyped as follows: Enteritidis (6; phage type 9c (3), 37 (1) and routine dilution non conformity (3)), Montevideo (31), Liverpool (9), Senftenberg (8), Tennessee (1), and Worthington (8). One (Tennessee and Worthington) or three (Enteritidis, Liverpool, Montevideo, and Senftenberg) PFGE patterns were identified within each serovar; most PFGE patterns were observed in more than 1 year of pistachio sampling.

Significance: *Salmonella* can be isolated from raw pistachios at low prevalence and levels, with distinct differences between sinker and floater streams.

P1-85 Drying and Decontamination of Pistachios by Sequential Infrared and Hot Air Drying

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Introduction: Sorting of dehulled pistachios in water results in significant moisture gain on the nut surface. Therefore, quick drying of pistachios is vital to avoid shell staining, decay and microbial contamination. The current hot air drying practices for pistachios have low drying rates, high labor and energy inputs, and do not guarantee safe products.

Purpose: The objectives of this research were to develop a sequential infrared and hot air (SIRHA) drying method for pistachios and evaluate its effectiveness to perform simultaneous drying and decontamination of pistachios.

Methods: Dehulled and sorted pistachios obtained from the Central Valley of California were dried in the SIRHA dryer using different combinations of time, and Infrared (IR) radiation and hot air drying to determine the optimum drying conditions. Pistachios were inoculated with *Pediococcus* and dried using the SIRHA dryer to evaluate the decontamination effect of SIRHA method.

Results: The results showed that the SIRHA drying of pistachios (IR heating for 2 h at a product temperature of 70°C followed by hot air drying at 70°C) reduced the total drying time by 25.93% to lower the pistachios moisture content from 38.14% (w.b.) to 8.90% compared with hot air drying at 70°C alone. Drying of pistachios in the SIRHA dryer using IR heating at 70°C for 20 min, 1 h, and 2 h and holding the pistachios at 70°C for 1 h followed by hot air drying to 8.90% MC resulted in *Pediococcus* population size reductions of 5.41, 5.60 and 6.39 log CFU/g, respectively, for pistachio kernels and 5.45, 5.42 and 5.29 log CFU/g, respectively, for pistachio shells. No significant difference in peroxide value and free fatty acid contents was observed for the pistachios dried with SIRHA method and hot air drying (70°C).

Significance: The results of this study confirmed the effectiveness of the SIRHA drying technology to achieve simultaneous drying and decontamination of pistachios.

P1-86 Thermal Inactivation of *Salmonella* and *Enterococcus faecium* during Pecan Conditioning Treatments

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❖ ***Developing Scientist Competitor***

Introduction: Validating the processes and equipment used by the nut industry is necessary under the Food Safety and Modernization Act. Currently, FDA recommends nut processors (except almonds) to use a process that is validated to reduce *Salmonella* by 5 log CFU/g. The use of non-pathogenic surrogate instead of the pathogen is recommended for validation studies.

Purpose: Our objective was to evaluate the suitability of *Enterococcus faecium* (ATCC 8459) as a surrogate of *Salmonella* during hot water conditioning treatment of pecans.

Methods: In-shell pecans (Southern Improved) were inoculated to ca. 7 log CFU/g after drying to the original moisture content and water activity, with either a 5-strain *Salmonella* cocktail or *E. faecium*. The cocktail of *Salmonella* included serotypes Enteritidis PT 30, Enteritidis PT 9c, Oranienburg, Seftenberg and Tennessee. In-shell pecans (50 g) were submerged in a water bath (n = 6) at 75 ± 1°C (20, 40, 80, 120 s), 80 ± 1°C (20, 40, 80, 120 s), 85 ± 1 (20 40, 80, 120 s), 90 ± 1°C (20, 40, 60, 80 s) and 95 ± 1°C (20, 40, 60, 80 s). Treated nuts were added to 100 ml of cold TSB, cracked with a hammer and stomached for 1 min. Populations were enumerated onto selective and non-selective media supplemented with nalidixic acid (50 µg/ml).

Results: Under all treatment conditions, log reductions (log N/No) of *E. faecium* were either not significantly different from ($P > 0.05$), or significantly lower than ($P < 0.05$), *Salmonella*. A maximum of 4.8-log reduction was observed following exposure to 95 ± 1°C water for 80 s. Reduction data did not fit into a linear or Weibull model.

Significance: *E. faecium* can be successfully used by pecan industry to validate hot water conditioning treatments. As no model to predict reductions during conditioning was developed, the pecan industry should validate individual protocols for achieved log reductions.

P1-87 Stability of *Salmonella* Inoculum on Hazelnuts

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Introduction: Due to a number of recent outbreaks associated with tree nuts, industries are in need of evaluating and validating effective processing steps to reduce *Salmonella.* The hazelnut industry desires the validation of effective processes for reducing *Salmonella* that could be easily implemented without being cost-prohibitive. Effective inoculation methods and inoculum stability has not been determined for hazelnut products.

Purpose: Develop consistent inoculation procedures for *Salmonella* on hazelnut products and determine stability under storage conditions.

Methods: *Salmonella* strains previously associated with nuts (n = 5; various serovars) were lawn harvested from tryptic soy agar (10 log CFU/ml). Harvests (25 ml) were mixed with in-shell hazelnuts (400 g; *Corylus Americana* var. Barcelona). Nuts were dried in a biological safety cabinet at ambient temperature for 24 h prior packaging and storage at 4°C. Inoculated hazelnuts were enumerated after 1 and 5 days of storage using standard dilution and plating methods on Xylose Lysine Desoxycholate (XLD) Agar following incubation (37°C, 24 - 48 hours).

Results: Immediately following inoculation, *Salmonella* levels on hazelnuts were 9.08 ± 0.23 log CFU/g. A 24-h drying time led to a modest decrease in the inoculum to a level of 8.56 ± 0.43 log CFU/g. Inoculum levels remained high (7.93 ± 0.33 log CFU/g) throughout 5 days of storage at 4°C. *Salmonella* Anatum and *Salmonella* Enteritidis PT9C levels were the most negatively impacted by storage.

Significance: Inoculation methods and subsequent storage at 4°C (up to 5 days) achieved and maintained suitably high concentrations for planned process validation studies.

P1-88 Effect of Water Activity on the Thermal Tolerance and Survival of *Salmonella enterica* Serovars Tennessee and Senftenberg in Milk Caramel

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Introduction: Ensuring the safety of water activity (a_w) controlled products is challenging due to the enhanced heat tolerance and survival mechanisms that *S. enterica* has shown in low a_w environments. Few studies regarding thermal tolerance and survival of *S. enterica* in foods with a_w ranging between 0.85 and 0.93 have been reported.

Purpose: We evaluated the effect of a_w on the D- and z-values, as well as survival of *S. enterica* serovars Tennessee and Senftenberg in milk caramel.

Methods: The decimal reduction time (D-value) at 76, 78 and 80ºC, change in temperature necessary to produce a 10-fold change in D-value (z-value), and survival at 20.0 ± 0.5ºC for 32 weeks of two *S. enterica* serovars were determined in goat milk caramel samples adjusted to three a_w values (0.85, 0.90 and 0.93). Experiments were performed in triplicate.

Results: A three-way ANOVA with log-transformed D-value as response ($r^2 = 0.9969$) showed a significant triple interaction between nominal factors serovar, temperature and a_w ($P < 0.0001$). Highest tolerance was observed at a_w 0.85 for *S. enterica* Senftenberg ($D_{76ºC}$ 2.9 ± 0.3 min), and lowest at a_w 0.93 for the Tennessee serovar ($D_{80ºC}$ 0.131 ± 0.007 min). A two-way ANOVA with log-transformed z-value as response ($r^2 = 0.9834$) showed a significant interaction between nominal factors serovar and a_w ($P < 0.0001$). Regardless of the tested serovar, no consistent trends were observed for the z-values at the evaluated a_w levels. A logistic decay model was fitted to all survival curves (r^2 ranged between 0.9857 and 0.9963). Although greater than 8-log reductions were observed at week 30 of storage, low levels of *Salmonella* were still found in the product at week 32.

Significance: Our findings contribute to the establishment of critical limits for the safe thermal processing of a_w controlled products. We also stress the relevance of maintaining good manufacturing and sanitation practices as a preventative action during processing of a_w controlled products.

P1-89 Heat Resistance of *Salmonella* Species in Confections Containing Sensitive Ingredients during Candy Processing

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Introduction: Candy making is an artisanal process with some modern recipes dating back decades and even centuries; long before the advent of modern food safety techniques. Candy processes are assumed to produce microbiologically safe foods due their high processing temperatures, generally greater than 100°C. However, this assumption is dangerous as candies often contain sensitive ingredients like, chocolate, flour, or whey,

which are known vectors for *Salmonella* and the low water activity of confections can contribute to the heat resistance of *Salmonella*. Since thermal processes vary between products and facilities these processes must be validated as a biological control step to ensure food safety.

Purpose: This study investigated the heat resistance of *Salmonella* spp. during typical confectionary thermal processing in model caramel and licorice matrices.

Methods: The model caramel and licorice ingredients were heated and mixed to mimic a pre-processed product. The caramel and licorice were then dry inoculated with two different *Salmonella* cocktails made on whey and wheat flour, respectively. Aliquots were sealed in Whirlpak bags which underwent thermal processing in an oil or water bath. Caramel and licorice samples were treated at temperatures between 80°C and 105°C. The data was analyzed to determine D-values and z-values.

Results: The water activities of caramel and licorice were between 0.4 - 0.6. The caramel $D_{85°C}$ and $D_{90°C}$ of *Salmonella* spp. were 196 and 98 seconds. The licorice $D_{80°C}$ and $D_{85°C}$ of *Salmonella* spp. were 29 and 24 seconds respectively. In both candies, regardless of A_w or inoculum type, instantaneous multi-log reductions of the *Salmonella* spp. at 100°C were observed.

Significance: The data suggests that if a minimum temperature of 100°C is reached in a confectionary process then the process can be can be considered a pathogen control step for caramel and licorice.

P1-90 Survival of *Salmonella* in Cookie and Cracker Sandwiches Containing Inoculated, Low-Water Activity Fillings

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Introduction: Low-Water activity (a_w) fillings in cookie and cracker sandwiches have not been considered as likely vehicles of foodborne pathogens. An outbreak of salmonellosis associated with a marshmallow confectionery and observations that *Salmonella* can survive for 8 months or more in fondant, halva, and dried fruits, however, have raised interest in learning more about its survival in high-sugar products.

Purpose: The purpose of this study was to determine the rate of inactivation of *Salmonella* in crème and non-crème fillings in cookies and cracker sandwiches.

Methods: Two types of cookies, crackers, and high-sugar fillings (a_w 0.18), along with peanut butter- and cheese-based fillings (a_w 0.25), were obtained from commercial sources. Sandwiches containing fillings dry- or wet-inoculated with *Salmonella* at high and low levels were stored at 25°C for 1 and 6 days and for 3, 5, 10, 16, and 26 weeks. Sandwiches were analyzed for populations and presence (by enrichment) of *Salmonella*.

Results: At initial populations of 3.4 and 3.6 log CFU/g of chocolate crème and peanut butter crème cookie sandwiches, *Salmonella* survived for at least 26 weeks; initially at 0.38 log CFU/g, the pathogen survived for 5 and 10 weeks, respectively. Initially at 2.9 and 3.4 log CFU/g of cracker sandwiches containing peanut butter- and cheese-based fillings, respectively, *Salmonella* survived for 26 and 16 weeks; initially at 0.53 log CFU/g, the pathogen survived in cracker sandwiches containing peanut butter-based and cheese-based fillings for 6 days and 5 weeks, respectively. Inactivation was more rapid in wet-inoculated peanut butter crème filling than in dry-inoculated filling but unaffected by type of inoculum in peanut butter-based filling.

Significance: The ability of *Salmonella* to survive for up to 26 weeks in cookie and cracker sandwiches demonstrates a need to assure that filling ingredients do not contain the pathogen and contamination does not occur during manufacture.

P1-91 Effect of Thermal Processing on the Survival of *Salmonella* Species, *L. monocytogenes*, and *E. coli* O157:H7 during the Baking Process of Fruit-filled Grain Bars

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Introduction: Recent recalls and outbreaks due to foodborne pathogens in thermally processed low moisture foods highlight the need for food industries to validate their thermal process.

Purpose: The purpose of this study was to evaluate the thermal inactivation kinetics of *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7 during the baking processes of fruit-filled grain bars.

Methods: Fruit-filled grain bar samples were obtained from an internal manufacturing facility. The samples were individually inoculated with cocktails of *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7 at the interface between the filling and the dough to achieve a target level of 10^8CFU/g. Following inoculation, samples were baked at oven settings of 300°F or 340°F for 9 min. The process parameters were chosen based on a worse-case scenario that is representative of the commercial processing conditions. Internal product temperatures were monitored and recorded during baking. Inoculated samples were enumerated using scientifically validated methods. All experiments included 3 replicates. Water activity analysis was also performed before and after baking. Log transformed data were analyzed using one-way ANOVA model with 95% prediction intervals to determine the minimum thermal processing parameters required to achieve a 4- or 5-log reduction of targeted pathogens.

Results: Statistical data analysis indicated that the internal product temperatures of 173.3°F and 189.9°F (for oven setting of 300°F and 350°F, respectively) at the end of 9 minutes baking process delivered a minimum 4- and 5-log reduction ($P < 0.05$), respectively, in *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7.

Significance: The study findings indicate that the thermal process employed by ConAgra Foods cereal bar manufacturing facility achieves an acceptable lethality of the vegetative pathogens evaluated in this study. The data also provide scientific basis to support the management of baking as an operational pre-requisite program (not a CCP) in the facility's food safety plan.

P1-92 A Pathogen Survival Kinetics Model for a Low-water Activity Environment and in Low-water Activity Foods

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❖ ***Developing Scientist Competitor***

Introduction: Food poisoning induced by pathogenic bacteria occasionally occurs through the ingestion of low water activity (a_w) foods. *Salmonella enterica* and pathogenic *Escherichia coli* are highly tolerant to desiccation compared with other bacterial species. Modeling the survival kinetics of both bacterial species as a function of a_w and temperature could be useful for microbial risk assessments for these bacteria.

Purpose: This study aimed to develop predictive models that can be used to estimate the number of bacteria in low a_w environmental conditions and low a_w foods.

Methods: We investigated the survival kinetics of 4 serotypes of *Salmonella enterica* (Stanley, Typhimurium, Chester, and Oranienburg) and 3 serotypes of *Escherichia coli* (O26, O111, and O157) when exposed to a combination of three temperatures (5°C, 15°C, and 25°C) and 5 a_w conditions (0.22, 0.43, and 0.93). The bacterial cultures (20 µl) were placed onto sterile plastic plates and dried in a safety cabinet for 4 hours. After drying, the plates were placed in plastic containers that were adjusted to each a_w condition using saturated salt solutions. In addition to these *in vitro* experiments, we examined the survival kinetics of these bacterial species on the surfaces of chocolate, cheddar cheese, almonds, and radish sprout seeds under various temperatures.

Results: Regardless of the a_w and the serotype, a rapid decrease in the number of viable bacterial cells was observed at 25°C compared with 5°C. The survival kinetics followed a Weibull distribution. The estimated Weibullian model parameters were described as a function of temperature but not of a_w.

Significance: The results of this study enable the prediction of the number of pathogenic bacteria in low a_w foods under various storage temperatures. This information could contribute to assessing the risk of these bacteria in low a_w foods.

P1-93 Potential Relationship of Membrane Fatty Acid Modification of *Salmonella* on Survival in a Low Water Activity Model

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❖ ***Developing Scientist Competitor***

Introduction: There is a lack of information regarding *Salmonella* long-term survival mechanisms in low water activity (a_w) foods raising challenges for minimizing or eliminating the microorganism in such products.

Purpose: To determine if there is a relationship between survival and fatty acid (FA) modification (as altered by exogenously supplied fatty acids) of *Salmonella* Tennessee at a_w 1.0 - 0.6.

Methods: Glycerol solutions (a_w 1.0 - 0.6) were inoculated with *Salmonella* Tennessee K4643 and incubated at 25°C. Total FA composition of parent strains on day 1 and 14 was determined by gas chromatography (GC). *Salmonella* Tennessee FA composition was modified by culturing in tryptic soy broth supplemented with 50 µg/ml oleic (C18:1) or linoleic acid (C18:2) or by incubating in peanut oil for 24 h. Modified *Salmonella* Tennessee cells were then inoculated in various water activity glycerol solutions and incubated at 25°C. Samples were taken over 14 days and plated on tryptic soy agar. Each experiment was repeated 3 times.

Results: The FA composition of the parent *S.* Tennessee cells did not differ ($P > 0.05$) at different a_w and no change was found over time. Exogenous oleic and linoleic acids were both incorporated into *Salmonella* Tennessee with a concomitant decrease of cyclopropane fatty acid (CFA, C17). However, incorporation did not influence survival in glycerol solutions over 14 days. In peanut oil, there was a 2% increase of CFA (C17) and 4% decrease of oleic acid. Cells suspended in peanut oil were more resistant at a_w 0.6 than parent cells (1.69 log vs. undetectable on day 14).

Significance: Reducing the a_w to 0.6 did not change the FA composition of *Salmonella* Tennessee. Exogenous oleic and linoleic acids caused incorporation of these fatty acids from growth media but had no effect on survival at low a_w. Cells incubated in peanut oil had improved survival at a_w 0.6 compared to controls.

P1-94 Physiological and Molecular Evaluation of *Salmonella* Strains Exposed to Salt Stress

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Introduction: Stresses exerted by the food environment can result in dramatic physiological changes in *Salmonella*, such as filamentation. Little is known about the impact of these changes on detection of this pathogen using traditional cultural and molecular testing methods.

Purpose: To examine the impact of filament-inducing conditions (reduced water activity) on physiological aspects of *Salmonella* relevant to existing detection techniques.

Methods: *E. coli* ATCC 25922, *Salmonella enterica* ser Typhimurium ATCC 13311 and two peanut butter outbreak-associated *Salmonella* spp. (*Salmonella* Typhimurium, *Salmonella* Tennessee) were grown under high osmotic conditions (Tryptic Soy Agar containing 8% NaCl) for up to two weeks at 37°C. Strains were examined at various intervals for growth on selective and non-selective media (XLT-4 agar and Tryptic Soy Agar, respectively) using light and fluorescence microscopy combined with Live/Dead staining (Invitrogen, Inc.) and using quantitative PCR or flow cytometry. Physiological responses to osmotic stress were compared across type and outbreak strains.

Results: Within four days of inoculation in TSB + 8% NaCl, all bacteria demonstrated a filamentous phenotype. Recovery of filamentous bacteria on selective or non-selective media was ~10-fold lower than expected from OD-adjusted cultures. Light microscopy and fluorescence microscopy (Live/Dead staining) indicated a greater persistence of a filamentous morphology and Live-staining (intact cell membrane) in outbreak-associated strains vs. *E. coli* and the *Salmonella* type strain. Both filamented and non-filamented cells were detectable via quantitative PCR.

Significance: Our results suggest a hardier phenotype for peanut butter outbreak-associated strains of *Salmonella* and demonstrate the utility of various physiological and molecular tools for characterization of differences between type and outbreak strains that may be important to understanding survival and persistence of *Salmonella* in foods having low water activity.

P1-95 Meta-Analysis of *Salmonella* Survival/Inactivation Kinetics in Low-moisture Foods

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Introduction: Empirical models often are used to describe the effects of various factors (e.g., temperature, water activity (a_w), and food matrix) on the survival and inactivation kinetics of *Salmonella*. Currently, the effects of a_w on the survival of *Salmonella* in low-moisture foods are incompletely understood. Despite widespread development of predictive models, there is non-uniform application of model selection criteria used to evaluate the appropriateness of reported models.

Purpose: The objective was to quantitatively evaluate the fit of primary and secondary models on the survival/inactivation kinetics of *Salmonella* in multiple low-moisture foods.

Methods: Isothermal and iso-moisture survival/inactivation data for *Salmonella* in low-moisture foods (> 1,000 data series; 8 different products; temperatures -20 - 120°C; a_w 0.11 - 0.95) were collected from prior studies and categorized. Log-linear and Weibull primary models were fit to single

and grouped replicate data series and were statistically compared using the Akaike Information Criteria (AIC). Secondary models describing the effects of temperature and a_w were similarly analyzed.

Results: Based on AIC, primary regression on single data series resulted in a 95% likelihood of the log-linear and Weibull models being the correct model in ~1 and 67% of the cases, respectively. Similarly, primary regression on grouped replicate series resulted in a 95% likelihood of the log-linear and Weibull models being the correct model in ~0.5 and 77% of the cases, respectively. The relative frequency of the log-linear model as the more likely correct model peaked in the 20 - 30°C or 0.90 - 0.95 temperature and a_w ranges, respectively.

Significance: Based on the primary model regression results, the Weibull model best described the survival/inactivation kinetics of *Salmonella* during isothermal and iso-moisture conditions. However, careful consideration is required for the development or application of secondary models for dynamic temperature or moisture processes.

P1-96 Murine Norovirus (MNV-1) Detection in Low-water Activity Foods by Real-time Reverse Transcription-Polymerase Chain Reaction

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Introduction: In Mexico, there is a lack of information regarding the incidence of pathogens in low water activity (a_w) food items. Most of these products are sold in bulk, and thus follow-up safety procedures during outbreaks become complicated. Human norovirus surveillance and studies on their ability to survive in low a_w foods and drying conditions applied in food industry are needed to determine transmission risk.

Purpose: The aim of this project was to rapidly detect murine norovirus (MNV-1, as an extraction control and surrogate for human noroviruses) from low a_w foods using real-time RT-PCR.

Methods: Individual 25-g samples of peanuts, pecans, raisins, and sun-dried tomatoes purchased from local supermarkets were kept under ultraviolet light for 10 min, and aseptically surface-spiked with 5 log PFU/ml MNV-1 (positive extraction control). Viruses were eluted using TRIzol™, RNA extracted and passed through a QIAshredder. SYBR green I-based RT-PCR was carried out on ten-fold diluted RNA extracts from MNV-1 stock and spiked samples. Agarose gel electrophoresis and Tm analysis were used to confirm product size. All experiments were replicated thrice.

Results: TRIzol RNA extraction followed by RT-PCR was able to detect MNV-1 stock up to -7 log RNA dilution (1 PFU/ml). Peanuts and pecans spiked with MNV-1 showed detection to -3 log dilution (~4 log PFU/ml or 4 log RT-PCR units) and to -5 log dilution (~2 log PFU/ml or 2 log RT-PCR units), respectively. Sun-dried tomatoes showed detection up to ~10 log PFU/ml (1 log RT-PCR units) and raisins up to 1 log PFU/ml.

Significance: TRIzol RNA extraction with RT-PCR detection showed that MNV-1 could be used as an extraction process control for human enteric virus detection from low a_w foods, though needs further optimization for improved detection.

P1-97 Thermal Resistance and Stability of *E. faecium* in Low-water Activity Foods after Inoculation by Atomization

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Introduction: Homogenous and stable inoculated matrices are needed for validating preventative controls required by the Food Safety Modernization Act (FSMA). At present, information is lacking on inoculation methods, thermal resistance and stability of microorganisms in low water activity foods.

Purpose: The objectives of this work were to assess homogeneity, stability and thermal resistance of *Enterococcus faecium* atomized into ground black pepper and oat flour over a 42-day storage period.

Methods: Plate harvested cells (10 ml) were atomized onto black pepper and oat flour (1,000 g) using a Sonic Model CV24 ultrasonic atomizer to achieve an initial inoculum level of approximately 8 log CFU/g. The inoculated matrices were kept in a 32% relative humidity chamber at ambient (23 ± 2°C) temperature. Triplicate samples of each matrix were obtained throughout the 42-day storage period to evaluate stability and thermal resistance of *E. faecium*. Thermal resistance was evaluated by immersing aluminum test cells containing the food matrices in an oil bath. Differences between treatments were verified with a two-tailed t-test.

Results: Initial *E. faecium* populations in black pepper and oat flour were 7.93 (± 0.13) and 8.08 (± 0.23) log CFU/g, respectively and after 42-days storage, were 7.88 (± 0.21) and 8.10 (± 0.19) log CFU/g, respectively ($P > 0.05$).The $D_{85°C}$-value for the organism decreased from 2.63 minutes to 1.81 minutes after 42 days of storage of inoculated black pepper, and decreased from a $D_{90°C}$-value of 2.56 minutes to 2.26 minutes after storage of inoculated oat flour ($P > 0.05$). There were no statistical differences in the stability and thermal resistance of *E. faecium* in black pepper and oat flour after the 42-day storage period.

Significance: Atomization of *E. faecium* into food matrices creates homogenous and stable test samples for use in validation of preventative controls for low water activity foods.

P1-98 Trends in Risk Factor Behaviors and Support Infrastructure in North Carolina Retail Food Facilities

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Introduction: The U.S. Food and Drug Administration (FDA) quadrennially publishes a model food code, based on the best available science, to aid state and local health authorities in regulation of retail facilities. To support the model code, the FDA established a non-regulatory retail food risk factor study to measure the occurrence of practices and behaviors commonly identified by the U.S. Centers for Disease Control and Prevention (CDC) as contributing factors in foodborne illness outbreaks.

Purpose: The purpose of this study was to compare retail food risk factors within four North Carolina counties using a nationally-recognized standardized instrument.

Methods: Risk factor data were collected during non-regulatory visits by local health officials. Data collectors observed and documented behaviors related to operational risk factors associated with foodborne illness. Descriptive statistics were used to characterize trends by county, facility type, and risk factor. Significant factors (facility type, county) and pairwise comparisons of observed risk factor rates were analyzed using a generalized linear model.

Results: A total of 1,354 establishments in three categories (institutional food service, restaurants and retail food stores) were included. Significant differences were seen across facility type ($P = 0.0063$) with full service restaurants having a significantly higher rate of outbreak contributing factors than all other facility types ($P < 0.001$). Improper holding/time-temperature had the lowest compliance rate (< 75%) with 47% compliance for cold

holding of potentially hazardous food (PHF); 58% for marking Ready-to-Eat (RTE), PHF after 24 hours; 43% for discarding RTE, PHF after 4 days (45°F) or 7 days (41°F), and 46% for marking dates on commercially processed RTE, PHF. Significant differences between observed risk factor rates were seen between counties.

Significance: This research is fundamental in understanding trends in food safety practices and behaviors, as measured by compliance with a standardized instrument to inform resource dedication, intervention development and policy evaluation.

P1-99 Employees Knowledge Associated with Handling Fresh Cut Leafy Greens in Retail Foodservice Operations

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Introduction: In leafy greens alone, there were approximately 100 U.S. foodborne illness outbreaks between 2000 and 2007. While changes in consumption patterns, production and processing technologies, and new sources of produce have all contributed to the increase in produce-related outbreaks, the question whether or not the retail foodservice industry kept pace with regard to food safety knowledge remains unanswered.

Purpose: The objective of this study is to determine foodservice employees and managers' food safety knowledge for fresh and fresh-cut produce.

Methods: A total of 16 grocery stores and 16 restaurants were recruited in Columbus, Ohio and Houston, Texas. Thirty-three managers and 77 employees (44% female and 56% male) participated in this study. Both employees and managers were given a food safety survey to measure knowledge in relation to cross contamination, personal hygiene, time and temperature control, foodborne illnesses, implementation of SOPs, and food preparation and storage. Descriptive statistics and independent *t*-test were used for data analysis.

Results: The majority of establishment (76%) had standard operating procedures for handling of fresh cut leafy produce. In terms of types of fresh leafy greens, 61% of respondents used head and precut, and prepackaged in their establishment. The level of food safety knowledge was found to vary depending on the working position (manager vs. employee), indicating that managers (M = 7.27, SD = 1.26) showed higher knowledge level than employees (M = 6.72, SD = 1.48); $t(108) = 1.90$, $P = .06$. With regards to temperature, foodborne illness and hand washing, the rates of incorrect answers were greater than of correct answers.

Significance: These findings will be used to develop a behavior-based food safety training program to ensure that proper food handling practices for fresh and fresh-cut produce. Behavior-based training may have a positive effect on reducing the number of foodborne illness outbreaks especially in regards to fresh and fresh-cut leafy greens.

P1-100 What's in Your Salad? An Observation of Food Handlers' Practices

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Introduction: There has been an increase in foodborne illness outbreaks associated with leafy greens in the past few decades. For example, there were approximately 100 outbreaks in the United States (U.S.) due to *Salmonella, E. coli* O157:H7, *Shigella* spp., and *Campylobacter jejuni* reported between 2000 and 2007 associated with leafy greens alone. The foodservice industry has worked with regulatory agencies diligently to develop and implement food safety training materials for supervisors; however, this material may be too generic for employees who work with fresh and fresh-cut produce.

Purpose: The goal of this portion of a larger study is to identify current food safety behaviors in restaurants and grocery stores in the South and Midwest region of the U.S. to develop effective behavior-based, task-specific food safety training materials.

Methods: Observations of standard operating procedures for fresh-cut produce were conducted in 34 establishments (restaurants and grocery stores). Previously developed observation tools were used to identify behaviors related to food handling, food preparation, food storage, and facility upkeep.

Results: The results demonstrated that although all of the 34 establishments observed had fully equipped hand-washing facilities, only 44% employees handling leafy greens were observed washing their hands at any point during the observation. Many employees relied on glove changing as an alternative to hand-washing. Specifically, of the 59% of employees handling leafy greens, only 56% of them washed their hands before changing their gloves. Additionally, only 59% of establishments record refrigeration temperatures of leafy greens, suggesting a need for improved temperature documentation of leafy greens.

Significance: The overarching goal of this study is to develop behavior-based, task-specific food safety training materials for fresh-cut produce for restaurants and grocery stores in an effort to reduce the number of foodborne illness outbreaks.

P1-101 Assessing Food Safety Messages Impacting Foodservice Workers' Handling of Leafy Greens in Facilities Serving Older Americans

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Introduction: Americans continue to frequently eat away from home, placing them at risk of illness due to retail foodservice workers' improper food handling. With an aging American population and the increasing prevalence of illness caused by fresh produce coupled with diversity within the retail foodservice workforce, development of effective education and training materials specific to fresh produce using visual, minimal text messages is needed to improve safe produce handling.

Purpose: The purpose of the study was to implement and evaluate the impact of minimal-text posters in different foodservice operations serving older Americans.

Methods: Hourly employees' food handling practices were observed and then employees were interviewed at 8 facilities (2 long term care, 2 hospitals, 2 assisted living, 2 restaurants). Compliance with safe food handling behaviors was evaluated using the Food Code. Based on findings from initial observations, minimal-text posters were developed and distributed. After intervention, observations and interviews were again conducted one month and three months later to assess short and longer-term compliance. Observational data were analyzed using SPSS 21.0 and interview data using Atlas.ti 7.0.73.

Results: Forty-five of 127 (35.4%) observed food handling behaviors (e.g., gloves changed as needed, refrigeration temperatures checked daily) improved after the food safety posters were displayed in facilities as evidenced by increased rates of compliance at either one month or three months post-intervention. During interviews, employees identified factors motivating their safe food handling behaviors including: knowledge acquired through on the job and food safety specific training, desire to keep food safe for customers, and management's emphasis on ensuring safe food handling practices.

Significance: Safe produce handling posters were helpful in changing observed food handling behaviors, thus mitigating risks of foodborne illness for a vulnerable population. Despite various language and literacy challenges presented by foodservice employees, visual, minimal-text messaging can be useful with a diverse workforce.

P1-102 Development and Evaluation of Visual-based Tools for Training Foodservice Workers about Safe Handling of Leafy Greens Using a Multi-pronged Approach

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Introduction: Foodborne illness resulting from unsafe handling of leafy greens is a concern in foodservice establishments. Formal food safety training is important, yet informal training tools such as posters and handouts can also communicate correct food safety information and serve as reminders of safe food handling practices. Workforce diversity has led to a need for visual-based food safety training tools to overcome language and literacy barriers.

Purpose: The purpose of this study was to use a multi-pronged approach to develop and evaluate minimal-text, visual-based posters depicting safe handling of leafy greens that would meet needs of a diverse foodservice workforce.

Methods: The poster development and evaluation process involved participants from Iowa and Kansas and multiple sets of data and reviews. The themes for the posters were identified through (a) microbial analyses of leafy greens and leafy greens contact surfaces at four types of foodservices establishments (n = 8 locations), (b) interviews with foodservice workers (n = 8), and (c) observations of leafy green handling (n = 8 locations). After a set of nine posters were developed in English, Spanish, and Mandarin Chinese. An expert panel (n = 5), foodservice workers (n = 8), foodservice managers (n = 4), and undergraduate foodservice students (future managers, n = 120) provided feedback on the posters.

Results: Food safety experts' reviews indicated posters were easy to understand, concise and delivered accurate *science-based* critical food safety messages. Foodservice workers and managers, and undergraduate students found the posters to provide information in a language that was understandable and liked the use of pictures depicting microbial growth on Petri dishes resulting from unsafe food handling practices.

Significance: Using a similar multi-pronged approach can aid in the development and evaluation of food safety training materials tools that can assist in conveying *"quick"* food safety messages that are *"to the point"* and promote safe handling of leafy greens in foodservice establishments.

Poster

P1-103 Cassava Flour at Street Markets of Salvador, Bahia, Brazil: Selling Practices, Preservation and Food Safety

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Introduction: Cassava (*Manihot esculenta* Crantz) is food for about 800 million people worldwide. In Brazil, cassava flour is one of the most consumed derivatives, although the production and distribution is within the informal food sector that poses health risks.

Purpose: To characterize the cassava flour commerce at street markets of Salvador, Bahia, Brazil, in the perspective of selling conditions, preservation and food safety.

Methods: A cross-sectional study was conducted, with a survey of 32 vendors, from 20 street markets – fixed and mobile, and collection of 62 samples of cassava flour – 46 of superior class and 16 of common one. The samples were subjected to microscopic and microbiological analysis.

Results: The lack of basic infrastructure – including availability of water, electricity and sanitation – was also observed. The main sources of contamination were understood to be trash (94%), animals (56%) and drains (22%). Regarding the storage of the product, at the fixed fairs, predominated the proper point of sale (78%) and at home (11%), while in mobile fairs, prevailed deposits (57%) and at home (29%). In 34% of the points, the flour was exposed during marketing, and at 62.5% of them we observed the practice of customers trying the flour directly. On microscopic examination, 94% of the samples had some physical contaminant. Mesophilic aerobic bacteria counts recorded between 2.48 to 5.77 log CFU/g, yeasts and molds did not exceed 2 log CFU/g. Counts of total coliform and *Bacillus cereus* were in compliance with the standards and *Escherichia coli* and *Salmonella* spp. were not identified.

Significance: The results highlighted the need for hygienic measures and basic infrastructure for the commerce of cassava flour in markets at Salvador, Brazil, and also indicate failures along this chain, which point out the need of supporting to farmers activities.

P1-104 Use of a Smartphone Application ("Food Safe Surveys") for Data Collection during Direct Concealed Observations

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❖ ***Developing Scientist Competitor***

Introduction: Observing food handling practices is an important aspect of food safety research. However, unintended behavioral changes by individuals who are being observed, referred to as the Hawthorne effect (HE), can occur. Direct concealed observations (DCOs) have been used by researchers to minimize the HE during data collection of food safety behaviors in various settings. However, there are limitations to data collection of DCOs, since researchers may have to memorize observations to record later, hindering their ability to capture information instantaneously.

Purpose: To solve this dilemma, a customized smartphone application, known as "Food Safe Surveys," was developed to record DCOs in real-time.

Methods: To evaluate public perception of smartphone usage, including activities related to DCOs, a web-based questionnaire, consisting of four different images of people using a smartphone or clipboard, superimposed on a retail setting or blank space, was developed and distributed online (n = 85) for feedback. Questions were open-ended and results were based on coded responses.

Results: Participants were 75% female and 25% male, primarily 18 to 34 years of age (69%), with an average of 32 years. Combined questionnaire responses indicated that the two images of an individual using a clipboard, with either background, suggested evaluative activities such as research, surveys, or inspections (48/192; 25%), while others stated the images revealed checklist-related (127/192; 66%) or other activities (11/192; 6%), respectively, and (6/192; 3%) did not know. Alternatively, (233/237; 98%) of the participants indicated that the two images of smartphone use, with either background, was primarily for phone or internet use.

Significance: These results suggest that the use of a smartphone in a retail setting may not be perceived as an evaluative activity by the public, and that the use of a smartphone application (ex. "Food Safe Surveys") for data collection, may minimize the HE during DCOs.

P1-105 Consumer Behavior towards Leftover Storage in American and European Countries

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Introduction: Proper food handling practices can potentially prevent foodborne illnesses. Handling food leftovers is an important subsection of preparing and storing food.

Purpose: The aim of this study was to investigate how people in North and South American and European countries manage food leftovers in their households.

Methods: Surveys were conducted in Argentina, Colombia, United States (USA), Estonia, Italy, Russia and Spain, with about 100 consumers in each country. Participants responded to questions related to issues such as length of time various types of food leftovers can be left refrigerated before spoilage; length of time that food is left to sit at room temperature before refrigeration, and storage conduct in relation to use of different containers.

Results: Most participants from each of countries indicated that they frequently saved food leftovers in containers different from the ones in which foods were originally prepared. Estonian participants often left the leftovers at room temperature for longer periods of time before refrigerating than Italian, Russian and Spanish participants. Age, education, and income were found to influence on how long some participants would let the food leftovers sit at room temperature. For example, people over 35 years old stored leftovers within 1 hour while participants under 35 had more varied results for letting leftovers sit out before storage. Gender was the influencing factor on how Colombian consumers stored food leftovers, i.e., females preferred containers specifically for leftovers while men used various containers.

Significance: These findings allowed identification of food leftovers handling and storing practices, as well as addressed issues such as food contamination which will allow tailing of food safety educational programs that can raise consumer awareness of proper food storage and foodborne illness.

P1-106 Observed Food Safety Behaviors among Consumers and Employees in Market-style Restaurants

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Introduction: Market-style restaurants (MSR) are becoming popular in that a variety of food choices are offered to customers in one location easily found in shopping malls, hospitals, and universities. While shared dining area of MSR may produce additional food safety hazards because of the greater risk of cross-contamination, limited research has examined the food safety practices in MSR.

Purpose: This study aims to assess observed food safety behaviors of consumers and employees in MSR that may contribute to the risk of foodborne illnesses.

Methods: One hundred one consumers (41% female; 65% Caucasian, 17% Asian, 6% African American, 2% other; 71% with co-eaters, 29% alone) and 34 employees (71% female; 56% Caucasian, 24% Asian, 18% African American, 3% other; 59% food preparation/cooking, 35% serving, 6% cashier) were observed at three market-style restaurants in a Midwestern university using Smartphone technology. For each observed individual, 30 transactions were recorded in a sequence of observed action, the object of the action, and observed hand sanitization practice. Fifteen percent of observations were conducted together by the observers to verify the inter-rater reliability, showing 77% of agreement.

Results: From a total of 3,030 consumer transactions, 73% (n = 2,222) were identified as behaviors that required hand sanitization practices but only 71 transactions (3%) were followed by cleaning hands with napkins. Particularly, the behaviors requiring hand sanitization were more commonly observed when consumers were eating with other people (22.5 ± 3.7 transactions) than when they ate alone (20.9 ± 4.0; $t(49) = -1.8$, $P < 0.1$). Male consumers were more often engaging in sanitization practices (4.7 ± 9.0%) than females (1.7 ± 3.9%; $t(86) = -2.3$, $P < 0.05$). Among 1,020 observed transactions of employees, 50% (n = 513) were identified as behaviors requiring hand sanitization practices, while only 4 transactions (1%) were followed by changing gloves.

Significance: The findings reveal poor food safety practices of consumers and employees, raising the needs of developing effective risk communication strategies for MSR.

P1-107 Understanding and Usage of Food Labels among Lebanese Supermarket Shoppers

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Introduction: Recently, Lebanese consumers have become increasingly interested in nutritional issues.

Purpose: This study aimed to assess the food label usage and understanding and factors affecting them among Lebanese supermarket shoppers.

Methods: A cross-sectional study was conducted among 748 supermarket shoppers from several branches of the two biggest supermarkets in Lebanon between December 2013 and February 2014. A pre-coded structured questionnaire was used at points of purchase to assess the use and perceptions of food labels.

Results: 29.3% of the shoppers check the food labels every time they buy a food product and 15.7% never do it. Shoppers who do not read food labels identified the long time needed in reading them as top reason (34.9%), while 15.1% thought that there is no need to read food labels and 9.8% answered that they do not understand them. 58.5% of the surveyed shoppers read the food labels at the supermarkets, 20.6% at home and 20.9% at both supermarkets and home. 42.9% of participants agreed that reading food labels is very important, while 30.3% read the food labels depending on the purchased product. 10.2% accept to purchase expired items for some food categories. 19.4% of participants complained that food labels contain too much information and 13.8% claimed that food labels are difficult to understand. 60.3% think that food labels have helped people in changing their eating habits, while health and nutrition claims affected the product selection among 59.8% of participants. The food label knowledge score average was 63.2%. Older, obese shoppers having kids, suffering from chronic illness or allergies, and following a specific diet scored significantly ($P < 0.05$) higher.

Significance: There is a need to create awareness about the various components of food labels and to influence the beliefs of Lebanese shoppers about the link between health and the benefits of following the food labels.

P1-108 Consumer Food Safety Perceptions and Practices in Common Areas in Market-Style Restaurants

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Introduction: Millions of Americans eat in market-style restaurants (MSR) everyday, yet food safety in MSR is poorly understood. The rise in instances of foodborne illnesses presents a need to further understand the role of consumers in food safety in MSR.

Purpose: The objective of this study was to determine the food safety perceptions, beliefs, and practices among MSR patrons.

Methods: A convenience sample of participants (n = 295; 20 ± 4.5 years of age; 59% female; 61% Caucasian, 21% Asian, 3.7% African American, 14% other) was collected in dining areas on an urban, Midwestern university campus. Questionnaires assessed consumers' perceptions about hygiene indicators, sources of contamination, measures to prevent contamination, safety of different cuisines, and their own or other stakeholders' role in food safety, using a five-point Likert-type scale. Questionnaires also assessed consumers' potential engagement in new food safety strategies.

Results: While 32% of consumers were concerned or very concerned with food safety in MSR, the majority (68%) had only moderate to no concern. Almost one half (46.8%) of consumers believed they were not responsible for food safety, and 92.5% believed that the owner of each restaurant unit has this responsibility. Most consumers (85%) perceived food handlers or other patrons to be important sources of contamination with human pathogens. Wiping tables is not a common practice among MSR consumers (9.5%), but the majority (75.6%) indicated they were very likely to do so if sanitary wipes were provided at their table in dining areas. The majority (65.6%) also indicated they would likely be enticed to use hand and surface sanitizers if they saw other patrons engaging in that behavior.

Significance: These findings highlight the importance of food safety culture in dining areas and will be used to develop effective interventions to improve behaviors related to prevention of foodborne disease in market-style restaurants.

P1-109 Risk Perceptions, Efficacy Beliefs, and Serving Practices of South Carolina School Foodservice Managers

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Introduction: Foodborne disease is rare in U.S. schools. However, the number of cases could increase as many schools now offer self-service, including salad bars and bowls of fresh fruit. Hand hygiene is one way to reduce illness in schools but compliance is low so understanding underlying factors for lack of compliance is critical.

Purpose: The aim of this study was to determine the relationship between hygiene risk perceptions, efficacy beliefs, and serving practices of school foodservice managers in South Carolina.

Methods: An invitation letter and link to a 35-item web-based survey was emailed to all South Carolina Child Nutrition Directors (N = 82) asking them to forward the email to all managers in their district (N = 1231). The survey, based on Extended Parallel Process Model (EPPM) and Risk Perception Attitude (RPA), measured hygiene risk perceptions, efficacy beliefs, and serving practices using a 5-point Likert-type scale. Descriptive statistics and hierarchical regression were performed using SAS version 9.

Results: Data from 403 surveys were analyzed. Most respondents were female (82.4%), aged 51 (26-73 years), and worked 15 years (1 - 42 years). Many (75.9%) were certified managers and most (85.6%) reported hand hygiene training. Respondents reported high level agreement with safe serving practices. Most were categorized into the *responsive* group of EPPM, indicating high risk perception and high efficacy beliefs associated with preventing illness. The relationship between risk perceptions and serving practices ($R^2 = 0.8$, $\Delta R^2 = 0.001$, $P > 0.05$) and the interactive effect of risk perception and efficacy beliefs on serving practices ($R^2 = 0.8$, $\Delta R^2 = 0.004$, $P > 0.05$) were not significant.

Significance: Because South Carolina schools receive federal funds, they must have a process HACCP Plan, which defines hand hygiene and serving practices. The HACCP Plan in combination with manager certification and hand hygiene training were believed to influence perceptions, beliefs, and practices suggesting their importance.

P1-110 Perceptions of Restaurants' Food Safety: An Application of Importance-performance Analysis

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Introduction: Importance-performance analysis (IPA) is beneficial to prioritize improvements to the quality of food and service in restaurant industry.

Purpose: This study identified the importance and performance of casual dining restaurant from the aspect of food safety in the U.S.

Methods: An online questionnaire was developed based on literature review, including respondents' demographics, importance of 18 food safety attributes and respondents' perceived performance of these attributes on a restaurant they visited. Market Research Company was used for data collection. Descriptive statistics applied to summarize the data. Grand means of the importance and performance ratings were used as crossing point to separate the IPA grid into four quadrants. The means importance ratings and mean performance value for each attribute have been calculated and plotted into these quadrants.

Results: Of 305 respondents, 28% (n = 85) of them visited a casual dining restaurant once a week. The three most important food safety-related attributes were cleanliness of serving utensils (4.74 ± .66) and table wares (4.70 ± .71) and employee hygiene (4.66 ± .75). The respondents perceived that the casual dining restaurants they visited performed well in cleaning the table wares (4.63 ± .62) and serving utensils (4.60 ± .65) and serving food at appropriate temperature (4.56 ± .63). Two attributes (cleanliness of employees' fingernails and not wearing gloves while handling Ready-to-Eat food) were captured in the "concentration" quadrant. Ten food safety attributes (i.e., cleanliness of serving utensils and table wares) were categorized as "Keep up the good work." Five restaurant attributes (i.e., wearing allowed jewelries, not providing ingredients and food allergy information) fell into the "low priority" quadrant. The "possible overkill" quadrant only included displaying "employee must wash hand" sign in the restroom, indicating it was rated as an "overdoing the job."

Significance: This study illustrates using IPA as a managerial tool to identify food safety areas which greater efforts should be taken in the restaurant industry.

P1-111 A State-by-State Assessment of Food Safety Regulations for Prevention of Norovirus Outbreaks

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Introduction: Noroviruses are the leading cause of foodborne illness in the United States. Foodborne norovirus transmission is commonly associated with contamination of food during preparation by an infected food service worker. The United States Food and Drug Administration's Food Code provides model food safety regulations to prevent transmission of foodborne illness in restaurants; however, adoption of specific provisions is at the discretion of state and local governments.

Purpose: To describe differences in adoption of norovirus-related provisions among jurisdictional food safety regulations and the 2013 version of the Food Code.

Methods: We analyzed the food safety regulations of all 50 states, the District of Columbia, and Puerto Rico. Key areas assessed were hand-washing procedures, prohibition of bare-hand contact with Ready-to-Eat (RTE) foods, management of ill workers, presence of a contamination event response plan, and requirements for certified food protection managers (CFPM). Data were abstracted from publically accessible state legislation and compiled for descriptive analysis.

Results: Of 52 jurisdictions assessed, 47 (90%) require hand-washing and 49 (94%) prohibit bare-hand contact with RTE food. In contrast, 22 (42%) jurisdictions require a CFPM and 7 (13%) require a response plan for contamination events, such as vomiting. There was wide variation in the adoption of criteria specifying when and for how long ill food service workers should be excluded or restricted from the workplace. Nine states (17%) do not provide any specific management criteria for ill workers.

Significance: Most states have adopted some form of the recommended provisions to reduce foodborne norovirus transmission, although there is variation in the adoption of specific regulations. Focus on enactment and improved compliance with recommended provisions may decrease incidence of foodborne transmission of norovirus. Future analyses could examine implementation and compliance with state regulations, and explore potential correlations between those regulations and characteristics of reported foodborne norovirus outbreaks.

P1-112 Prevalence of Human Noroviruses in Commercial Retail Food and Foodservice Establishments without a Recently Reported Outbreak of Acute Gastroenteritis

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Introduction: Human noroviruses (NoV) are a leading cause of acute gastroenteritis (AGE), causing most foodborne disease in the U.S. Diarrhea and vomit are concentrated sources of NoV. Diarrhea and vomiting events often take place in bathrooms.

Purpose: The aim of our study was to determine the prevalence of NoV on select bathroom surfaces in retail food and foodservice establishments in South Carolina (SC).

Methods: A total of 120 foodservice establishments were randomly selected from a list of licensed food establishments in 9 SC counties. Four surfaces (toilet seat, flush handle of same toilet, bathroom door handle, and sink faucet handle) were swabbed in male and female bathrooms using macrofoam swabs pre-moistened with a solution of PBS and Tween 80 (0.02%) at a pH of 6.5. The RNA samples extracted from swabs were concentrated and analyzed by real-time RT-PCR. GI and GII NoV transcripts were used as positive controls and nuclease-free water was used as a negative control.

Results: In total, 15/681 (2.2%) swabs from 13 (10.8%) establishments were presumptively positive for NoV. Factors significantly associated with NoV presence included gender of bathroom, mechanism of toilet flush, door handle type, sink faucet type, paper towel dispenser material, and trash can type. We found borderline association between gender of bathroom and type of establishment.

Significance: Our results suggest bathrooms can be a source of NoV in food establishments so greater emphasis needs to be placed on cleaning and disinfecting bathroom surfaces.

P1-113 Assessing Cross-contamination of Ready-to-Eat Roast Beef by *Listeria monocytogenes* during Mechanical Slicing

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Introduction: Cross-contamination of foods with undesirable microorganisms, such as *Listeria monocytogenes*, caused by direct or indirect contact with contaminated surfaces and handlers, can have serious consequences for the consumer. Slicing of Ready-to-Eat foods at retail level can be a source of cross-contamination and be hazardous, as no killing step is applied before consumption.

Purpose: To investigate the transfer of *Listeria monocytogenes* (cross-contamination) during slicing of Ready-to-Eat roast beef.

Methods: Experiments were carried out with Ready-to-Eat roast beef pieces purchased in local supermarkets and checked for the absence of *Listeria monocytogenes* using the ISO 11290-2:1998 method. To start, a meat matrix was created in a manual meat slicer by slicing a *L. monocytogenes*-negative piece of roast beef. Another piece of roast beef was experimentally contaminated with *L. monocytogenes* by immersion in a suspension containing 8 log CFU/ml of the pathogen and sliced, causing the experimental contamination of the slicer. Subsequently, new pieces of non-contaminated roast-beef were sliced, until 200 slices were obtained. To assess the extent of the pathogen transfer (cross-contamination), counts of *L. monocytogenes* were carried out in the first slice, in every 5th slice up to the 50th slice and in every 10th slice up to the 200th slice. The experiment was repeated three times.

Results: Average counts of *L. monocytogenes* in first cross-contaminated slice were 4 log CFU/g and remained the same up to the 25th slice. After the 30th slice, a long tailing effect was observed until the 105th slice, with counts around 2 log CFU/g. From the 110th slice, counts of *L. monocytogenes* were below the detection limit (< 10 CFU/g)

Significance: The study demonstrated that careless handling of slicing machines can result in cross-contamination of Ready-to-Eat products, to an extent that can hazardous for the consumer.

P1-114 Prevalence of Susceptible and Resistant *Campylobacter* in Retail Ground Beef During the Spring of 2013 in Lubbock, Texas

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Introduction: Antibiotic resistant foodborne pathogens are a threat to public health. Some studies have found antibiotic resistance in *Campylobacter*. Therefore, it is important to monitor this microorganism throughout the food chain. The investigation of antimicrobial resistance at retail level will help to further understand the efficacy of current meat interventions and may also lend insight into the downstream effects of agricultural antibiotic use.

Purpose: To determine the prevalence of susceptible and resistant *Campylobacter* in retail ground beef in Lubbock, Texas.

Methods: A total of 178 ground beef samples were collected from seven stores across the city of Lubbock, Texas. Samples were rinsed and enriched with Bolton broth and processed in accordance with standard BAX protocols (Dupont Qualicon) for the detection of *Campylobacter*. Recovered isolates were subjected to the National Antimicrobial Resistance Monitoring System protocol utilizing the SWIN software. A multiple proportion test was performed using the statistical analysis software R to determine relationships between susceptible and resistant *Campylobacter* isolates.

Results: There were 88 recovered, positive *Campylobacter* isolates were resistant to four out of nine antibiotics (erythromycin, gentamicin, ciprofloxacin, and tetracycline). *Campylobacter* isolates expressed resistance as follows: 4.54% (n = 4) for ciprofloxacin, 91.9% (n =68) for erythromycin, 15.3% (n = 13) for gentamicin, and 4.60% (n = 4) tetracycline. There was a significant difference ($P < 0.05$) in the prevalence of resistant *Campylobacter* observed among the four antibiotics. Only 3.41% (n = 3) of the isolates were multidrug resistant as defined by the isolate exhibiting resistance to three or more classes of antibiotics.

Significance: The highest amount of resistance was from erythromycin, a macrolide. Though erythromycin is not used in the cattle industry, other macrolides are administered to cattle to prevent liver abscess. It is important to consider resistant pathogen contamination at the retail level in order to better understand the need for pathogen interventions from farm to fork to protect public health.

P1-115 Assessing the Occurrence of *Salmonella* spp. in the Peel and in the Pulps of Avocado (*Persea americana*) and Custard Apple (*Annona squamosa*)

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Introduction: In recent years an increase in occurrence of cases and outbreaks of foodborne illness associated with the consumption of fruit have been reported. Among the pathogens that can contaminate tropical fruits, *Salmonella* has been the pathogen most frequently associated with foodborne disease. Avocado and custard apple are exotic and/or tropical fruits produced in Brazil, whose consumption and exports have increased in recent years. This increase has been related to due to their exotic flavors, variety of nutrients, antioxidants compounds and consumers' search for healthier foods. Despite this, there are no data on the microbial ecology of these fruits regarding pathogenic bacteria such as *Salmonella*. This fact is of great concern, since it is known that the low acid fruits constitute optimal substrates for the multiplication of microorganisms, especially pathogens.

Purpose: To assess the incidence of *Salmonella* spp. in avocado and custard apple marketed/produced in Brazil; and characterize phenotypically and genotypically the *Salmonella* strains isolated from these fruits.

Methods: A total of 400 samples of fruits (n = 200 for each fruit), acquired in sales centers (supermarkets and/ or production centers) located in the city of Campinas, SP, Brazil, were analyzed for the presence of *Salmonella* according to ISO 6579:2002. Both the peel and pulp of each fruit (avocado and custard apple) were analyzed separately. The results further characterized by serotyping and pulsed field gel electrophoresis (PFGE).

Results: Of the 400 samples analyzed, 3 samples of custard apple acquired in the same local were positive for *Salmonella*. The strains were isolated from the pulp of the fruits and identified as *Salmonella*Typhimurium. The three isolates presented 100% similarity, according to PFGE patterns.

Significance: The results showed that tropical fruits such as custard apple can harbor pathogens in their inner tissues, offering risk to public health.

P1-116 A Multistate Outbreak of Multiple *Salmonella* Serotypes Linked to Organic Sprouted Chia Powder Products

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Introduction: In May 2014, the Food and Drug Administration (FDA), the Centers for Disease Control and Prevention (CDC), the Canadian Food Inspection Authority (CFIA), and state and local partners investigated an outbreak of illnesses caused by multiple serotypes of *Salmonella* linked to consumption of organic sprouted chia powder products.

Purpose: The purpose of the investigation was to identify the vehicle responsible for the outbreak and take appropriate actions to protect public health.

Methods: A case was defined as an infection with the outbreak strains of *Salmonella* Newport, *Salmonella* Hartford, or *Salmonella* Oranienburg occurring between 1/21/14 – 7/22/14. Food exposure questionnaires were examined to help determine a food vehicle. FDA and state partners conducted an informational traceback investigation to identify common manufacturers and sources of chia seeds. A total of 216 samples were collected including retail product samples, consumer samples, and investigational samples consisting of environmental swabs and product samples.

Results: A total of 31 cases from 16 states were identified, while 19/21 (90%) of cases with available information reported exposure to a chia seed product. A Canadian manufacturer was the source of the final sprouted chia powder product, obtaining the suspect lot of chia seeds from two farms in Argentina. Thirty-four samples from multiple lots of sprouted chia seed powder products yielded multiple serotypes of *Salmonella*. The investigation resulted in voluntary recalls by multiple firms as well as increased import surveillance. The recalled products were distributed to numerous countries and as a result, an International Food Safety Authorities Network (INFOSAN) alert was issued by the World Health Organization.

Significance: FDA and CFIA were not able to confirm whether the source of the *Salmonella* contamination originated with chia seeds or the processing thereof. This outbreak involved a novel, raw food product that has a small but growing population of consumers.

P1-117 Microbial Diversity of Fruit Flies and Fruit Fly Breeding Sites at Multiple Restaurant Sites in Minnesota

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Introduction: More than 50% of food handling establishments in the US experience fruit fly (*Drosophila* spp.) infestations. State restaurant inspectors vary in their assessment of small flies as food safety risks. No surveys of microbial diversity of small flies and their breeding sites have been carried out to date.

Purpose: In order to assess the microbial diversity of small flies in restaurant environments a survey of restaurants in Minnesota was undertaken. Both flies and breeding sites were sampled and analyzed.

Methods: Fruit flies and swabs of fruit fly breeding sites and from multiple restaurant locations in Minnesota were aseptically gathered and processed on the same day as collection. Flies were exterminated by freezing (-70°C for 3 min), diluted in buffered peptone water, stomached and plated on Tryptone Glucose Extract Agar (TGE). Swabs were also plated on TGE and both sample types incubated at 32°C for 48 h. Isolates were identified using a Vitek II system.

Results: Seventy separate bacteria were isolated from fruit fly and breeding site sample from restaurants visited. Of the breeding site isolates 80% were Gram negative bacteria, 10% Gram positive and 10% yeast. Flies harbored 53% Gram negative bacteria, 40% Gram positive (mainly *Bacillus* spp.) and 7% yeast. Species of biofilm-forming *Sphingomonas* and *Chryseobacterium* were the most commonly found bacteria in all samples and were most commonly found on both flies and in corresponding breeding sites. Captured flies were found to carry between 1×10^3 and 1×10^5 CFU bacteria/fly.

Significance: Little is known of the risk posed by small flies in the food handling environment. This is the first study of the microbial diversity of fruit flies in a food service environment and highlights vector potential between breeding and landing sites of these flies.

P1-118 Environmental Sanitation in Food Preparation Areas in Long-term Care Facilities in South Carolina

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Introduction: Most outbreaks of noroviruses (NoV) (60%) occur in long-term care facilities (LTCF). The Centers for Disease Control and Prevention (CDC) recommend hand hygiene, exclusion/isolation, and environmental sanitation to prevent and control NoV outbreaks.

Purpose: The purpose of this study was to identify environmental factors associated with transmission of NoV in food preparation areas of LTCF in South Carolina (SC).

Methods: As part of a larger study, all LTCF in SC that met inclusion criteria (163) were contacted and those that agreed to participate (26) were visited during July - November 2013. Two trained observers assessed sanitary conditions in food preparation areas using a checklist. Equipment cleanliness and condition, three-compartment sink set-up, type of sanitizer used, handwashing sinks, and worker hygiene were evaluated. Answers were hand-recorded as "yes" or "no" and comments noted. Categorical responses (yes/no) were digitized and comments categorized into themes. Relative frequencies were calculated.

Results: All work tables, cutting boards, and preparation and three-compartment sinks were clean and in good repair. A total of 23 LTCF used quaternary ammonia as a sanitizer; only 3 used chlorine bleach. All handwash sinks had warm water, soap, and an appropriate drying device. Most (18) used antimicrobial soap; 7 used plain soap. Most (24) had paper towels for drying and handwashing signage (22). Food workers appeared to be healthy and wearing clean clothes (26), hair restraints (25), gloves (21), and had no jewelry on hands or forearms (23).

Significance: Most LTCF adhered to recommended environmental sanitation practices in food preparation areas. However, SC state regulations require LTCF to use quaternary ammonia and not chlorine bleach to sanitize/disinfect surfaces. Quaternary ammonia is not effective against NoV even at disinfection concentrations. Therefore, regulations concerning sanitizing/disinfecting must be changed to better control the spread of NoV in food preparation areas in LTCF.

P1-119 Cross-contamination Prevention Practices in Retail Delis: An Observation Study

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❖ ***Developing Scientist Competitor***

Introduction: *Listeria monocytogenes (L. monocytogenes)* is a persistent public health concern for the United States. Epidemiologic investigations have found that ingestion of contaminated food is the primary vehicle of transmission of this pathogen. Additionally, data indicate that of 23 Ready-to-Eat foods linked to *L. monocytogenes*, deli meats pose the greatest risk of Listeriosis per year and per serving. Data also suggest that cross-contamination is a significant contributor to contaminated Ready-to-Eat foods in retail delis. We need to identify gaps in retail deli practices and policies that may contribute to cross-contamination of *L. monocytogenes*.

Purpose: To describe retail deli food safety practices and policies associated with cross-contamination prevention.

Methods: This study was conducted by the Environmental Health Specialists Network (EHS-Net), a collaborative forum of federal, state, and local environmental health specialists working to understand factors associated with foodborne illness. EHS-Net environmental health specialists collected data in delis (N = 298) by interviewing the manager about deli characteristics, practices, and policies and observing the deli kitchen environment.

Results: In 22% of delis, data collectors observed at least one handwashing sink being used for other activities, such as rinsing raw vegetables or washing dishes, utensils and equipment. Raw foods were cooked in an area separate from but next to the deli area in 49% of delis. In 24% of delis, an in-use sanitizing solution was not at the proper concentration. There was no written policy concerning handwashing or disposable glove use in 27 and 45% of delis, respectively. In 34% of delis, there was no written policy for fully cleaning the food slicers.

Significance: The results indicate that some delis are engaging in practices that could contribute to cross-contamination. To reduce *Listeria* rates, these cross-contamination prevention gaps must be addressed.

P1-121 The Influence of Soap Characteristics and Food Service Facility Type on the Degree of Bacterial Contamination of Open, Refillable Bulk Soaps

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Introduction: Soap in open, refillable bulk soap dispensers in public restrooms may become colonized with high levels of bacteria. Little is known about the influence of the type of food service operation or soap formulation characteristics on the degree to which open refillable bulk soap dispensers can become colonized.

Purpose: The purpose of the study was to survey the microbial quality of open refillable bulk soap sampled in three different states and four different food establishment types, and to determine the influence of formulation factors on the degree of contamination.

Methods: Laboratory personnel visited 100 locations in each of three states: Arizona, Ohio, and New Jersey to collect 300 soap samples from public restrooms in convenience stores, fast food restaurants, grocery stores, and sit-down restaurants. Samples were tested for total plate count (TPC), coliforms, pH, percent solids, water activity, and the presence of two common antimicrobial ingredients.

Results: More than 12% of samples contained high level of bacteria (typically > 10^7CFU/ml) Samples with high TPC tended to have higher coliform counts. Many of the contaminated soaps contained antibiotic resistant organisms. Solids content was correlated with high TPC and all samples with < 4% solids had detectable TPC. More than 16% of samples with no detectible antimicrobial were contaminated, while 8% of samples with triclosan and 3% of samples with chloroxylenol were contaminated. Bacteria were more prevalent in bulk soaps in grocery stores (16.7%) and fast food locations (15.6%) than in sit-down restaurants (9.7%) or convenience stores (3.6%). Little difference in bacterial prevalence between soap in men's and women's restrooms ($P = 0.58$) or between states ($P = 0.81$) was noted.

Significance: Bulk soap dispensers are frequently (> 10%) contaminated with bacteria, often at very high levels (10^7). Washing hands with bulk soaps in food service facilities may represent a significant, avoidable safety risk, especially to immunocompromised individuals.

P1-122 Assessment of Microbial Quality at Four Food Courts in the Greater Montreal Area

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Introduction: Few studies have evaluated the microbial food safety in food-courts, where increased food safety risks may be present in comparison to the average restaurant because of smaller kitchens and higher volumes of food and people.

Purpose: The purpose of this study was to evaluate food courts as potential harbors of foodborne bacteria.

Methods: Four food-courts in the Greater-Montreal area were sampled for the presence of bacteria within a two month period in late 2014 – early 2015. At each food-court, a total of twelve food trays (front, back and sides), six tables, and garbage flaps on food garbage receptacles were sampled by sponging for various bacteria including total aerobic bacteria, *Escherichia coli*, *Listeria* spp., Methicillin-Resistant *Staphylococcus aureus*, and *Enterococcus*. Bacteria were identified using classical microbiological methods including assessment of colony morphology, Gram reaction, and biochemical reactions. In addition to bacterial testing, adenosine triphosphate (ATP) testing was also conducted.

Results: No bacteria of foodborne concern were isolated, with the exception of a single isolate of *Staphylococcus aureus*. Most of the isolated bacteria were Gram positive and belonged to bacterial genera (*Staphylococcus*) that are regular inhabitants of human skin, indicating potential cross contamination during cleaning. Similar bacterial species were found on trays and garbage flaps in the same food-court, indicating the potential spread of bacteria from the garbage to the trays when customers discard leftover food. Among Gram negatives, bacteria belonging to *Pantoea* spp. and *Pseudomonas* spp. were regularly isolated, regardless of the shopping mall. ATP testing indicated high levels of contamination on tables and trays, and this was generally consistent with the microbial counts observed.

Significance: These results demonstrate the lack of foodborne pathogens in the eating areas within food-courts. Still, the high levels of potentially opportunistic bacteria isolated from tables and food trays necessitates improvements in hygiene.

P1-123 Developing the 4 C's Approach for Food Safety Culture in a Catering Business as a Tool to Assess and Improve Food Safety Standards

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❖ ***Developing Scientist Competitor***

Introduction: *"Developing a world class food safety system that will help provide safe food to the customers and several millions that visit the exhibitions each year"* is Dubai World Trade Center's commitment towards their customers which drives them to ensure a positive food safety culture is developed & maintained.

Purpose: The aim of the study was to develop a method to assess the hygiene culture for each of the outlets and find out areas that needs to be improved in the existing hygiene culture

Methods: Four major components which strongly impact the food safety culture were identified and a questionnaire was developed accordingly for the staff (not including supervisors or managers) to fill out. This method was termed as 'The 4 C's method to analyze food safety culture' namely Commitment; Control (or leadership); Communication and Competence.

Results: The 4 chosen outlets were ranked according to their cumulative year end hygiene audit scores and the cumulative score from the 4 C's questionnaire. The ranking of the outlets according to their audit inspection scores matched exactly the ranking based on the 4 C's questionnaire. Further ranking of the outlets according to the commitment, control, communication & competence sections brought to light that the competence scores of the highest and lowest audit scoring outlets were equal (83%) thus implying that staffs of the lowest audit scorer had a high caliber and competence skills, however the fall in their audit was due to the low scoring 'Control' section (approx. 50%).

Significance: This 4 C's approach developed by DWTC is a cost effective as well as cost saving innovative research initiative that exposes the exact root cause of the problem and aims at improving upon that target factor to improve and maintain a high food safety culture across all their outlets.

P1-124 *Listeria monocytogenes* Isolated from a Ready-to-Eat Fish-processing Environment Differ in Surface Adherence, Sanitizer Resistance, and Adapt Quickly to Cold and Salt Stress

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❖ ***Developing Scientist Competitor***

Introduction: Certain strains of the foodborne pathogen *Listeria monocytogenes* (*Lm*) can persist in food-processing environments (FPE) for reasons that are not fully understood. A recent survey in British Columbia (BC) detected *Lm* in fish facilities and in RTE fish products, yet little is known regarding the potential persistence of strains from these sources.

Purpose: To phenotypically characterize *Lm* isolated from a RTE fish FPE in BC to determine if strains possess traits that may allow persistence in the FPE and proliferation in RTE products.

Methods: *Lm* (n = 28) isolates were subjected to PCR-based lineage typing and phenotypic characterization as described. Surface adherence was determined at 20°C by a crystal violet microtiter plate assay. Minimum inhibitory concentration (MIC) of quaternary ammonium compounds (QACs) was determined by agar dilution methods. Salt and cold stress were assayed in 6% NaCl-BHI at 30°C or BHI at 4°C, respectively. Optical densities or plate counts were taken at regular intervals and lag phase duration (LPD) was computed with DmFit. ANOVA was used to assess statistical differences.

Results: Lineage II isolates (n = 17) had increased AC surface adherence compared to Lineage I isolates (n = 11) on both polyvinyl chloride (PVC) and polystyrene (PS) ($P < 0.005$). Although adherence to PS was increased relative to PVC, a positive correlation was seen between adherence to PS and PVC ($r = 0.827$; $P < 0.001$). Three isolates showed increased resistance to QACs (MIC ≥ 15 µg/ml). LPD in salt ranged from 3 - 6 h and 28 - 53 h at 4°C. No differences in stress adaptation were observed between lineages ($P > 0.25$).

Significance: The ability of *Lm* recovered from a BC FPE to adhere to surfaces, resist sanitizers, and quickly adapt to stress may allow persistence of these isolates and growth in contaminated food. These results confirm the need for comprehensive sanitation measures to eliminate *Lm* from the FPE and reduce potential for RTE food contamination.

P1-125 Inhibition of *Morganella morganii* Growth and Histamine Production by GRAS Products

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Introduction: Histamine-producing bacteria (HPB; e.g., *Morganella morganii* [Mm]) are in saltwater/estuarine environments and on gills and within intestines of scombroid fish (e.g., tuna) where they can proliferate and produce histamine (Hst) over a wide temperature range. Hst production can occur under moderate (e.g., 7.2°C) abuse conditions and in raw unfrozen fish.

Purpose: This study evaluated use of GRAS (generally recognized as safe) products to inhibit growth and Hst production by Mm on raw tuna at various storage temperatures.

Methods: Two GRAS products (2% and 4% DV™ [vinegar-based] and1% ProTek™ [NaCl:acetate:diacetate]) were evaluated using Bioscreen C (TSB + 0.1% yeast extract [YE], 30°C, 18 h) for effectiveness against pure cultures of Mm (3 strains; 5 log CFU/ml). Subsequently, 25-g portions of raw tuna (9 replicates) were surface-inoculated with Mm strain 11 at 4 log CFU/g and treated with sterile deionized water (DI, control) and 15% DV™ solution (≤ 2% pick-up) using a prototype sprayer. Mm numbers were determined by MPN real-time PCR immediately following treatment, weekly during cold storage (4°C, 3 weeks), and daily during temperature abuse (18°C, 3 days; 25°C, 2 days). Hst (ppm) was determined by the modified AOAC 977.13 fluorometric method.

Results: With Bioscreen C, the greatest reduction in Mm growth (turbidity), 90 - 107%, was produced by 4% DV™; growth was reduced 47 - 64% by 2% DV™ and 31 - 43% by 1% ProTek™. Mm numbers on DV™- and DI-treated tuna did not increase during 3 weeks at 4°C. Mm numbers on DV-treated fish were 1.6 - 3.6 and 1.3 - 3.5 log CFU/g lower than on DI-treated controls during the incubation periods at 18°C and 25°C, respectively ($P < 0.001$). DV™ reduced Hst production by 300 ppm after 2 days at 25°C.

Significance: DV™ could be used as a control measure to restrict growth and Hst production by Mm during processing and storage of raw tuna.

P1-126 Isolation and Identification of *β*-hemolytic *Aeromonas* Species and *Vibrio* Species Potentially Virulent in Seafood Sold in Bogota, Colombia

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Introduction: Seafood can transmit *Aeromonas* spp. and *Vibrio* spp., which survive refrigeration and freezing, and may cause foodborne illness. In Colombia, it is mandatory to report positive *V. cholerae* tests.

Purpose: The purpose of this study was to: 1) determine presence of *β*-hemolytic *Aeromonas* spp. and *Vibrio* spp. as indicators of microbial contamination in seafood; 2) identify isolates using biphasic method, and 3) determine potential virulent strains of *Aeromonas* spp. by presence of the *hlyA* gene.

Methods: Isolation was performed with protocol of the National Institute of Health in Colombia. Strains were identified by 9 biochemical tests, CrystalTM kit, and analysis of 16S rDNA gene. *Vibrio* spp. recovery was validated with standardized dilution plate, and a multiplex-PCR the *dnaJ* gene was standardized. To assess the virulence of *Aeromonas* spp., primers were designed and standardized PCR to *hylA*. *Vibrio* spp. recovery was validated using Thiosulfate Citrate Bile Sucrose (TCBS) agar.

Results: No sample was positive for *Vibrio* spp. Seventy percent of the strains of *Aeromonas* spp. phenotypically identified were confirmed by Crystal™ and 63% with sequencing 16S, with no significant differences between the methods. One hundred percent of *Aeromonas* spp. were *β*-hemolytic and 70% were confirmed by amplified and sequencing of *hlyA.*

Significance: *β*-hemolytic *Aeromonas* spp. isolated in seafood represents a potential risk to consumers. No sample was positive for *Vibrio* spp., but the selectivity of the medium was a point of interest. Adequate refrigeration promotes seafood safety. Although pathogens can grow in refrigerated products, satisfactory phenotypic and molecular characterization helps strengthen the quality and safety of fishery products.

P1-127 Determination of Histamine in Fried Milkfish Stick (*Chanos chanos*) Implicated in a Foodborne Poisoning

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Introduction: Histamine is the causative agent of scombroid poisoning and a foodborne chemical hazard. An incident of foodborne poisoning causing illness in 37 victims due to ingestion of fried fish sticks occurred in September 2014 in southern Taiwan.

Purpose: To elucidate the causative agent, two suspected fried fish sticks were collected from the suspected kitchen. Additionally, the 16 other raw fish stick samples were purchased from seven retail stores in southern Taiwan and processed for analyses.

Methods: The leftovers of the victims' fried fish sticks and the 16 raw fish stick samples were tested to determine the occurrence of histamine, chemical and microbiological quality, and histamine-forming bacteria. Moreover, the fish species of suspected samples were identified as milkfish (*Chanos chanos*) by using PCR direct sequence analysis.

Results: The two suspected fried fish sticks contained 86.6 and 235.0 mg/100 g of histamine, levels which greater than the potential hazard action level (50 mg/100 g) in most illness cases. In addition, four of 16 tested raw milkfish stick samples (25%) had histamine levels greater than the FDA guideline of 5.0 mg/100g for scombroid fish and/or product. Ten isolates isolated from raw milkfish stick samples were proven to be prolific histamine-formers with ability to produce 373-1261 ppm of histamine in trypticase soy broth (TSB) supplemented with 1.0% L-histidine (TSBH).

Significance: Given the allergy-like symptoms of the victims and the high histamine content in the suspected fried milkfish sticks, this foodborne poisoning was strongly suspected to be caused by histamine intoxication.

P1-128 The Effect of Storage Temperature on the Outgrowth and Toxin Production of *Staphylococcus aureus* in Freeze-thawed Pre-cooked Tuna Meat

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Introduction: Staphylococcal poisoning is caused by ingestion of enterotoxins produced in food by certain strains of *Staphylococcus aureus* that can be transmitted during manual handling of food. While staphylococcal poisoning has not been associated with canned tuna, potential contamination is possible when significant manual handling of pre-cooked tuna loins occurs prior to canning. While the canning process will destroy the pathogen, its enterotoxins are thermo-stable. Therefore, to control for this potential hazard, time/temperature control measures should be considered as part of a robust risk management process that also includes effective sanitation and Good Manufacturing Practices.

Purpose: The aim of this study was to determine the time for a 3-log outgrowth and toxin production of *S. aureus* on previously frozen, thawed pre-cooked tuna meat (albacore or skipjack) held at either 21 or 27°C.

Methods: A five-strain cocktail of enterotoxin-producing *S. aureus* was inoculated with the level of ~ 3.0 log CFU/g onto commercially pre-cooked tuna samples defrosted overnight at 4°C. After 3-h incubation at 37°C, inoculated samples were individually vacuum-sealed and stored at -20 ± 2°C for ~4 weeks. Following the frozen storage, samples were thawed to the target temperature and incubated aerobically at 21 or 27°C. Growth of *S. aureus* in tuna was monitored using Baird-Parker plate; simultaneously, aerobic plate counts and enterotoxin production were determined.

Results: Time for a 3-log CFU/g increase was > 20 h at 21°C and 10 h at 27°C for albacore, and the toxin production was observed at 14 h at 21°C and 8 h at 27°C. A 3-log CFU/g increase for skipjack occurred in 22 h at 21°C and 10 h at 27°C. The toxin production in skipjack started at 20 h at 21°C and 8 h at 27°C.

Significance: The data developed from this study can be used by the tuna industry for modeling the growth and enterotoxin production of *S. aureus* and to design manufacturing controls that ensure food safety.

P1-129 The Positive Impact of Research on Policy in the FOODSAFE Food Handler Training Program in British Columbia, Canada

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Introduction: The FOODSAFE food safety training program has been operating in British Columbia (BC), Canada since 1985. In 2000, food safety training (FOOSAFE, or its equivalent) became a legislated standard in BC. The FOODSAFE program is a self-funded non-profit organization with a steering committee that includes representatives from academia, public health and the food service industry. When training was legislated, no expiry date on FOODSAFE certificates was issued, bringing into question the training effectiveness for older FOODSAFE program graduates.

Purpose: The purpose of this research was to evaluate the overall effectiveness of the FOODSAFE program and determine if refresher training and certificate expiration dates were required.

Methods: Two surveys were conducted in 2009 and 2012. They ascertained whether food safety training improved food safety knowledge, when retraining should occur, and the effectiveness of retraining food handlers. An on-line food safety refresher training course was developed to meet the needs of over 790,000 FOODSAFE graduates whose certificates would expire if new policy was introduced. Enrollment statistics for FOODSAFE and refresher training courses were examined.

Results: We found food safety knowledge scores significantly decreased in FOODSAFE trained food handlers over a 15 year period post-certification (linear regression, $P = 0.02$), that most food handlers would fail a FOODSAFE exam 5 years after taking training, and that retraining food handlers was effective (paired difference *t*-test, $P = 0.038$). Both surveys found knowledge scores were significantly higher in trained compared with untrained food handlers ($P < 0.0001$). Enrollment statistics found no significant increase in routine FOODSAFE training ($P > 0.05$), and 545 food handlers have taken the refresher course.

Significance: This research supported the program and a significant policy change: five year expiry dates on FOODSAFE certificates were implemented on July 29, 2013. Industry associations supported food safety training certificate expiry dates and retraining for food handlers whose certificates expire.

P1-130 Issues Identified in Sous Vide Style Cooking in Restaurants That Led to Development of Guidelines for Chefs and Health Inspectors in British Columbia, Canada

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Introduction: Sous vide (fr.) means under vacuum. Sous vide style cooking of vacuum packaged foods is typically done in a water immersion circulator for longer times and lower temperatures than conventionally cooked foods. Widely used in food manufacturing, this technique has become mainstream in restaurants. However, questions about safe cooking temperatures and observed practices were a shared concern of chefs and health inspectors in British Columbia (BC), Canada.

Purpose: Our purpose was to examine sous vide practice safety and create guidelines for chefs and inspectors that would address food safety issues.

Methods: A working group was established to develop food safety guidance using a consensus driven approach. Student projects were conducted in restaurants under the mentorship of chefs and supervisors that illuminated safety issues with this food preparation technique.

Results: Some of the issues identified during discussions and student projects included (1) existing times and temperatures in guidelines and regulations did not address sous vide style cooking; (2) sous vide cooking and finishing steps did not meet log reduction objectives for *Salmonella*; and (3) practices in restaurants such as adding cold pouched foods to immersion circulators already in use adversely affect water temperature recovery. The working group developed guidelines, released in September 2014, to explain food safety issues associated with sous vide style cooking. The guidelines provide food flow charts, CCPs, time and temperature requirements and guidance for creating and evaluating food safety plans, creating shared expectations for sous vide style cooking between chefs and inspectors.

Significance: The importance of describing both the process come-up-time and process lethality (described as equilibrium and hold-at-temperature cooking by chefs), addressed a significant gap in our food safety knowledge of sous vide style cooking. BC chefs and inspectors are actively applying the guidelines.

P1-131 Socio-psychological Factors Affecting Food Safety Practices among Middle School Students

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Introduction: Although various food safety education programs for adolescents have been implemented in Korea, adolescents are still exposed to food safety risks because their attitudes and behaviors have not changed. To develop effective food safety programs for adolescents, personal factors that affect their food safety practices need to be identified.

Purpose: This study aimed to identify socio-psychological factors that drive food safety practices among middle school students.

Methods: Data were collected from 438 middle school students in Daegu, South Korea, using a self-administered questionnaire in December 2013. The questionnaire consisted of 63 items with the following categories: general information, self-efficacy, self-resilience, social support, and food safety practices. Statistical analyses to determine frequency, average, ANOVA, factor analysis, reliability analysis, and regression analysis were performed using SPSS 21.

Results: Food safety practices score was 3.67 ± 0.49/5.00 on average and differed significantly by gender ($P < 0.05$), grade ($P < 0.001$), school records ($P < 0.001$), and BMI ($P < 0.05$). Self-efficacy comprised of self-confidence, difficulty preference, and self-regulation. The results of factor analysis

indicated that self-resilience was classified into challenge, self-perseverance, and positive outlook, and social support was sub-grouped into positive and negative types. Food safety practices score was significantly affected by self-confidence ($P < 0.001$), difficulty preference ($P < 0.001$), challenge ($P < 0.001$), self-perseverance ($P < 0.001$), positive future outlook ($P < 0.001$), and positive social support ($P < 0.001$). Four areas (hand washing, food purchasing and consumption, food storage, and cooking equipment cleanliness) which were subgroups of food safety practices were associated with factors such as self-confidence, difficulty preference, challenge, self-perseverance, positive future outlook, and positive social support. Only purchasing and consumption were inversely related to negative social support.

Significance: This study suggests that food safety education programs should account for socio-psychological factors such as self-efficacy, self-resilience, and social support according to gender, grade, school records, and BMI to be effective for middle school students.

P1-132 Food Safety Knowledge among Undergraduate College Students: A Questionnaire-based Survey

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Introduction: The threat of foodborne illness remains a serious issue and it affects public health. Consumer education is the primary means for decreasing the potential for improper food handling and food safety issues. Preventing foodborne illness and promoting safe food practices among college students is one of the priorities. There are limited studies on the prevalence on the knowledge of food safety on a university campus.

Purpose: The purpose of this study is to assess food safety knowledge of various undergraduate students on the University of Central Oklahoma campus.

Methods: Each student was given a 10-question survey that included an assortment of questions about food safety. After the survey was taken, students were given an answer sheet that gave a detailed explanation of each question. There were also educational handouts for each student who took the survey.

Results: A total of 163 university students participated in the survey. There were 24.5% male and 74.50% female, 24.4% nutrition majors and 54.6% non-nutrition majors, 59.5% Caucasians, 17.2% Asian, 6.7% African-American, and 16.6% Other. The demographic profile indicated that 29.4% were ≤ 20 y/o, 47.9% were between the ages of 21 and 24, and 22.70% were ≥ 25 y/o. Based on the Food Safety Knowledge Score Test the results are as follows: males scored 7.45 (n = 40), females scored 7.33 (n = 123), nutrition majors scored 7.9 (n = 74), non- nutrition majors scored 6.9 (n = 89), Caucasians scored 7.7 (n = 97), Asians scored 6.4 (n = 28), African-Americans score 7.0 (n = 11), Others scored 7.4 (n = 27), ≤ 20 y/o scored 7.0 (n = 48), ages of 21 and 24 scored 7.4 (n = 78), and ≥ 25 y/o scored 7.7 (n = 37).

Significance: Food safety education is an important topic that needs to be educated to all sexes, ages, and majors. The findings concluded that females, Caucasians, mid twenties, and nutrition majors are more educated about this topic. A need for relevant and motivating food safety education exists in other groups.

P1-133 The Implementation of Interactive Case Study Videos

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Introduction: There is opportunity to decrease the frequency of foodborne illnesses by improving training programs. The association between food safety knowledge and behavior is weak; therefore, training programs should focus on improving students' behaviors in addition to their knowledge.

Purpose: The objective of this study was to better prepare distance education students for a food safety career and to improve their planned food safety behaviors related to implementing HACCP by challenging them to a case study consisting of a series of interactive videos.

Methods: An interactive game and three documentary videos were produced to portray the Howling Cow dairy foods manufacturing facility. Students interacted with these videos through discussion forums, interactive questions, and a gamification module. Participants in a senior-level introductory HACCP course (n = 18) were the target audience. A pretest and posttest survey research instrument was developed and distributed electronically.

Results: Students experienced significantly higher gains in knowledge, attitude and intention ($P < .05$). One hundred percent of students agreed to some extent that the interactive videos aided in their understanding of a food safety concept.

Significance: These results suggest integrating case studies with interactive videos into food safety training programs enhances food safety behaviors and could therefore reduce the occurrence of foodborne illness and recall incidents in the food industry.

P1-134 Shaping Health Perceptions: Effectively Communicating about Chemicals in Food

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Introduction: Public understanding of chemical risks, and also of benefits associated with food production and chemicals, is one area where efforts to reach a mutually acceptable consensus have been largely unsuccessful. This study provides an initial step in bridging the communication divide between scientists and consumers.

Purpose: This research establishes a baseline understanding of how consumers view the potential risk of chemicals in the food supply.

Methods: This study assessed belief, attitudes and behaviors of mothers. The study employed a two-pronged approach to data collection. Phase I of the study implemented small group discussions (focus groups) to generate basic knowledge of how participants perceive food, food risks, and eventually chemicals. Focus groups consisted of 4 individuals in each group; 2 groups in 4 cities for a total of 32 participants in Phase I. Phase II used a quantitative, on-line survey to assess perceptions about specific chemicals in food. Survey participants totaled 1,000.

Results: Chemicals in food are not top of mind. When probed, results from Phase I of the study confirm that, in general, attitudes about chemicals are negative especially for those mothers who are very sensitive to the issue. Our study found these mothers are typically young (18 – 34), have a higher household income, are knowledgeable about food topics, and are engaged with most aspects of food and shopping. They are also more likely to use social media as a source of information.

Significance: Results from this survey indicated both constraints and opportunities that exist in the future development of educational materials explaining the role of chemicals in the food supply. Finally, recommendations from this survey highlight communication challenges surrounding these risks, and offer further insight on more effective and accurate consumer health communication surrounding chemicals in food.

P1-135 Development and Evaluation of a Food Safety Survey for the Deaf and Hard of Hearing

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❖ ***Developing Scientist Competitor***

Introduction: Food safety practices in private homes in the United States do not align with food safety recommendations, even with adequate food safety knowledge, thereby putting individuals at risk for foodborne illness. Deaf individuals are at an increased risk for lack of food safety knowledge due to communication barriers and lack of educational approaches culturally appropriate for Deaf culture.

Purpose: Develop and assess the usability of standard survey design techniques including: paper and online formats and two forms of Likert scale 5-point and 3-point response options with American Sign Language among Deaf adolescents.

Methods: Cluster sampling technique recruited 28 participants, ages 18-21 years old, from six schools for Deaf and hard of hearing in Idaho, Oregon, Washington, Arizona, Texas, and California. The forty-five *Food Safety Survey* pilot survey questions assessed the following constructs: general food safety knowledge, food preparation frequency specifically fresh produce, food handling practices, hand washing knowledge and practices corresponding to key food safety recommendations, factors influencing hand washing frequency, and familiarity with common food safety related words and phrases. Validity, reliability, and item difficulty assessments, in addition to participant feedback were used to assess pilot study's ability to measure food safety constructs for this population.

Results: Survey participants' understood the importance agreeing the standard food handling practices including washing fresh fruits and vegetables before eating or serving them (100% agreed a lot); believed it is important that food is prepared in a safe way (96% agreed a lot); and washing hands before eating (86% agreed a lot). However participants were less likely to recognize the safest method for: thawing meat (14% disagree a lot that "Thawing meat on a countertop is the best way to thaw frozen meat"); washing hands for 20 seconds or more (43% every time); and wash hands after blowing their nose (61% every time). The food safety words and phrases respondents most frequently "know what word means" were bacteria (55.6%), virus (48.1%) and food preparation (40.7%) while the least familiar were handling produce (37.0%), foodborne illness (22.2%), and cross contamination (22.2%).

Significance: Reaching target audiences with unique communication challenges, such as with deaf and hard of hearing teenagers, requires development of tailored surveillance tools. Lessons from this study can be applied to the development of a food safety survey for Deaf and hard of hearing teenagers and research in other populations with low health literacy.

P1-136 What Makes a Difference for Consumers: Evaluating the Effectiveness of Food Irradiation Messages

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Introduction: Food irradiation is a promising food safety technology that can eliminate disease-causing organism in foods. However, nearly half of consumers reported hesitancy to buy irradiated food. Previous research found that insufficient information about the risks, benefits, and safety were major factors driving consumers' reluctance to buy.

Purpose: To determine the impact of information about food irradiation on consumers' perceptions about and willingness to buy irradiated food products.

Methods: Three information statements about food irradiation were developed, based on FDA and USDA websites. They were 'benefits of food irradiation,' 'consumer's most frequent questions' and 'authorities approving food irradiation.' The effects of these messages individually and in combination were evaluated through an on-line web survey. Participants (n = 791) were randomly assigned into seven groups with about one hundred persons in each.

Results: Information related to 'benefits of food irradiation,' including reducing harmful bacteria, was the most effective in changing consumers' perception of irradiated food. The information of 'consumer's most frequent questions,' including not inducing radioactive and no significant nutrition loss, was less powerful in changing perceptions, but better than information as to 'authorities approving food irradiation.' The combination of all three messages generated the largest number interested in selecting irradiated food. After exposure to information, 27% (n = 205) of total participants chose not to 'buy' irradiated food, even if it was 10% cheaper than non-irradiated food, with reasons like: 'I need more information before deciding' (77%), and 'I already cook my food properly' (60%) . Twenty-two percent of consumers who received all three messages chose not to buy.

Significance: Irradiated food provides extra protection to consumers from foodborne illness. Information presented in this study was effective in helping consumers make informed food purchasing decisions. Findings can be used as a guide by policy makers, educators, and marketers when describing irradiated food.

P1-137 Food Safety Knowledge and Self-reported Practices of Chemotherapy Patients and Associated Caregivers

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Introduction: Safe handling/storage of foods consumed by immunocompromised cancer patients is critically important to reduce risks of foodborne diseases. Currently, relatively little is known about what cancer patients/caregivers know and think about food safety and how they prepare food at home. Targeted education is required to increase implementation of risk-reducing behaviors.

Purpose: This study aims to determine chemotherapy patients/caregivers cognitive behavioral influences relating to food safety. Data will be used to inform targeted educational strategies/interventions to decrease the risk of foodborne illness for this 'at-risk' consumer group.

Methods: Chemotherapy patients (n = 63) and caregivers (n = 39) responsible for food-preparation completed a self-complete questionnaire (online/paper-based) to determine knowledge, self-reported practices and attitudes towards foods safety in the home.

Results: Although a large proportion of cancer patients/caregivers were knowledgeable (97 - 99%) and self-reported (88%) food safety practices associated with preventing cross-contamination in the home, many also lacked knowledge and indicated malpractices that could increase risk of illness. Although 86% were aware that inadequate cooking of food increases the risk of food poisoning to cancer treatment patients, 79% reported to 'never' check meat products are thoroughly cooked by using a meat-thermometer. Some confusion existed regarding date markings on foods indicating food safety, with only 79% believing it to be the 'use-by' date. Although 63% reported to 'always' follow the 'use-by' date on food products, only 54% 'never' ate/served Ready-to-Eat food beyond its 'use-by' date during chemotherapy. The majority (99%) believed their home refrigerator to be

cold enough, only 20% reported to 'always' use a thermometer to check the operating temperature and only 30% reported refrigerator-thermometer ownership.

Significance: Although chemotherapy patients/caregivers reported awareness of practices that can increase cross-contamination, knowledge and self-reported practices relating to adequate temperature control and 'use-by' dates were lacking. Such practices need to be the focus of targeted food safety education strategies for cancer patients/caregivers to reduce associated risks.

P1-138 Meat and Poultry Processing Employee Perceptions of Food Safety Pictograms

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Introduction: Pictograms are commonly used in the transportation, pharmaceutical, and retail food service industries to portray safety messages. Most signs in food processing facilities focus on occupational safety, thus, the use of pictograms to convey a food safety message is limited. This research explored the use of food safety signs in the meat and poultry processing industry.

Purpose: Involving the target population can provide valuable insight into worker preferences and design of pictograms. Employee perceptions of pictogram semantics provide crucial information before effective messages can be created.

Methods: Mailed and telephone food safety needs assessment surveys were sent to meat and poultry HACCP coordinators in the mid-Atlantic region. Focus groups with employees from several facilities that responded were conducted in both English (5) and Spanish (5) based on language availability, geographic location, and size. Participants were asked about training practices, pictogram semantics, and sign preferences.

Results: Of the 308 surveys sent to meat and poultry processing facilities, 47 (15.3%) were completed and returned. Only 53.3% (24/45) currently have pictograms and the most important food safety topics were hand washing (60.9%), cleaning/sanitizing (78.3%), and preventing cross contamination (69.6%). Overall, employees believe color, instructional text, and multiple language options increase employee recognition and retention of intended sign messages. Many Spanish speaking employees preferred red/yellow food safety colored signs, while English speaking employees preferred differently colored signs. While Spanish speakers preferred a horizontal orientation, English speaking employees had no preference for sign orientation.

Significance: Understanding the food safety needs of meat and poultry processing facilities combined with knowing employee preferences can help create relevant food safety pictograms. Pictograms may enhance worker compliance with basic food safety practices. Based on the survey and focus groups' feedback, a new handwashing sign with minimal English and Spanish text was created and is undergoing evaluation for effectiveness.

P1-139 Knowledge of Certified Restaurant Managers in Northern Illinois: Are Some Certification Programs More Effective Than Others?

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❖ ***Developing Scientist Competitor***

Introduction: Food handlers need accurate knowledge of food safety principles. Food safety certification is an important source of food safety knowledge.

Purpose: The purpose of this study was to describe the common certification training methods utilized in Illinois and how this training relates to food safety knowledge.

Methods: Certified managers (n = 460) from 279 participating restaurants in Chicago and surrounding Suburbs (Suburban Cook, Kane, Lake and DuPage Counties) were interviewed with a food safety knowledge survey. Managers self-reported whether their certification training course was provided by a local health department, restaurant/corporation, ServSafe, college/university, other or unknown. We used a multivariate mixed-effects regression analysis to examine the relationship between certification training type and overall food safety knowledge score.

Results: The mean food safety knowledge score among the certified managers was 79.5% (35 correct of 44 questions). Seventy-six percent (350) were unable to correctly provide the temperature range of the danger zone. Twenty-two percent (102) of the managers were certified by a restaurant/corporation, 20% (90) by a college/university, 18% (83) by a health department, 9% (43) directly through the ServSafe program, and 21% (142) by an 'other' or unknown program. By training program, knowledge scores ranged from 77% for those who did not know their training program to 84% for ServSafe. While controlling for other factors, managers with ServSafe training scored significantly higher than those reporting health department, restaurant/corporation or unknown training ($P = 0.05$, $P = 0.03$ and $P = 0.003$, respectively).

Significance: These data suggest that some food safety certification programs in Illinois may be more effective than others in educating food managers. However, these are observational data and not derived from a comparative randomized trial. Certified managers did not score very high overall on the knowledge survey although they are often oversee food safety for restaurants which may present a public health risk.

P1-141 Tools for Improving On-farm Food Safety Workshops and Survey of Food Safety Costs

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Introduction: Challenges of developing farm food safety plans and implementing best practices can exclude growers from being able to sell to certain markets such as farm-to-school or retail/restaurant buyers, who often require verification of the farmer's ability to grow, pack, and deliver a safe product. Gaining familiarity with food safety issues can be key in instilling producer confidence to identify and mitigate on-farm contamination risks. In addition producers need to plan for the costs of employing food safety plans to maintain financially viable operations.

Purpose: To assess the situation in Colorado, a food safety cost survey was conducted and the curriculum for *Tools for Improving On-Farm Food Safety* workshops was developed, delivered, and evaluated by a team which included agri-business, food safety, and outreach professionals.

Methods: A 19-question survey was distributed to growers (n = 52) and three regional 1-day workshops were offered, divided into 7 sessions: On-Farm Food Safety Planning, Food Safety Landscape, Worker Health and Hygiene, Irrigation Water Management, Harvest and Post-harvest Operations, Traceability and Recall, and Preparing for Audits.

Results: Survey results indicated no correlation ($P > 0.05$) between operation size and having a plan; 40% of growers with 10 to 100 acre farms were operating without a functional and complete food safety plan. Seventy-nine per cent of workshop attendees reported an increase in their confidence about on-farm food safety, rated as 'considerably' or 'to a great extent,' while 44% indicated they intended to start developing a plan. Only 17% of participants reported keeping records of on-farm food safety costs. In a 6-month post workshop survey, 67% of participants reported making changes to their food safety plans.

Significance: To improve fresh produce safety and insure viability of direct marketers, farm managers and workers need education and training in best food safety practices and associated financial record-keeping.

P1-142 Enhancing the Safety of Locally Grown Produce – Outcomes and Challenges of Food Safety Education for Small-scale Farmers and Market Managers

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Introduction: Research has identified risks of foodborne illness associated with locally grown produce. Education is needed for small-scale farmers and market managers, who traditionally lack access to food safety educational programs.

Purpose: Objectives were to conduct trainings for small-scale farmers and market managers, evaluate increases in knowledge of factors affecting produce safety, identify practices to be changed, conduct follow-up evaluations to determine actual changes and conduct surveys with Extension Educators to verify outcomes.

Methods: Trainings using the Enhancing the Safety of Locally Grown Produce curriculum were conducted. Workshop evaluations measured knowledge gained and identified practices that would be changed as a result of training. Participants were emailed or mailed follow-up surveys nine to 12 months after trainings to determine actual changes. Extension Educators conducting sessions were surveyed to verify outcomes and actual changes.

Results: Data from 328 farmers and 114 market managers indicated significant improvement in knowledge of all covered topics related to enhancing produce safety ($P < 0.0001$). Specific practices were identified for change by up to 64% of farmers and up to 73% of market managers at the end of sessions. However, low response rates to follow-up surveys made meaningful analysis of actual changes impossible. This is indicative of challenges faced by food safety educators attempting to evaluate effectiveness of education programs when observations or audits are not options. Limitations include participants not providing contact information, information not being legible, email addresses changing before the follow-up timeframe, etc. Extension Educator surveys, however, indicated receiving requests for additional information from participants and, in some cases, observing changes in practices among those trained.

Significance: The curriculum increased knowledge and helped participants identify practices to change to enhance produce safety. Outcomes of evaluation indicate challenges of measuring success of food safety education programs and verifying changes in practices as a result of education.

P1-143 Assessment of Food Safety Practices for Small Scale Fruit and Vegetable Growers in the Midwest

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Introduction: With increasing numbers of recalls and outbreaks associated with fruits and vegetables, there is a critical need to educate growers at all production levels on food safety practices. An assessment of growers' current food safety practices can be used to develop Good Agricultural Practices (GAP) programs that will lead to changes in knowledge and improved on-farm behaviors.

Purpose: The objective of this study was to assess food safety practices of small scale fruit and vegetable growers in the Midwest prior to participating in GAP workshops.

Methods: Before each of seven Basic Good Agricultural Practices and On-Farm Food Safety Plan Preparation workshops held throughout the state of Iowa, 129 growers completed a survey about current on-farm food safety practices and desired areas of change. The survey included questions related to water use and testing, worker hygiene, facility accommodations, worker training, wildlife exclusion practices, manure/composting, written procedures, and record-keeping.

Results: Prior to the first GAP workshop, more than 47% of participants had hand washing sinks for use and trained workers about food safety fundamentals while less than 20% had written protocols about cleaning of surfaces; worker health; cleaning of produce; eating, drinking, and smoking; worker attire; and product holding during storage and transportation. After the first GAP workshop, half of the participants indicated a desire to improve all of the areas reported as deficient with 80% identifying plans to develop protocols for wildlife dropping and sanitizer preparation and use.

Significance: Growers have demonstrated a desire to provide safe, high quality and wholesome fruits and vegetables to consumers by implementing GAP; yet much of available resources are focused on larger production farms. Educators of small scale produce growers can use these findings to identify needed areas of emphasis and raise awareness about food safety practices that can be improved.

P1-144 Michigan Rapid Response Team (RRT): Improving Multi-disciplinary and Multi-agency Collaboration during Food and Feed Emergency Responses

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Introduction: Reducing response time to food and feed contamination incidents is critical in reducing the burden of illnesses and deaths associated with each incident. The Food and Drug Administration' Rapid Response Team (RRT) Program increasing multi-agency collaboration in Michigan and is an effective method used to control the magnitude of the incident. Partnerships by federal, state and local agencies, reduces duplication of effort and disseminates pertinent information in a synergistic manner. Multi-disciplinary participation provides expertise in toxicology, epidemiology, regulatory, laboratory and emergency management.

Purpose: Rapid Response Teams (RRTs) serve to coordinate multi-disciplinary and multi-agency emergency personnel in a foodborne outbreak or other emergency situation.

Methods: Tabletop Training Exercises and After Action Reports involving all response partners improve working relationships and communication, while preparing the RRT for future incidents. RRT environmental assessments and environmental sampling provide ongoing training for RRT staff.

Results: Foodborne outbreaks responses to *Salmonella,* STEC and cyclospora*,* pesticide and toxin contamination of animal feed, and sulfa drug residue contamination of dairy products highlighted the MI RRT involvement in 2014.

Significance: Preparation and partnerships provided effective responses by multi-disciplinary and multi-agency emergency personnel in limiting the size and scope of numerous incidents in the state of Michigan.

P1-145 Evaluating the Effectiveness of Food Safety Trainings by Determining Factors That Influence Grower Adoption of Food Safety Certification Practices

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Introduction: Fresh produce growers are under increased pressure to abide by federal food safety guidelines and obtain third-party Good Agricultural Practices (GAPs) certifications. Food safety trainings have been offered in Maryland for several years, although only about one quarter of attendees implement food safety plans and receive GAP certification.

Purpose: To increase the number of trainees implementing recommended practices, this study aimed to define the factors influencing grower's opinions of food safety practices and their reluctance to obtain GAP certification.

Methods: Attendees filled out a pre-training questionnaire at full-day food safety trainings to capture their perceptions of food safety regulations and abilities to implement recommended practices. A second questionnaire was given post-training to self-assess skill level, training satisfaction and estimated barriers to certification. A multiple-choice factual test on training content was given pre- and post-training to provide a quantifiable measurement of learning. Nine months post-training, attendees were sent a follow-up questionnaire to measure implementation of food safety practices on their farms.

Results: Over 62 participants completed the questionnaires and factual tests. Participants were involved in a range of markets (retail to wholesale), and 80% (45/56) had never attended a previous GAP training. After the trainings, 76% (47/61) of participants planned on implementing GAPs and receiving a certification. Perceived difficulties to receiving certification were listed as: "none" (58%), "paperwork" (16%), "labor" (8%), "not enough time" (5%), " lack of infrastructure" (3%), "lack of equipment" (2%), and "no incentive" (2%). An analysis of factual tests demonstrated improved comprehension of food safety concepts (7.3% scored 75% or higher on pre-test, 50.9% scored 75% or higher on post-test).

Significance: These results will allow trainers to address barriers to certification and increase grower adoption of food safety practices and documentation. These results and evaluations will be incorporated into alternative training methods in 2015.

P1-146 Teaching Molecular Epidemiology in Latin America: An International Collaborative Effort to Tackle Foodborne Pathogens

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Introduction: A successful global food safety system depends on reliable, cost-effective foodborne pathogen detection and characterization technologies that can be readily applied to various sectors across the world. Currently, there are major disparities in the methods used between developed and developing countries, which resulted in knowledge gaps in data comparison and regulation standards. For example, the application of next generation sequencing and geographical information systems (GIS).

Purpose: With the goal of training grad students and food safety professionals in Chile, we conducted a 4-day workshop in collaboration with Virginia Tech, The University of British Columbia, and Universidad Andres Bello.

Methods: The workshop was focused on the application of cutting-edge technologies to study foodborne pathogens. Teaching methods as project and problem learning activities, along with exercises and paper discussions were used.

Results: A total of 38 students participated in the workshop. Participants were from the Academia (18), Industry (7) and the Government (13). Activities as outbreak investigations, bacterial genome sequencing analysis, and analysis of GIS applications were successfully conducted.

Significance: This collaborative effort was a success. It provided an overview of epidemiology and molecular subtyping methods, with emphasis on the application of whole genome sequencing and geographical information systems, to detect disease outbreaks and their sources in Chile.

P1-147 Framework for Stakeholder Engagement to Develop Water Quality Monitoring Guidelines to Protect Public Health

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Introduction: Gaining multi-stakeholder support for investing in public health (PH) protection can be a daunting challenge. Frequently, stakeholders do not recognize the larger implications of not addressing PH risks. For example, neglecting food safety risks can have detrimental impacts on a business sector.

Purpose: A demonstrated framework developed to promote multi-stakeholder engagement for the protection of PH at recreational beaches can be adapted to promote improved water quality (WQ) practices within the food industry. Through targeted outreach and communication activities, we established multi-stakeholder support for a coordinated WQ monitoring program to protect PH at recreational beaches. This process led to the development of a stakeholder engagement framework that can be applied to other emerging areas of PH concern.

Methods: As an independent organization, RTI worked with public and private sector entities to establish a working group of WQ stakeholders focused on protecting PH. Stakeholders were engaged by holding one-on-one meetings to gain buy-in. A subsequent meeting was held to bring together all stakeholders to discuss WQ issues, agree on monitoring program guidelines, and determine roles and responsibilities.

Results: Four out of six invited stakeholder organizations were present at the initial stakeholder meeting. Guidelines were developed based on a pilot study and review of international best practices. Ensuring consistent and effective implementation, stakeholder agreement on monitoring guidelines was reached giving consideration to feasibility, resource constraints, and the need to protect PH. Roles and responsibilities were established for monitoring activities and adherence to guidelines.

Significance: The context of this initiative was protecting public health at recreational beaches but the general framework has broader applications, particularly in situations where both public and private sector entities have a stake in protecting public health (e.g., ensuring food safety). This framework provides an approach to facilitate multi-stakeholder support for protecting PH through environmental monitoring programs.

P1-148 Tracking Trends in Research on Foodborne Pathogens of Public Health Concern through USDA's Food Safety Research Projects Database

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Introduction: The Food Safety Research Information Office (FSRIO) at the USDA ARS National Agricultural Library was mandated by the United States Congress to support the needs of the research community. FSRIO meets its congressional mandate through collecting, organizing and

disseminating food safety research information via its website which features the Research Projects Database (RPD). The RPD is a publicly accessible and searchable database that showcases more than 8,800 food safety research projects funded by both United States and International government agencies and other non-government organizations. In an effort to better meet the information needs of the researchers, FSRIO is exploring the possibilities of utilizing the RPD to showcase food safety research trends worldwide.

Purpose: The purpose of this study was to quantitatively estimate the number of research projects in the RPD (1980-present) that are associated with foodborne pathogens of public health concern and track trends in research on foodborne pathogens.

Methods: Key foodborne pathogens of public health concern were selected based on the data available from the Centers for Disease Control and Prevention 2014 report. To determine the number of research projects for each selected pathogen, the search fields were created on FSRIO's RPD interface. Each search sequence contained the name of the pathogen in the "full-text" field combined with "exact phrase" or "all words" field. The search was conducted separately for each pathogen.

Results: Among the pathogens searched, RPD contained highest number of research projects on *Salmonella* (1,671) followed by *Listeria* (737), *Escherichia coli* O157 (619), *Campylobacter* (598), *Vibrio* (247), *Cryptosporidium* (170), *Shigella* (133), *Yersinia* (105), STEC non-O157 (60) and *Cyclospora* (17).

Significance: These results indicate that *Salmonella* is the principal pathogen of interest among food safety researchers worldwide followed by *Listeria* and *Escherichia coli* O157. This trend may provide important information to researchers in their research planning process.

P1-149 A Review of School-linked Norovirus Outbreaks: Lessons Learned

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Introduction: Norovirus is estimated to be the leading cause of viral gastroenteritis in school settings and increasingly, those affected by outbreaks are going online to discuss. Online conversations about outbreaks in schools, through news articles and social media, provide insight into attitudes, knowledge, misconceptions and outrage of participants. Methods are needed to engage in these conversations, in order to better educate the public on preventing and controlling norovirus.

Purpose: The objective of this study was to characterize the online discussion related to norovirus school outbreaks in order to better understand what messages are currently being communicated.

Methods: Using an online search engine, 64 outbreaks and 312 associated media articles were found from 2006 to 2014. Inclusion criteria included mention of norovirus in a school setting, availability of document in English and ability to locate document through internet searches. A mixed-methods approach, content analysis focusing on artifact text, was carried out. Articles were coded by two researchers and a list of themes was established through an iterative approach.

Results: Of the 64 outbreaks, 76% also had social media activity. The most used social media platforms were on Facebook and Twitter. Major themes found in both news articles and social media activity included the use of drama and humor, placing blame on others for the outbreak and discussing preventative measures. Some preventative measures referenced were incorrect or misleading, including discussions on using hand sanitizer and vaccines.

Significance: This is one of the first studies to analyze online conversations around norovirus, and provides important information as new methods of communication about the virus are developed. The lessons learned from analyzing how people discuss norovirus, and what information they are receiving, can guide new intervention methods that are more targeted to an online audience and address identified gaps.

P1-150 Profiling Food Sector Businesses in the UK According to Growth, Innovation, Food Safety and Technical Compliance

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Introduction: Sustainable growth in the UK food sector is considered to be vital in aiding economic recovery with > 30% increased turnover required by Government strategies by 2020. To achieve this, food businesses need to achieve/maintain food safety and technical 3rd party accredited standards (e.g., British Retail Consortium - BRC) and innovate with new-product-development (NPD) and novel production methods to expand/retain customer markets. A profile of UK Welsh businesses is lacking and is required to inform a pathway to growth and innovation.

Purpose: This study aims to benchmark the current position of UK food and drink manufacturers/processors (FDMP) and identify opportunities for sector development in line with Government growth targets.

Methods: A telephone survey (with follow-up online survey) was conducted with FDMP in Wales (UK) (n = 784) using a staged approach. Responses were collated to determine food sector, current food safety and technical compliance, innovation and growth as well as current challenges and barriers encountered.

Results: Cumulatively, 108 FDMP businesses responded to the survey (14% response rate). Findings indicated that in Wales 99% of FDMPs are small-medium-sized-enterprises and main activities in the food sector include dairy/liquid egg (21.5%), bakery (18.5%) and raw, red meat (18.5%). Three-quarters of FDMPs reported business growth during 2012 - 2013. Results showed 43.5% FDMP businesses reported obtaining technical 3rd party accreditation standards (only 13.8% had achieved BRC standard); 73.1% FDMPs considered they were not functioning at full capacity and 37.9% reported aspiration for business growth. Barriers/challenges encountered for business growth/development were associated with international export, packaging, labelling, legislation, licensing and lack of required technical knowledge. Fifty-five percent of FDMPs reported innovation in their businesses; 54.6% companies reported development and launching between 1 - 10 new products and 1.8% between 11 - 20 and 2.7% > 20 new products in the past year. Innovative processes reported included improvement in production efficiency and waste reduction.

Significance: Increased understanding of the FDMP food sector will aid targeted support to improve technical development and subsequent innovation resulting in increased and sustainable business growth.

P1-151 Impact of an Educational Sign on Consumer Use of Plastic Bags for Raw Poultry

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Introduction: Thousands of cases of foodborne illnesses caused by *Salmonella* and *Campylobacter* from poultry products are reported each year. The consumer is the last defense against cross-contamination and possible spread of these bacteria. An earlier study reported that the use of plastic bags often provided in poultry sections eliminates cross-contamination during transport and storage of raw chicken.

Purpose: This project examined shoppers' use of plastic bags when purchasing raw poultry and the influence of educational signage.

Methods: Customers (100) were observed while purchasing raw poultry in two grocery stores. Following initial observation, a sign "For your safety and convenience bag your meat" with a picture was posted to inform shoppers to use bags provided. Shoppers were observed again after the sign was displayed. Another 100 shoppers who purchased raw poultry were surveyed concerning their use of bags and impact of the sign.

Results: Fewer than 10% of shoppers were observed placing raw poultry in bags provided before the sign was posted. After the sign was posted, none of the observed shoppers used bags for raw poultry. About half of those surveyed reported they used bags for raw poultry in the past, but only 9 used bags on the day surveyed. Reasons for not using the bags included: not noticing them, not thinking they were necessary, and thinking it was too much trouble. Forty-one shoppers noticed the sign displayed in the poultry section; 22% of those reported it had an impact on their decision to use the bag. However, most could not remember the displayed message (66%).

Significance: Further research is needed to assess the effectiveness of messages posted in grocery stores. This study demonstrated that consumers are increasing their risk of foodborne illness by not using the bags provided in grocery stores for their raw poultry.

P1-152 Consumer Awareness of and Response to Safety Issues upon Delivery of Meat, Fish and/or Seafood Purchased Online

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Introduction: Approximately 500 US companies market fresh or frozen meat, fish, and/or seafood directly to consumers online, with home delivery using common carriers such as FedEx and UPS. The perishable nature of these products and their vulnerability to temperature abuse in transit increases the potential for foodborne illness. It is important for consumers to be aware of food safety issues upon delivery and to report problems to vendors.

Purpose: This study was conducted to determine problems reported by consumers when receiving products ordered online, and the subsequent actions taken by the vendor.

Methods: A nationally representative sample of 951 US adults completed a Web-based survey. Within the prior year, all respondents had purchased or received gifts of perishable meat, fish, and/or seafood products ordered from online vendors and were delivered using common carriers.

Results: Only 6% of consumers who recalled seeing contact information on company websites (53.3%) contacted the company to report a problem with their order. Most common problems included: the order did not arrive at scheduled time, order was incorrect, and recipient was unhappy with the products (16.7%). A common action taken by the company was to replace the unsatisfactory items (46.7%). Four percent of consumers believed the delivered items were not cold enough for safe consumption. Only 21.2% used a thermometer to test the temperature. Other methods used to determine the safe temperature of the products included visual inspection (51.5%), touching the items (45.5%), and/or smelling the product (30.3%). Most consumers kept the items they believed were not cold enough and placed them into the refrigerator/freezer (63.6%). Only 18% contacted the company or returned products.

Significance: Consumers place themselves at risk by not checking temperatures of products upon delivery. Findings suggest consumers believe they have encountered few problems with ordering and receiving perishable products from online companies.

P1-153 Transcriptomic Response of *Salmonella* Newport in Raw Tomato Using RNA-seq Technology

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Introduction: The consumption of fresh tomatoes has been linked to numerous foodborne outbreaks involving various serovars of *Salmonella enterica*, specifically *Salmonella* Newport. Despite the acidic interior of tomato, *Salmonella* is still able to grow and survive, which presents a unique set of challenges to food safety.

Purpose: The underlying molecular mechanism for *Salmonella*'s growth and survival in acidic environment inside raw tomato was investigated using RNA-seq approach.

Methods: mRNAs of *Salmonella* Newport strains grown to mid-log phase in hand-expressed tomato juices from different tomato varieties (round and Roma) and those in tryptic soy broth (TSB) at pH 7.0 and pH 4.1 were extracted and subjected to RNA-seq. Three biological replicates of RNA-seq from each sample were used to identify the differences among the transcriptomes (average 5 million 151-bp reads per sample). After mapping the paired-end reads to the 4,912 unique features within the closed annotated *Salmonella* Newport reference genome (CP001113), the R packages DESeq2 and EdgeR were used to identify genes with significantly different expression levels ($P \leq 0.05$).

Results: Growth curves showed that *Salmonella* Newport strains grew much better in tomato juices (pH 3.8 - 4.0) than in TSB at pH 4.0, where *Salmonella* Newport strains survived poorly at pH below 4.0. Although the transcriptional profile of *Salmonella* Newport was not affected by tomato varieties (round versus Roma tomatoes), a principle component analysis showed that distinct transcriptional profiles existed between *Salmonella* growth in tomato juice and in TSB with pH 4.1. Different from response to growth medium, sigma 28 and its regulated operons as well as *glp* operon in *Salmonella* Newport were suppressed in responding to acidic environment in tomato.

Significance: Results here presented a global view of *Salmonella* Newport acid adaptation in tomato, which will guide further research efforts to control the unique challenge of *Salmonella* Newport contamination of raw tomato.

P1-154 Shiga Toxin-Producing *Escherichia coli* (STEC) in Organic Vegetables Produced in the Area of Sao Paulo City, Brazil

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Introduction: Shiga Toxin-producing *E. coli* (STEC) strains are among the most common pathogens involved in outbreaks of foodborne diseases due to consumption of vegetables worldwide. However, there is no report on the presence of STEC in vegetables in Brazil.

Purpose: This study aimed at investigating the presence of STEC in organic vegetables in the area of Sao Paulo City, Brazil, characterizing the virulence factors *stx1, stx2, eae* and *ehx* as well as identifying the serotype.

Methods: A total of 200 samples of organic vegetables (leafy green), obtained from three organic producers located in the area of Sao Paulo City, SP, Brazil, were analyzed for the presence of STEC strains. Tryptic Soy Broth supplemented with vancomycin (8mg/l), cefixim (50µg/l) and potassium telurite (2.5mg/l) was used in the pre enrichment step (incubation at 37°C/24 h), followed by plating onto Sorbitol-MacConkey (SMAC) and CHROMagar STEC (CHROM) incubated at 37°C/24 h. Presumptive colonies were confirmed by biochemical tests and submitted to PCR targeting *stx1, stx2, eae* and *ehx* virulence genes.

Results: Among the 200 organic vegetable samples analyzed for STEC strains, 29 were positive for *E. coli*, but none of them showed the virulence genes studied.

Significance: Our findings show that STEC strains were not detected in any organic vegetable sample, indicating a low risk of infection due the consumption of these fresh produce in Sao Paulo, Brazil. However, more research is required with a larger number of samples, since this is, most likely, the first study to investigate STEC in vegetables reported in Brazil.

Acknowledgement: CNPq

P1-155 Microbial Quality of Surface Agricultural Water and the Presence of Pathogen Genes in Central Florida

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❖ ***Developing Scientist Competitor***

Introduction: How the microbiological quality of surface water relates to, and should be evaluated, in regard to produce safety requires further evaluation.

Purpose: The purpose of this study is to determine populations of indicator organisms and the presence of *Salmonella* and Shiga Toxin-producing *Escherichia coli* (STEC) genes in agricultural water.

Methods: Water samples (500 ml) from six agricultural ponds were collected during the 2012/2013 and 2013/2014 growing seasons (46 and 44 samples, respectively, 540 total). Microbial indicator populations (total coliforms, generic *Escherichia coli*, and Enterococci) were enumerated. A microbial water quality profile (WQP) was established for all ponds. Water (150 ml) was filtered and filters stored at -20°C until pathogen analysis by PCR. For STEC, filters were enriched in modified buffered peptone water with pyruvate at 35 ± 2°C for 24 h, DNA extracted, and multiplex PCR for detection of six genes *(hly*, *fliC*, *eaeA*, *rfbE*, *stx-I*, and *stx-II*), run. For *Salmonella,* the presence of the *invA* gene was evaluated following a subsequent enrichment in Rappaport-Vassiliadis 42 ± 1°C for 48 h and DNA extraction.

Results: All ponds met the current FDA WQP recommendations 100.0% of the time. All STEC genes were detected in 2.6% of the samples. Individual STEC genes varied in the number of samples they were detected in: *hly*-83.3%, *fliC*-51.8%, *eaeA*-17.4%, *rfbE*-17.4%*, stx-I*-32.6% *stx-II*-9.4%. The *invA* gene was detected in 26/540 (4.8 %) samples, in all ponds and both growing seasons. However, 57.7 % (15/26) of the *invA* positive samples were from ponds 2 and 4, where the WQP was the poorest.

Significance: Surface waters tested in Central Florida meet the FDA recommendations for microbial water quality, however at least one *Salmonella* or STEC gene was detected in 91.3% of samples. Understanding the relationships between indicator microorganisms and pathogens presence allows a greater understanding of agricultural water risks.

P1-156 Quantification of Native Microbiota on Surface of Leafy Green Produce Commodities Grown in South Texas

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❖ ***Developing Scientist Competitor***

Introduction: Knowledge of types and numbers of native microbiota on produce surfaces can assist the development of antimicrobial interventions utilizing the antagonism of contaminating pathogens by microbiota.

Purpose: The objective of this study was to quantify differences in numbers of native microbiota on leafy green produce grown and harvested in south Texas.

Methods: Samples (25.0 g ea.; n = 6) were collected from field-harvested produce from two farms in the Rio Grande valley. Aerobic bacteria, *Pseudomonas* spp., streptococci, enterococci, coliforms, *Escherichia coli*, homofermentative and heterofermentative lactic acid bacteria (LAB), yeasts and molds were enumerated on appropriate plating media. Differences between means of log-transformed counts of each microbial grouping from produce commodities by farm were determined by Student's *t*-test ($P < 0.05$).

Results: *Pseudomonas* spp., homofermentative and heterofermentative LAB mean numbers on lettuce from farm 2 (Temp = 60°F, ERH = 51%) were lower than those on lettuce from farm 1 (Temp = 75°F, ERH = 81%) ($P < 0.05$). Lettuce from farm 2 bore higher counts of fungi and streptococci versus lettuce from farm 1 ($P < 0.05$). On spinach, higher counts of aerobic bacteria, pyocyanogenic pseudomonads, yeasts and molds, streptococci, and *E. coli* were observed from farm 2-obtained samples (Temp = 72°F, ERH = 81%), compared to samples from farm 1 (Temp = 65°F, ERH = 90%) ($P < 0.05$). Counts of fluorescein-producing pseudomonads, coliforms, and LAB did not differ between these farms ($P > 0.05$). Parsley from farm 1 (Temp = 76.5°F, ERH = 74%) bore greater numbers ($P < 0.05$) of homofermentative and heterofermentative LAB versus farm 2 (Temp = 67.5°F, ERH = 48%). No differences between counts of other microbial groupings were observed in parsley ($P > 0.05$).

Significance: Environmental harvest conditions, including ambient temperature and relative humidity, may have impacted differences in numbers of epiphytic microbiota on leafy greens, which would be expected to affect the efficacy of pre-harvest biocontrol methods using pathogen antagonists. This research suggests need for further analysis of the correlations between harvest conditions and microbiological profile on leafy green produce.

P1-157 Pathotype and Genotype Determination of Isolates of *Escherichia coli* Obtained from the Production Chain of Jalapeño Pepper, Tomato and Melon in Northeast Mexico

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Introduction: While most *Escherichia coli* strains are commensal in nature, some are pathogenic. Six major pathotypes exist, as classified according to metabolic characteristics and virulence factors, including ETEC: enterotoxigenic and EPEC: enteropathogenic. Another way to classify *E. coli* strains, typically used for microbial source tracking, is to analyze phylogenetic cluster characteristics, where A, B1, B2 and D are the main genotypes. Furthermore, to increase the discrimination power of *E. coli* population analyses, the use of subgroups A_0, A_1, B1, $B2_2$, $B2_3$, D_1 and D_2, has been proposed.

Purpose: To identify pathotypes and genotypes from 345 isolates of *E. coli* obtained from the production chain of jalapeño pepper, tomato and melon in Northeast Mexico.

Methods: Genotypic subgroups (A_0, A_1, B1, $B2_2$, $B2_3$, D_1 and D_2), were determined by analysis (presence/absence) of a combination of the genetic markers using TSPE4.C2, *chuA* and *yjaA* primers by PCR. Pathotypes were determined by multiplex PCR according to Vidal *et al*., 2005 using *stII*, *virF*, *ipaH*, *daaE*, *aafII*, lt, stx_1, stx_2, *eae* y *bfp* primers.

Results: Relative to genotype, most of the isolates belonged to phylogenetic group A (297 isolates or 86 %). Twenty-five isolates (7.2%) belonged to genotype B1; 20 (5.8%) to genotype D; and only 3 (0.9%) to genotype B2. These isolates were grouped into seven phylogenetic subgroups: 58% A_0, 27% A_1, 7% B1, 0.3% $B2_2$, 0.6% $B2_3$, and 5.8% D_1. No isolates were genotyped as D_2. The high prevalence of genotype A and B1 suggests that most of those isolates were commensals from gut microflora and therefore non-pathogenic. Only four isolates (1.2%) were positive for pathotype grouping, three for EPEC and one for ETEC.

Significance: Although at low levels, potentially pathogenic *E. coli* strains were present in the production environment of these crops and could represent a health risk.

P1-158 Fate of Avirulent *Salmonella* Mutant Strains in Soil, Water, and on Lettuce Leaves

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Introduction: Non-pathogenic surrogate organisms have been validated for certain manufactured food processes. Validated *Salmonella* surrogates for use during pilot food plant production to evaluate survivability in complex production matrices are limited.

Purpose: The aim of this study was to compare survival of *Salmonella* mutant strains to wild types in soil, water, and on lettuce leaves.

Methods: Eight *Salmonella* Typhimurium strains (derived from ATCC 14028), including: a series of five *Salmonella* Pathogenicity Island (SPI) mutants, from SPI-1 to SPI 5; one strain which contains the entire series of SPI 1-5 mutations (potential avirulent surrogate); and two wild type strains, were evaluated for survivability. The eight strains were individually inoculated at ca. 7 log CFU into i) 250 g freshly collected soil samples; ii) 250 ml sterile EPA "worst-case" water samples; and iii) onto the mid-rib of fresh-cut romaine lettuce leaves (n = 6). Soil and water samples were incubated at 25°C; lettuce samples were incubated at 4 and 10°C. *Salmonella* populations were enumerated at 0, 1, 2, 5, 7, 14, and 21 days or 0, 1, 2, 5, and 7 days for lettuce at 10°C. Significant differences were determined using the Tukey-Kramer test.

Results: All strains declined significantly (ca. 1 to 4 log CFU/g reduction) in soil over 21 days ($P < 0.05$). *Salmonella* populations in soil at day 5 and 21 were significantly lower for the potential surrogate strain containing five SPI mutations than the two wild type strains. *Salmonella* populations in water and on lettuce leaves remained steady for most strains (ca. 6 log CFU), including the wild type strains and the potential surrogate strain containing five SPI mutations.

Significance: The strain of *Salmonella* with attenuated virulence (five SPI mutations) may be useful as a surrogate in future pilot plant production environment studies.

P1-159 Attachment of Various Serovars of *Salmonella enterica* to Vegetable Seeds with Different Surface Characteristics

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❖ ***Developing Scientist Competitor***

Introduction: Numerous outbreaks of foodborne illness have been linked to the consumption of contaminated fresh produce and contaminated seed is a potential source of foodborne pathogens such as *Salmonella*.

Purpose: To assess the attachment abilities of *Salmonella* to vegetables seeds with different surface characteristics.

Methods: Chemically treated *vs*. untreated and intact *vs*. mechanically damaged seeds of alfalfa, fenugreek, tomato and lettuce were artificially contaminated with nalidixic acid resistant cells of 4 *Salmonella* strains at 20°C for 5 h. Contaminated seeds were rinsed twice with 10 ml of sterile water and then soaked overnight in 5 ml of phosphate buffered saline (pH 7.40) at 4°C. Seeds were then vortexed vigorously, and seed soaking solutions were plated onto bismuth sulfite agar, tryptic soy agar (TSA) and TSA supplemented with nalidixic acid. The ratio of the number of attached cells to the number of inoculated cells was reported as attachment rate. Student *t*-tests were performed to determine the significance of differences among each pathogen attached to each type of seeds.

Results: The attachment rate of *Salmonella* on different types of seeds varied from 0.1% - 38.2%. Overall, the attachment rate of *Salmonella* was the highest (18.7%) on lettuce seed, followed by tomato seed (13.2%), alfalfa seed (11.3%) and fenugreek seed (6.0%). *Salmonella* attachment to mechanically damaged seeds (17.4%) was significantly higher ($P < 0.05$) than to intact seeds (7.2%). Pathogen attachment to untreated seeds (13.4%) was significantly ($P < 0.05$) higher than to chemically treated seeds (11.2%). Each *Salmonella* strain had a unique attachment pattern although the overall attachment abilities of the 4 strains were not significantly different.

Significance: Data suggest that *Salmonella* can attach to vegetable seeds and pathogen attachment rate varies with seed type and surface characteristics. Mechanically damaged and non-chemically-treated seeds are more vulnerable to *Salmonella* contamination.

P1-160 Investigating Metrics Proposed to Prevent the Harvest of Leafy Green Crops Contaminated by Flood Water

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Introduction: Produce crops have been associated with foodborne disease outbreaks in the US. Pathogens in manure deposits can be transported by water through macropores in soil to contaminate distant leafy greens. The California Leafy Green Products Handler Marketing Agreement (LGMA) states that leafy green crops within 30 ft (9 m) of the edge of a flooded field should be destroyed due to potential contamination.

Purpose: This experiment investigated if a 30-ft 'buffer zone' after flooding is sufficient to prevent the harvest of contaminated leafy greens.

Methods: One end of a spinach bed (Beltsville, MD) was flooded with contaminated water containing 6 log CFU/ml *Escherichia coli*. Surface and subsurface samples of bulk and rhizosphere soil, along with foliar samples, were taken 0, 1.5, 5, 15, and 30 ft from the flood edge. *E. coli* prevalence was determined by MPN analysis, and direct comparisons between soil samples, and between spring and fall trials, were made using *t*-tests (significance level 0.05).

Results: No significant differences in *E. coli* populations were found between bulk, rhizosphere, surface, or subsurface samples. No *E. coli* were detected on plants outside the flood zone after 14 days. On day 60, *E. coli* populations in the flood zone soil were significantly higher in the fall (2.77

log MPN/g) than in the spring (0.4 log MPN/g). Populations of *E. coli* in soil 1.5-ft from the flood zone were greater than at any other distance from the flood in both seasons, but were significantly lower in the fall than in the spring. By day 60, no *E. coli* were detected beyond 5ft from the flood in the spring, and populations were <0.1 log MPN/g in the fall.

Significance: These data suggest the 30-ft buffer zone is appropriate to prevent the harvest of contaminated leafy greens after a flooding event.

P1-161 An Assessment of the Potential for Movement of Microorganisms from a Poultry Operation to an Adjacent Almond Orchard

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Introduction: *Salmonella* is found in raw unprocessed almonds at a prevalence of about 1%. Although contamination likely occurs in the orchard, sources of the *Salmonella* are not well understood.

Purpose: To evaluate the potential for transfer of microorganisms from a poultry operation to an adjacent almond orchard.

Methods: Orchard surface (drag swab), air (microbiological air sampling device), soil, and dust (almond leaf surface) samples were collected in an orchard adjacent (< 35 m) to a commercial poultry operation ("poultry orchard") and two control orchards (surrounded by other almond orchards) over a 3-year period. Dry solids rinsed from leaf surfaces, aerobic plate count, presence of *Salmonella* and *Escherichia coli,* and bacterial community analysis through 16s rRNA next-generation sequencing were determined.

Results: *Salmonella* was isolated from one of the 804 samples evaluated. *E. coli* was isolated from 64 of 186 (34%) and 1 of 207 (0.48%) air samples in the poultry and control orchards, respectively. On average, the amount of dry solids collected from almond trees closest to the poultry operation was more than two-fold greater than from trees 120 m into the orchard or in the control orchards. The microbiota identified in leaf dust samples collected from the poultry orchard was more diverse than those from the control orchards. Members of the Staphylococcaceae family – often associated with poultry – were, on average, more abundant (12%) in the phyllosphere of trees closest to the poultry operation than in trees 120 m into the orchard (2.1%) or in control orchards (0.39%).

Significance: Poultry-associated microorganisms from animal operations may transfer a short distance into adjacent orchards; the food safety implication of this movement is unknown.

Poster

P1-162 Behavior of *Listeria monocytogenes* on the Surface of Yellow Peaches Stored at Refrigeration Temperatures

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Introduction: *Listeria monocytogenes* has been associated mostly with dairy and Ready-to-Eat meat but in recent years fresh fruits have emerged as a major concern and have been linked to outbreaks, sporadic cases and recalls. In the summer of 2014, a nationwide recall of stone fruits (yellow peach, white peach, nectarines) was issued due to the contamination of *L. monocytogenes*. Therefore, an understanding of the potential risk of *L. monocytogenes* on in-stone fruits is needed.

Purpose: Investigation of the fate of *L. monocytogenes* in yellow peaches stored at refrigeration temperature for 4 weeks.

Methods: Yellow peaches purchased from a local grocery store were used in the study. Fruits were inoculated by submersion into water containing a *L. monocytogenes* strain isolated from a nationwide stone fruits recall in 2014. Two inoculation levels were used. Peaches were air dried for 30 min at room temperature and then stored at 4°C for 4 weeks. Peaches were enumerated over a 4-week period by a rinsing and direct plating method developed in our FDA laboratories. Counts were obtained by plating onto ALOA and RAPID' *L. mono* agars.

Results: *L. monocytogenes* was not able to grow in the surface of the peaches but able to survive for the duration of the experiments. Averaged counts for peaches inoculated at high levels declined from 3.2×10^3 CFU/peach to 45 CFU/peach after 26 days of storage and for peaches inoculated at low levels, averaged counts declined from 340 CFU/peach to 68 CFU/peach. We have estimated that the average recovery rate of the rinsing method is 50%.

Significance: *L. monocytogenes* did not grow on yellow peaches under dry refrigerated storage conditions, but survived for an extended period of time, demonstrating that yellow peaches can carry a potential risk for causing Listeriosis in susceptible populations.

P1-163 Monitoring Presence of Shiga Toxigenic *Escherichia coli* (STEC), *Salmonella* and Indicator Microbes within Rainwater Catchment Systems

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✦ ***Undergraduate Student Award Competitor***

Introduction: Rainwater catchment systems serve to lower irrigation costs; however, contaminated irrigation water can be a significant source of foodborne pathogens and contaminate crops that come in contact with the soil.

Purpose: Identifying and monitoring the presence of coliform bacteria, generic *E. coli*, *Salmonella* and Shiga toxigenic *E. coli* (STEC) in rainwater catchment systems.

Methods: Over multiple growing seasons beginning in September 2013, water for irrigation of lettuce and tomatoes was collected weekly and then a twice monthly from three rainwater catchment tanks located at the Organic Crops Unit of the University of Tennessee Knoxville, which supply water to three respective high tunnels with drip irrigation systems. Coliform bacteria and generic *E. coli* populations were determined via most probable number assessment using Colilert® Quanti-Tray 2000. STEC and *Salmonella* presence was also monitored by filtering 50 and 100 ml samples through 0.45 µm membranes, which were then plated on STEC *CHROMagar*™ or XLT4, respectively.

Results: From September 2013 to October 2014, average total coliform counts between the three tanks were 2.53 log CFU/100 ml, with an average generic *E. coli* population at 0.65 log CFU/100 ml. Average STEC populations were 0.84 log CFU/100 ml. A weak correlation between STEC and generic *E. coli* populations ($R^2 = 0.51$; $P < 0.02$) as well as between STEC and total coliform count ($R^2 = 0.44$; $P < 0.05$) was shown. *Salmonella* were not detected in any water samples.

Significance: Coliform bacteria, generic *E. coli*, and STEC were detected in a rainwater catchment system evaluated over the course of a year, indicating the potential for produce contamination. The presence of pathogens demonstrates the need for routine water testing and implementation of mitigation strategies with these systems. A weak correlation between STEC and both indicator organisms suggests that improved water testing methods are necessary.

P1-164 Evaluation of Pathogen Survival in Fresh Water Sediments and Implications for Irrigation Water Quality

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Introduction: Potential contamination of fresh produce with human pathogens via irrigation water has long been recognized. One area of uncertainty involves the transport of pathogens in fresh water sediments to the water column.

Purpose: This study aims to evaluate the relationship between pathogens and fecal indicator bacteria (FIB) in fresh water sources over time and the role sediments have in harboring and distributing pathogens in water resources.

Methods: Aquatic mesocosms were used to evaluate the persistence of 14 different pathogens, 5 surrogates, and 2 FIB. Mesocosms were designed to simulate the natural environment and diurnal variations (UV radiation, ambient temperature). Both lake and river systems were used as sources of water and sediments. Microorganisms (MO) were inoculated at 10^5 per ml for each mesocosm set up (7 liters). Water and sediment samples were collected for up to 28 days to determine MO concentrations. To evaluate the attenuation and release of FIB and surrogate MO in fluvial systems, an indoor fluvarium was used at both base (1 l/s) and high (2 l/s) flow rates.

Results: Mesocosm results suggest that all MO steadily declined in the water column regardless of season; however, winter mesocosms had a less rapid decline overall – most MO detectable out to 14 days – when compared to other seasons when most MO declined by < 7 days. Attenuation and persistence of MO in sediment was greater in the cooler seasons – fall and winter. Finally, *Salmonella enterica* serovars were more persistent in both water and sediment samples when compared to other MO, and generic *E. coli* followed a similar pattern as *Salmonella* whereas enterococci trended with the *Listeria monocytogenes* strains. Fluvarium results indicate that *E. coli* persisted at greater concentrations in water for both the base and high flow input phase. Analysis of MO in fluvarium sediment at 24 h showed a significant difference ($P = 0.0117$) between base and high flow attenuation of MO. During the flushing phase, more MO were resuspended in the water during base flow as opposed to high flow after 4 h. Moreover, *E. coli* was resuspended into the water column at higher concentrations followed by *Salmonella* Typhimurium (ATCC 53647) and PRD1 bacteriophage.

Significance: This study will inform the design of risk-based sampling programs and formation of pathogen fate and transport models that better predict potential health risk related to irrigation water quality and fresh produce.

P1-165 Reducing *Listeria* Species and *Escherichia coli* O157:H12 on Cantaloupes at Pre-harvest Level by Octenidine Hydrochloride

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Introduction: Cantaloupes have been increasingly implicated in outbreaks of human illnesses in recent years. The cantaloupe industry has traditionally relied on various chemical wash treatments to reduce pathogens at post-harvest level. Effective interventions applied at the pre-harvest level could also decrease pathogens on the fruit, and reduce the risk of foodborne outbreaks.

Purpose: To determine the efficacy of a potent and safe bispyridinamine compound, octenidine dihydrochloride (OH) was applied as a pre-harvest spray for reducing *Listeria* spp. and *Escherichia coli* O157:H12 on cantaloupes.

Methods: Cantaloupe plants grown for 3 weeks in a growth chamber were transplanted in a high-tunnel. Mature fruits (n = 60) were dip-inoculated for 10 s in a fecal slurry containing *L. innocua* or *E. coli* O157:H12, allowed to air-dry for 15 min, and then sprayed with 15 ml of 0.1% ethanol (n = 20), 0.1% OH (n = 20) or 0.2% OH (n = 20). Three cantaloupes from each group were harvested on days 0, 14, and 28; 2-cm dia cores (n = 21) were sampled from each cantaloupe and sub-grouped into three samples of 7 cores each. These core samples were analyzed for surviving *Listeria* and *E. coli* O157:H12 by direct-plating on selective media and MPN.

Results: Initial *Listeria* and *E. coli* O157:H12 populations on control cantaloupes were 6.4 and 4.6 log CFU/cm^2, respectively. Spraying cantaloupes with 0.1% ethanol did not result in significant reductions in pathogen counts. All OH treatments reduced *Listeria* and *E. coli* on cantaloupes by 1.0 and 1.5 log CFU/cm^2, respectively, on day 0. On day 28, Listeria was reduced by ~ 2 log CFU/cm^2, whereas *E. coli* populations were reduced to undetectable levels on OH-treated cantaloupes.

Significance: The results of this study indicate that OH is effective in reducing *E. coli* and *Listeria* spp. and could potentially be used for decontaminating cantaloupes at the farm level.

P1-166 Understanding *Salmonella*-tomato Plant Host Interactions and Development of Novel Effective Control Strategies to Reduce *Salmonella* Burden in Tomato Production

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❖ ***Developing Scientist Competitor***

Introduction: Understanding and anticipation are important steps in the management of foodborne outbreaks. This is especially true since the discovery in 2006 of asymptomatically colonized tomato plants and fruits by *Salmonella* Typhimurium (ST). Contamination can result in internalization of the pathogen thus rendering it immune to subsequent decontamination.

Purpose: Comprehension of environmental factors controlling the interaction between tomato plants and *Salmonella* Typhimurium, and identification of effective control methods are a priority for preventing new epidemics.

Methods: Three-week-old tomato plants were sprayed with a suspension of ST, or cotyledons were clipped with scissors contaminated with the pathogen. Grown at high relative humidity (80%) and low temperature settings (20°C/15°C), survival *in planta* of ST populations was studied weekly until the ripening stage through bacterial quantification in plant tissues.

Results: The first week following spray inoculation, ST survived in the phyllosphere of all inoculated tissues; however, the pathogen was detected internally only in foliar tissues. Fourteen days post-inoculation, ST was detected on the surface and inside inoculated leaves, and its population stabilized at a density of 10^6 CFU per plant, without migration of the bacteria into the stem. Fourteen days after cotyledon inoculation by clipping, the bacterial population stabilized at a density of 10^5 CFU per plant. ST was still restricted to the inoculation point despite a significant increase of the internal population 21 days after the introduction of the pathogen ($P < 0.01$). A library of 4,182 small molecules was screened against ST. Eleven compounds killed ST with a minimal inhibitory concentration between 5 µM and 400 µM, and seven of these were bactericidal against multiple *Salmonella* serotypes.

Significance: This is the first investigation to demonstrate the inability of ST to colonize tomato at high relative humidity and low temperature. Further, despite the broad range of resistance of ST to antimicrobials, promising anti-ST candidates were found.

P1-167 Role of Extracellular Structures of *Escherichia coli* O157:H7 in Primary Attachment to Biotic and Abiotic Surfaces

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Introduction: Bacterial cell attachment to plant and abiotic surfaces is influenced by many factors. Cell surface proteins and structure may play an important role in this process.

Purpose: The objective of this study was to determine the role of selected major surface structures of *E. coli* O157:H7 in adherence to biotic and abiotic surfaces.

Methods: A set of isogenic deletion mutants lacking major surface structures was generated. The wild type and the mutant strains were inoculated on fresh produce (spinach leaves) and glass surfaces, and the capability of bacteria to adhere to biotic and abiotic surfaces was assessed by adherence assays and fluorescent microscopy methods. The expression of the genes encoding for the cell surfaces proteins was determined following shifting cell cultures from 37°C to 25°C and 4°C by RT-qPCR.

Results: Most of the surface structure deficient strains bind to the spinach leaves and glass surface less strongly compared to the wild type strain. The reduction in adherence to spinach leaves was especially prominent in the cases of the flagellar filament structural protein (fliC) and outer membrane protein A (ompA) deletion mutants, which retained approximately 17% and 12%, respectively, of that of the wild type strain. The deletion of several other cell surfaces proteins, including curli, perosamine synthetase (per, an enzyme essential for the production of O antigen), long polar filament A (lpfA) and other filamental structures also led to reduced adherence to spinach leaves, but the effect was less significant. The upregulation of the expression of target genes upon temperature shift also underlines the importance of filamental structures in adaptation of *E. coli* to the new environment.

Significance: We conclude that pili, flagella and other filamental structures are important factors in the process of initial attachment and in the establishment of biofilms.

Poster

P1-168 Influence of Plant Pathogenic and Commensal Bacteria on the Uptake of *Escherichia coli* and *Listeria innocua* in Salad Vegetable Plants

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Introduction: Produce-related outbreaks continue to be on the rise. *Escherichia coli* O157:H7 and *Listeria monocytogenes* are important zoonotic or geonotic pathogens often associated with fresh produce. Although uptake of these human pathogens into vegetable plants has been extensively investigated, the potential role of plant microbiota on internalization has garnered less attention.

Purpose: The study aimed to investigate the potential transfer of *Escherichia coli* ATCC 25922 and *Listeria innocua* ATCC 33090, surrogate microorganisms for *E. coli* O157:H7 and *L. monocytogenes,* respectively, from artificially contaminated soil into the edible portion of vegetables, as affected by the presence of phytopathogen *Ralstonia solanacearum* or plant commensal *Pseudomonas fluorescens.*

Methods: Tomatoes, bell peppers and green onions were cultivated in sterile soil (a_w ~ 0.98, pH ~ 7.45) in a greenhouse for approximately 60 days. Each potted vegetable was soil-inoculated with a suspension (ca. 10^8 CFU/ml) of nalidixic-acid resistant *E. coli* (EC) or *L. innocua* (LI). In addition, selected vegetables were co-inoculated with *R. solanacearum* (RS) or *P. fluorescens* (PF) on alternate days. A total of six inoculation treatments were considered: EC, EC+PF, EC+RS, LI, LI+PF and LI+RS. Mature tomatoes (110), peppers (86) and green onions (63) were harvested and analyzed by traditional culturing methods using selective media supplemented with 50 µg/ml of nalidixic acid.

Results: EC and LI were undetectable in tomato and pepper fruits analyzed by both plating (< 1.7 - 2.2 log CFU/g) and enrichment methods. EC and LI were isolated from the foliar parts of green onions at a maximum population density of 3.4 and 3.7 log CFU/g, respectively. Uptake of these microorganisms in green onions was not significantly affected by the presence of RS or PF ($P > 0.05$).

Significance: Findings point to the possible transference of zoonotic or geonotic pathogens from soil into onion plants and the health risks associated with the consumption of raw green onions.

P1-169 Diversity of O-Serotypes and Virulence Markers of STEC Isolates from Feedlot Cattle and Wildlife in the Desert Southwest

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Introduction: Although over 100 serotypes of Shiga Toxin-producing *Escherichia coli* (STEC) have been associated with human disease, many are not associated with human illness.

Purpose: To determine the diversity of O-serotypes and virulence markers of STEC isolates from feedlot cattle, wild birds, wild rodents, and feral pigs in a major vegetable production region of the desert southwest. Differentiation of clinically relevant STECs from those not considered a significant health risk is critical to sound testing policy.

Methods: Purified STEC strains were O-serotyped and characterized for virulence genes (*stx1*, *stx2*, *eaeA*, *hlyA*, *fliC* and *rfbE*) using conventional PCR. A subset of 36 strains was tested with STEC G2 combo (Roka Bioscience) and for putative virulence markers (*ehxA*, *aggR*, *saaD* and *subAB*).

Results: Overall, 130 *E. coli* O157 and 299 non-O157 STEC were detected from 750 domestic cattle and 1212 wildlife in the desert southwest during 2011-2013. PCR revealed that 94 (22%) isolates carried *stx1* genes, 226 (53%) possessed *stx2* genes, and 109 (25%) possessed both targets. Both *hlyA* and intimin *eaeA* were detected in 190 (44%) isolates and alone in 67 (16%) and 12 (3%) of the isolates, respectively. Multivariate logistic regression analysis of these isolates showed significant associations ($P < 0.005$) between the presence of both *hlyA* and *eaeA* with the presence of *stx*1 or *stx*2. Among 199 non-O157 STEC isolates, 64% belonged to six serotypes: O136 (19.2%), O156 (15.2%), O171 (8.1%), O109 (7.6%), O2 (7.1%) O160 (6.6%). Approximately 50% of the isolates analyzed with Roka were classified as STEC. One strain (feral pig) was found to carry a putative virulence gene (*saaD*).

Significance: The majority of STEC isolates obtained in this study were not among the "Top 6" non-O157 serotypes (O26, O111, O103, O121, O45 and O145). Rapid discrimination of clinical relevance would improve risk management decisions and, potentially, avoid unnecessary destruction of crops.

P1-170 Food Safety Risks at the Fresh Produce-animal Interface: Identifying Pathogen Sources and Their Movement on Diversified Farms

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Introduction: Diversified farming systems, which promote rearing livestock and growing produce within the same agricultural environment, are a recognized strategy to increase farm income stability across the US. Identifying the potential pathogen transmission routes within animal-produce interfaces will aid in developing control measures to improve produce safety in local markets.

Purpose: To study the movement of indicator (fecal coliforms, *E. coli*, and *Enterococcus* sp.) and pathogenic (*Salmonella*, and non-O157:H7 STEC) organisms at the animal-produce interface.

Methods: Between the months of March - December 2014, 770 samples comprising of manure, various vegetable crops, soil, air, and water were collected from two independently managed farming systems rearing Dairy or Poultry next to vegetable fields. Samples were collected from the animal source and at 30, 200, and 400 feet away from animal enclosures. The population of indicator microorganisms and the presence of *Salmonella* and non-O157:H7 STEC was determined from all sample sources and confirmed via probe-based PCR.

Results: STEC organisms were consistently isolated from produce in close proximity to dairy (30%) and poultry (24%) enclosures irrespective of setback distance. No *Salmonella* was isolated from produce within a 400-foot setback distance from dairy or poultry operations. *Salmonella* was only isolated from one (soil) out of 770 samples. Average populations of *E. coli*, coliforms, and *Enterococci* from all produce samples were 2.01, 3.33, and 2.58 log CFU/g, respectively. Significantly higher populations of these microorganisms were isolated during the summer months ($P < 0.05$). No significant differences in the population of indicator microorganisms were observed at varying setback distances from the selected animal enclosures ($P < 0.05$).

Significance: Consistent levels of STEC and indicator microorganisms were isolated from produce and other matrixes within a 400-foot setback distance from various animal operations. These findings suggest that new metrics for buffer setbacks within diversified farming systems should be assessed to improve fresh produce safety.

P1-171 Agricultural Biodiversity within North Carolina Tomato Production Systems Associated with Serotypes and Environmental Reservoirs of *Salmonella* Species

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Introduction: Tomato (*Solanum lycopersicum*) production environments have been linked with recurring multistate outbreaks of *Salmonella*. Agricultural ecosystems may serve as reservoirs for contaminants, allowing *Salmonella* and other pathogens to survive and persist within food systems. Limited microbial data on these reservoirs prevent application of effective management schemes.

Purpose: Characterize *Salmonella* serotypes isolated from three biodiverse tomato farms to determine environmental reservoirs, potentially leading to remediation strategies.

Methods: Environmental samples were collected (2012 – 2014) during tomato production seasons from 3 N.C. farms. Field (tomato fruit, blossom, leaf, weeds, soil) and water samples (n = 1547) were analyzed for *Salmonella* by enrichment using a modified BAM method as well as by real-time PCR. Isolates were serotyped and genotyped by pulse-field gel electrophoresis. In addition, generic *E. coli* was enumerated in water samples using the IDEXX Colilert and quanti-tray 2000 system.

Results: *Salmonella* was isolated (n = 169) in June (23 isolates), July (46 isolates), August (55 isolates), and September (45 isolates). Environmental sources for these isolates were water (58%; 98/169), sediment (30%; 50/169), tomato fruit (10%; 17/169), and soil (2%; 4/169). Of the serotypes identified through PulseNet with potential clinical significance (87/169), 22% (37/169) were Paratyphi B (monophasic from water and sediment); 11% (19/169) were Newport (water and sediment); 7% (12/169) were Typhimurium (water and sediment); 5% (8/169) were Hartford (sediment); 3% (two sets of 5/169) were Agona and Montevideo (sediment and tomato, respectively); and 0.6% (1/169) were Berta (sediment).

Significance: This project establishes three years of correlation between N.C. tomato production environments and the occurrence of clinically significant *Salmonella* serotypes. Recurring agricultural niches for this human pathogen were source water and sediments in each of the production seasons. Determining relationships between raw agricultural commodities, pathogen serotypes, and environmental reservoirs can assist in developing strategies to reduce commodity-pathogen risks for our farming communities.

P1-172 Whole Genome Sequencing of *Salmonella* Newport Clone JJPX01.0061 Reveals Phylogenetic Evidence for Endemic Persistence and Extensive Microevolutionary Diversification among Eastern Shore Surface Waters

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Introduction: Recurrent outbreaks of *Salmonella* Newport, *Xba*IPFGE pattern JJPX01.0061, have been linked to the consumption of tomatoes grown along the Eastern Shore of Virginia (VES) at least 6 times since 2002. Environmental surveys of this region suggest that this subtype is endemic, persisting in surface waters.

Purpose: The genomic diversity of a large population of JJPX01.0061 isolated from surface waters across the VES was investigated using whole genome sequencing (WGS) approaches.

Methods: More than 70 environmental JJPX01.0061 isolates spanning seven years from the VES were subjected to whole genome shotgun sequencing, assembled, and aligned using a reference-based mapping approach to a closed Newport genome (CFSAN024225). A maximum likelihood tree was then constructed based on total SNP variation and evaluated in light of geographic variation based on the location from which each isolate was collected.

Results: Genomic diversity within this PFGE subtype clustered the strains into four distinct clade separated by a range of only 5 to more than 100 SNPs. Two clades (C and D) sorted isolates uniquely based on specific creeks and were identical (C, 0 SNPs intraclade variation) or nearly identical (D, 1 SNPs). Two additional clades (A and B) were polyphyletic with respect to creek location suggesting two independent introductions of JJPX01.0061 variants into each of these locales. Finally, clade B, comprised largely of Newports from 2007, was separated from more recent Newport groupings by

at least 100 SNPs suggesting that substantial microevolutionary change has accrued within this lineage, a find consistent with its establishment and prolonged environmental persistence.

Significance: Genetic diversification of JJPX01.0061supports its long-term and endemic persistence within this regional microcosm. Occasional reintroduction of distinct genomic variants into common creek environments is also seen pointing to a potential role for geese or other water fowl species in the local mixing of isolates.

P1-173 Contamination of Soils with *Escherichia coli* by Deer Feces

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Introduction: Deer (*Odocoileus virginianus*) may excrete foodborne pathogens in their feces. Thus, deer intrusion into fields where fruits and vegetables are grown can result in food safety risks.

Purpose: The purpose of this study was to determine the extent to which *E. coli* transfers from deer feces to soils where crops are grown in Ohio.

Methods: Total coliform and *E. coli* counts were assessed in soil and surface debris samples collected from five areas of a vegetable production field naturally contaminated with deer feces and compared with counts present in soil and debris from the same field away from any visible signs of fecal contamination. Samples were collected again 60 days after feces removal and field fencing and assessed similarly. In separate experiments, fresh deer feces was deposited on soils in vegetable production fields and *E. coli* counts present in surrounding soils was determined at multiple distances and time intervals.

Results: Initially the soil and surface material from the visually contaminated areas had a higher generic *E.coli* than the uncontaminated area ($P = 0.01$). Sixty days later there was no difference in *E. coli* counts between the soils collected from the two areas ($P = 0.35$). *E. coli* counts in soils decreased sharply with distance from point of application ($P < 0.05$) within the first 30 cm. *E. coli* counts in soils 1 m away from the point of fecal deposition did not differ significantly from counts 5 m away.

Significance: Remediation of fields contaminated by deer 60 or more days prior to harvest can minimize risk of transfer of microorganisms from feces to soil. Moreover, refraining from harvesting fruits and vegetables within the proposed 5-foot (1.5 m) no-harvest zones around areas of fresh deer fecal contamination may also minimize the risk of transfer of fecal-origin microbial contaminates in soils to produce.

P1-174 Survival of Key Shiga Toxin-Producing *Escherichia coli* and *Salmonella* Serotypes in Various Domestic Animal Feces

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Introduction: Shiga Toxin-producing *Escherichia coli* (STEC) and *Salmonella enterica* have undergone a rapid epidemic spread in animal waste, therefore providing an efficient mechanism for pathogen amplification and dissemination into the environment through manure spreading on agricultural land.

Purpose: The objective of this study was to determine and compare the persistence of important produce-related STEC and *Salmonella* serotypes/serovars in various types of animal manures which commonly contaminate vegetable fields.

Methods: Two Rifampicin resistant-bacteria cocktails, one consisting of six *Salmonella* serovars (Typhimurium, Montevideo, Anatum, Javiana, Branderup, and Newport) and the other consisting of five STEC serotypes (O103, O104, O111, O145, and O157), were each applied to manures of domestic cattle, deer, wild pig, raccoon and waterfowl at level of 10^3 to 10^4 CFU/g feces, respectively. The inoculated manures were stored at room temperature (22°C), and tested periodically for Rifampicin resistant STEC and *Salmonella*. Molecular typing (PCR and PFGE) of recovered bacterial colonies were done to determine the bacterial serotypes/serovars surviving longest in manure.

Results: Under 22°C, STEC survive the longest in cattle feces for 12 months, followed by raccoon feces (10 months), deer feces (6 months), pig feces (4 months), and waterfowl feces (2 months). In comparison, *Salmonella* survived same long in feces of cattle, deer, and waterfowl, but lived longer in raccoon (12 months) and pig feces (5 months). Among the serotypes/serovars tested, STEC O104, *Salmonella* Anatum and Javiana totally out-competed others in feces of cattle, deer, raccoon and waterfowl. *Salmonella* Branderup survived as well as Javiana in pig feces for 5 months.

Significance: The study justifies no harvest stipulations and one year prohibition of cultivating on fields when incompletely composted or non-thermally treated manure has been applied, and facilitates understanding of how feces type may affect pathogen fate in the environment.

P1-175 Survival of *E. coli* in Biological Soil Amendments Applied to Soils in Southeastern Pennsylvania

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Introduction: Farmers utilize raw animal manure to improve soil fertility but may introduce bacterial pathogens to soils used to grow produce crops. The recent FDA "Produce Rule" currently proposes a 9-month interval between the application of manure and harvest of the crop.

Purpose: The study examines persistence of *E. coli* in manure-amended soils in Pennsylvania.

Methods: Inoculum containing attenuated rifampicin-resistant *E. coli* O157:H7 (*att*O157) and non-pathogenic *E. coli* (*g*Ec) was spray-applied (1,000 ml) at low (3.9 log CFU/ml) or high populations (6 log CFU/ml) to the surface of 2 m^2 plots containing poultry litter (PL), dairy solids (DS), or no manure treatment (UN). Additional PL, DS and UN plots were tilled after spray application. Surface samples of DS and UN plots and core samples of tilled PL (TPL), DS (TDS), and UN (TUN) plots were collected up to 113 days post-inoculation (dpi). *E. coli* populations were determined by enumeration on sorbitol MacConkey agar with rifampicin or by mini-MPN.

Results: High populations of *att*O157 in DS and UN declined to < 1 log CFU/gdw (gram dry weight) within 14 d; however, low populations of *g*Ec in DS and UN and of *att*O157 in DS increased between 0 – 2.5 log CFU/gdw between 0 -14 dpi before consistently declining to the detection limit by 56 dpi. Both low and high populations of *g*EC in TPL increased by up to 2.5 log CFU/gdw between 0 -14 dpi before declining; other *g*Ec and *att*O157 populations in TPL, TDS, and TUN, declined to < 1 log CFU/gdw by 56 dpi. *g*Ec populations declined more slowly in TPL than in TDS or TUN.

Significance: *E. coli* populations initially (0 - 14 dpi) fluctuated after the application of manure to soils. Microbial competition, nutrient limitation and/or physiological stress potentially affected *E. coli* population declines in manure-amended soils.

P1-176 Field-validation of Minimum Application Intervals for Use of Raw Animal Manure as a Soil Amendment in the Central Valley, California

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❖ ***Developing Scientist Competitor***

Introduction: Biological soil amendments of animal origin have been identified as a potential source of contamination of fresh produce with enteric pathogens. The original FDA Produce Safety Rule proposed a 9-month interval between application of raw manure and crop harvest; in contrast, the USDA National Organic Program (NOP) standard requires 120- and 90-day intervals for crops with and without soil contact, respectively.

Purpose: A 12-month experimental field trial was conducted to examine the survival of a three-strain cocktail of rifampicin-resistant generic *Escherichia coli* applied to soil amended with different animal manure types in California's Central Valley.

Methods: High (10^7 CFU/ml) and low (10^4 CFU/ml) inoculum were separately applied by spraying the *E. coli* cocktail onto 4 untreated horse-, cattle-, goat-, and chicken litter- amended soil and control plots (2 m x 1 m) at the UC Davis vegetable crop field station. Soil samples were collected from November 2013 to October 2014 to determine the generic *E.coli* population by direct plating and MPN methods. The study was initiated during a time when the region was experiencing extreme drought conditions.

Results: We observed a 7.16 log reduction after 120 days from manure application. The generic *E. coli* populations survived longest in untreated chicken litter followed by horse, cattle and goat manure. *E. coli* populations increased after heavy rains by 5.87 and 5.61 log CFU in high and low inoculums plots, respectively. Time and manure type were statistically significant ($P < 0.0001$) and predicted the concentration of indicator *E. coli* in a linear regression model.

Significance: Although die-off was observed in soil by day 120 during a fall-winter period, resuscitation was observed for all manure types following heavy spring rains. The findings suggest that generic *E. coli* experiences multiple log reductions over 120 days, but exposures to rain fall can contemporarily reverse these reductions.

P1-177 Fate of *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica* in Soil is Not Influenced by Mulching Treatment

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Introduction: Mulching is a common cropping practice used in the cultivation of fresh produce. The influence of mulching on bacterial community composition and the fate of human pathogens is not well investigated. Extended survival of human pathogens within mulch-covered soil could increase the risk of produce contamination.

Purpose: The fate of three foodborne pathogens, *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica*, in soil previously exposed to four different mulches was examined over a three-month period.

Methods: Soil was collected from lettuce plots covered with polyethylene plastic, biodegradable plastic, paper, or straw mulch, or bare ground (n = 4/mulch). Soil was transferred to potting trays containing 72 separate pots, and pathogen cocktails were applied separately. Trays were placed inside a growth chamber maintained at 21°C and 60% relative humidity. Pathogen populations were enumerated by spread plating or MPN enrichment.

Results: Biological replications were not significantly different. At time zero, no significant differences ($P > 0.05$) between populations (2.92 ± 0.5 log CFU/pot *E. coli* O157:H7, 6.42 ± 0.09 log CFU/pot *L. monocytogenes*, and 6.63 ± 0.2 log CFU/pot *Salmonella*) existed among mulch treatments or bare ground. Populations, declining over time in each mulch treatment, were either not significantly different or significantly different at only one time point; on days 3 and 28 biodegradable plastic had significantly higher *E. coli* O157:H7 and *Salmonella* populations, respectively. By three months, populations had decreased to -0.68 ± 0.16 log MPN/pot, -0.19 ± 0.09 log MPN/pot, and 0.18 ± 0.13 log MPN/pot for *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella*, respectively. *Salmonella* populations declined significantly less than those of the other pathogens.

Significance: *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* may persist for durations exceeding three months in mulched soil and bare ground. Choice of mulch treatment does not have a significant effect on pathogen fate within soil.

P1-178 Population Dynamics of Generic *E. coli* and Naturally Occurring *Listeria* in Manure-Amended Soils in the Northeastern U.S

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Introduction: Proposed U.S. FDA standards for use of manure as a biological soil amendment originally stipulated a 9-month waiting period between soil application and harvest to reduce the risk of pathogen contamination on fresh produce. Survival of bacterial pathogens and concomitant risk of produce contamination are impacted by many factors including climate, manure source, soil type, geographical location and bacterial populations.

Purpose: To compare population dynamics of non-pathogenic *E. coli* (*gEc*) and *Listeria* spp. in surface and tilled plots in two soil types amended with untreated dairy manure in the northeastern U.S.

Methods: A three-strain inoculum of rifampicin-resistant *gEc* (6 log CFU/ml) was sprayed onto field plots (2 m^2) of either Loamy sand (L) or Sandy (S) soils amended with dairy-manure solids (DS) or no manure (NM). Manure was tilled into the soil in half the plots. Survival of inoculated *gEc* and native *E. coli* in DS in surface and tilled plots was determined over 56 days post-inoculation (dpi) by colony count or MPN. The presence of *Listeria* spp. was assayed in selected plots.

Results: *gEc* and native *E. coli* populations in tilled L plots declined less slowly (by 1.1 - 2.7 log MPN/gdw) compared to in surface L plots (by 4.0 - 4.3 log MPN/gdw) at 56 dpi, . However, *gEc* populations increased in tilled S plots between 28 and 56 dpi while *gEc* populations declined (3.4 - 4.0 log MPN/gdw) in surface S plots. In general, *gEC* and native *E. coli* populations increased after rain events between 0 - 14 dpi regardless of soil type or manure application. *Listeria* spp. was present in uninoculated NM and DS plots.

Significance: These results suggest that tillage and soil type may affect the survival of *E. coli* in soils as much as the presence of manure.

Poster

P1-179 Emission and Deposition of Bioaerosols Generated during Mechanical Handling (Mixing) of Composts

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Introduction: Produce in fields that are in close proximity to concentrated animal feeding operations (CAFO) can become contaminated through airborne transport and deposition of manure with fecal pathogens. It is unclear to what extent fecal pathogens in animal manures are aerosolized, transported, and deposited by air during mechanical operations conducted during compost production.

Purpose: An experimental study was conducted in Beltsville, MD, to determine the emission and deposition of bioaerosols generated during feedstock mixing and windrow turning, common mechanical handling operations in composting processes.

Methods: Bioaerosols generated during mixing/turning of dairy manure solids (DMS, compost feedstock) and 2-week-old DMS-compost windrows were collected 3 m downwind of materials handling operations using 6-stage Anderson Impactors and SpinCon air samplers, operating at 28.3 l/min and 450 l/min, respectively; 30 samples from two experiments were analyzed. Samples (n = 14) of basil leaves from plants exposed to bioaerosol deposition at 3 m downwind were collected. Total aerobic bacteria (tAB), fecal coliform and *E. coli* concentrations were determined by conventional plating and MPN procedures.

Results: Populations of tAB, coliforms, and *E. coli* in for dairy solids and composts were not significantly different ($P > 0.05$). Airborne tAB concentrations were 4.29 and 6.39 log CFU/m^3 and *E. coli* were 0.133 and 1.52 MPN/m^3 during mixing and turning of DMS and compost, respectively. Particle-size distribution of tAB was not significantly different ($P > 0.05$) from that for composts. Both coliforms and *E. coli* were primarily associated with coarse particles (> 2.1 µm). Calculated deposition of *E. coli* on basil leaves at 3 m downwind = -3.48 and -4.31 log MPN $cm^{-2} \cdot s^{-1}$, for DMS and compost, respectively.

Significance: These data will be useful in atmospheric dispersion modeling to predict, refine, and validate projected downwind concentrations of bacterial aerosols generated during dairy manure solids handling and composting operations under a variety of atmospheric conditions across various terrain.

Poster

P1-180 Validation of Waiting Intervals for the Incorporation of Untreated Biological Soil Amendments of Animal Origin into Soil Where Specialty Crops are Grown in Ohio

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Introduction: Biological soil amendments of animal origin provide a valuable source of fertilizer for growing fruits and vegetables. At the same time, animal manures may contain foodborne pathogens that can result in contamination of produce. The survival of pathogens in soil-applied manure is dependent upon a number of factors including the composition and characteristics of the manure and the soil and environmental conditions.

Purpose: The purpose of this study was to model the survival, under environmental and management conditions in Ohio, of microbial indicators of fecal contamination (*Escherichia coli*) and pathogens (*Escherichia coli* O157, *Listeria monocytogenes, Campylobacter jejuni, Salmonella* spp.) in soils amended with bovine manure.

Methods: Untreated bovine manure (mixture of feces, urine and used bedding materials) was applied at a rate of approximately 2 ton/acre on fields (n = 3) intended for growing produce with predicted harvest dates to coincide with 270 days, 180 days, 120 days and 90 days prior to harvest. Prior to planting, the soil was turned following traditional Amish cultivation practices. *E. coli* counts and pathogen prevalence was monitored monthly following application of manure.

Results: Results from the first season demonstrated decline generic *E. coli* counts in soils following application and tillage. Counts did however increase after tillage without that application of additional soil amendments. Total *E coli* counts in soils at the time of harvest did not vary with the time since prior manure application. Pathogens were infrequently recovered from both amended and non-amended soils.

Significance: The survival rate of *E. coli* and pathogens calculated from this study provide valuable data to populate risk models critically needed to predict food safety risks associated with the application of untreated biological soil amendments of animal origin, especially under the conditions typical of specialty crop production in Ohio.

P1-181 Characterizing Changes in the Soil Microbiota that Can Alter Natural Suppression of *Escherichia coli* O157:H7 in Ohio Specialty Crop Soils

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Introduction: Understanding foodborne pathogen persistence in the soil is a crucial link for minimizing pre-harvest contamination of vegetables and small fruits entering the food chain.

Purpose: A longitudinal survey was conducted to quantify the extent to which soils from different fields suppress survival of *E. coli* O157 and to characterize important microbial groups in the soil that may be responsible for altering this suppression.

Methods: Soil was collected at planting and during harvest from twelve specialty crop fields in Ohio. Heat-treated and unheated subsamples were assayed for anti-*E. coli* O157 effects by inoculating the soil with *gfp*-labelled *E. coli* O157 and following the fate of these bacteria over 48 h. In order to understand the biological basis for pathogen suppression, differences in microbial community structure associated with *E. coli* O157 suppressive soils were determined by sequencing of the V4 region of the 16S rRNA gene and ITS-1 of fungal targets by Illumina MiSeq platform. Analysis was performed using QIIME.

Results: Heat-sensitive microorganisms were largely responsible for the suppression of *E. coli* O157 regardless of the season, region, or chemical composition of the soil. Soil pH, moisture content, and levels of soil organic matter (SOM) of the samples varied widely across regions and seasons. Likewise, relative suppression of the samples varied within each region and season. Although not statistically significant, this suppression appeared to be more frequently observed at harvest than at planting. Across the different fields, the composition of the microbiota varied and, within a single field, seasonal changes within the soil microbiota were also observed.

Significance: The results of this project support that a novel biocontrol soil additive to enhance natural foodborne pathogen suppression in specialty crop soils has potential as a means of controlling foodborne pathogens on small fruits and vegetables.

P1-182 Effect of Top-dressing Compost and Heat-treated Poultry Litter Pellets on Survival of *E. coli* in Dairy Manure Compost-amended Soils

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Introduction: Animal manure composts (biological soil amendments, BSAs) are used to maintain quality and fertility of soils. When produced by validated processes, composts are approved for use by USDA-National Organic Program rules and current FDA Produce Safety rules. Likewise, heat-treated poultry litter pellets (htPLP) produced by a validated process are approved BSAs. Nutrient-rich, microbiologically unbuffered htPLP top-dressed on compost-amended soil poses a potential risk of rapid colonization and survival by pathogenic bacteria inadvertently introduced to BSAs and then subsequently transferred to crops.

Purpose: A randomized complete block (n = 4) field experiment conducted in Beltsville, MD, examined survival of non-pathogenic *E. coli* (*gEc*) and attenuated *E. coli* O157:H7 (*attO157*) in soil amended with and without composted dairy manure solids (CDM), and top-dressed with or without either CDM, htPLP, or fertilizer.

Methods: Plots (2 m^2) surface-amended with CDM or not were spray-inoculated with a multi-strain cocktail of rifampicin-resistant *gEc* and *attO157* (~4.92 log CFU/ml) prior to tillage incorporation at USDA-ARS-BARC. Plots were seeded to spinach 1 day post-inoculation (dpi) and top-dressed with either CDM, htPLP, or fertilizer according to nitrogen requirements 35 dpi. Soil samples (n = 160) were collected/analyzed 0 - 62 dpi by direct plating and MPN procedures to determine survival of *E. coli* populations.

Results: The *attO157* populations declined from ~2.0 to ≤ 0.02 log MPN/gdw in CDM-amended plots by day 3 in the fall and did not recover after top-dressing with CDM, htPLP, or fertilizer. Populations of *gEc* survived better in all BSA treatments than *attO157* populations ($P < 0.05$). Rainfall during 1 - 29dpi sustained *gEc* populations to 2.5 - 3.8 log MPN/gdw, but after top-dressing, rainfall, and below freezing temperatures, populations ranged 0.95 (htPLP only) - 1.7(CDM only) log MPN/gdw on 62 dpi.

Significance: These results indicate that *attO157* populations decline more rapidly than *gEc* populations in soil amended at agronomic rates with quality CDM even with adequate soil moisture and subsequent top-dressing with CDM, htPLP, or fertilizer.

P1-183 The Effect of Mechanical Damage, Heat and Flooding on Human Norovirus, Tulane Virus and Murine Norovirus Survival on Pre-harvest Lettuce Plants

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Introduction: Leafy greens are often implicated in human norovirus (HuNoV) foodborne outbreaks. On the farm, leafy green plants may experience various stressors including mechanical damage, heat and flooding which may affect virus survival.

Purpose: To assess the effect of damage, heat and flooding on HuNoV survival on pre-harvest lettuce plants.

Methods: Murine norovirus (MNV) and Tulane virus (TV) were used as surrogates to assess HuNoV infectivity. Two days prior to viral inoculations, mature lettuce plants were subjected to the following treatments: mechanical damage of outer leaves, heat at 36°C daytime, or flooding with water. All plants, including controls, were then spot inoculated with HuNoV [6 log genomic equivalent (GE)/ml)], MNV and TV [6 log 50% tissue culture infectious dose ($TCID_{50}$)/ml] and maintained in a growth chamber at 20°C daytime. RNA viral titers were determined after RNase treatment using real-time reverse transcription-PCR. Infectivity titers for TV and MNV were determined using cytopathic effect-based assays. Each treatment was replicated three times per virus type. Titers were determined on post-inoculation days (PID) 1 and 7. Data were analyzed using two-way ANOVA with time and treatment as factors. Statistical significance was defined at $P < 0.05$.

Results: None of the treatments significantly affected HuNoV RNA titers, MNV or TV infectivity and RNA titers on any date. For all treatments and controls: HuNoV, TV and MNV showed no significant difference in RNA titers on PID7 as compared to PID1, whereas TV and MNV infectivity titers decreased significantly (average 2.87 ± 0.19 $TCID_{50}$/g). For MNV and TV, time exerted significant effects on infectivity titers accounting for 94.7 and 93% of total variance, respectively.

Significance: Our results suggest that damage, heat and flooding had no effect on HuNoV viral RNA titers on lettuce plants. However, time had a significant negative effect on the survival of infectious MNV and TV.

P1-184 Binding Dynamics of Human Norovirus to Berries and Berry-associated Microflora

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Introduction: Fresh berries, including raspberries and strawberries, have been associated with human norovirus (HuNoV) outbreaks. It is likely that HuNoV bind to berries, but the dynamics of such binding are unknown. Candidate mechanisms of binding include histo-blood group-like antigens (HBGAs) or unknown ligands present in foods; native berry microflora; biofilms; and/or electrostatic interactions.

Purpose: To elucidate the binding dynamics of HuNoV to berries.

Methods: Three varietals of strawberries and five of raspberries were collected and concentrates prepared. Natural berry microflora was isolated, whole colony sequencing performed, and biofilm production of selected strains was quantified using a crystal violet biofilm assay. Affinity of HuNoV GI.6 and GII.4 virus-like particles (VLPs) binding to berries and biofilm-producing bacteria was determined by ELISA, as was the presence of HBGA-like moieties (A, B, H, Le^a, Le^y). Virus capture efficiency of GI.6 and GII.4 HuNoV to berries and isolates was determined by RT-qPCR.

Results: A total of 172 (n = 98 from raspberries; n = 74 from strawberries) bacterial isolates were sequenced, with microbial profiles differing by berry type. Biofilm production also varied greatly, but strains of *Pantoea*, *Pseudomonas*, *Klebsiella,* and *Enterobacteriaceae* were the strongest biofilm producers ($A_{405} > 2.2$). ELISA results indicated minimal binding of GI.6 and GII.4 VLPs to berry concentrates (+/- absorbance ratio of 0.2 - 0.7). Only low levels of Le^a HBGA-like moieties were present in strawberries ($P < 0.05$), and a minimal level of A, B, H and Le^y moieties were detected in biofilm-producing bacteria isolates. Capture efficiency of GI.6 and GII.4 HuNoV to berries and selected isolates was ~1 log (91.7%) or less.

Significance: HuNoV bind weakly to raspberries and strawberries, berry microflora, and associated biofilms. Berries and the bacterial isolates do not contain significant levels of HBGA-like moieties. We hypothesize that fecal material may facilitate HuNoV binding to berries and further studies to confirm this are underway.

P1-185 Survival of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, Murine Norovirus, and Tulane Virus on Foliar Surfaces of Spinach Plants

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Introduction: The persistence of *E. coli*, *Salmonella* spp., and norovirus in "non-host" environments, including on contaminated spinach plants, is poorly understood. The presence of the *rpoS* gene in *E. coli* O157:H7 and *Salmonella* spp. may affect survival on foliar surfaces.

Purpose: To compare persistence of *E .coli* O157:H7, *Salmonella* Typhimurium, murine norovirus (MNV) and Tulane virus (TV) on the foliar surface of spinach plants.

Methods: Five week-old spinach (Menorca, semi-savoy) plants were co-inoculated with 6 log CFU/plant of: wild-type *E. coli* O157:H7 (wtO157), wt *Salmonella* Typhimurium (wtSal), and *rpoS*-deficient mutants of *E. coli* O157:H7 (rposO157) and *Salmonella* Typhimurium (rposSal). MNV (6 log PFU/ml) and TV (4 log PFU/ml) were inoculated on the same spinach plants in study 2. Bacteria from homogenates were enumerated on selective media or by MPN. Five plants/day on days 0 - 2 and three plants/day on days 0 - 4 were analyzed in study 1 and study 2, respectively. MNV and TV titers were determined by quantitative real-time PCR on days 0 - 4. T-tests were used to determine statistical differences ($P < 0.05$) between wt and *rpoS*-deficient strains.

Results: From day 0 to 1, population declines of wtO157 (2.79 log CFU/plant) and rposO157 (4.49 log CFU/plant) were not significantly different ($P = 0.1344$); however, the decline of rposSal populations (4.22 log CFU/plant) was significantly ($P = 0.0010$) greater than that of wtSal (2.83 log CFU/plant). By day 4 (study 2), populations of rposO157 and rposSal were 0.16 log MPN/plant, while wtO157 and wtSal populations were 0.51 and 1.01 log CFU/plant. MNV titers did not decline from day 0 - 2 (1.8 - 2.1 log genomic copies/plant), and TV was only detected plants on day 2.

Significance: The *rpoS* gene slows the decline of *E. coli* O157:H7 and *Salmonella* populations on spinach plants. Norovirus surrogates may persist at constant levels longer than bacterial populations on spinach plants.

P1-186 Plant-microbe Interactions Associated with Noroviruses and Leafy Greens

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❖ ***Developing Scientist Competitor***

Introduction: Norovirus is the leading cause of acute gastroenteritis, including illness linked to contaminated produce. Methods by which norovirus comes into contact with and persists within the phyllosphere and rhizosphere of leafy greens is a complex issue that remains unsolved. The fields of food safety and plant science are merging to address plant-norovirus interactions to develop control strategies to reduce the number of illnesses associated with norovirus and leafy greens.

Purpose: The purpose of this study was to determine if norovirus elicits an immune response in plants. We also sought to determine if plant signaling hormones affect the persistence of internalized norovirus.

Methods: Whole *Arabidopsis thaliana* plants or Romaine lettuce leaves were pooled (n = 4) and frozen in liquid nitrogen prior to RNA extraction and semi-quantitative PCR. Gene expression was quantified using markers for plant immune response pathways. Salicylic acid (SA) persistence studies were performed at 22°C for 20 min. SA was neutralized with FBS and total viral RNA extraction was performed prior to qPCR.

Results: In *Arabidopsis thaliana*, murine norovirus (MNV) was able to elicit an immune response through both the SA and jasmonic acid pathways shown through increased (> 2-fold) expression of PR-1, PDF1.2 and VSP2a at 6 hpi. Human norovirus was able to induce a 4-fold increase in the expression of the NPR-1 gene in lettuce 12 hpi indicating stimulation of the SA pathway. MNV and Tulane virus (TV) were shown to survive for 20 min in 500 mM (pH 2.4) SA indicating the ability of internalized norovirus to survive in plants ($P = 0.80$) although TV was able to persist at significantly higher levels in SA compared to MNV ($P = 0.029$).

Significance: This is the first study to show the biological recognition of human norovirus by plants as well as the ability of internalized virus to survive within plants during an immune response.

P1-187 Bacteria Associated with Tomato Microbiomes: Ripe vs. Unripe

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Introduction: The majority of tomatoes that have been implicated in *Salmonella* outbreaks associated with Virginia grown tomatoes were harvested when they were green (a common agricultural practice). Enhanced understanding of bacteria associated with tomato microbiomes may shed light on how pathogens such as *Salmonella* become associated with the fruits and result in foodborne outbreaks. Different stages of ripening may harbor distinct microbial communities that play currently un-described, yet significant roles in plant responses to introduction and persistence of human pathogens on tomato surfaces.

Purpose: The purpose of this study is to describe microbial communities associated with tomato fruits at mature green and red ripe stages to better understand microbial dynamics of different ripening stages that may be significant to food safety.

Methods: Green and red tomatoes were aseptically collected in the summer of 2014 from the Agricultural Research Station in Painter, Virginia. Culture-independent DNA was extracted from washes of fruit surfaces. PCR amplicons of V1-V3 region of 16S rRNA gene were used to characterize bacterial communities associated with green and red fruits. Bioinformatic analyses were performed using QIIME.

Results: Ripe and unripe tomato surfaces supported distinct microbial communities in principal coordinates analyses ($P < 0.001$). Red ripe tomatoes supported a higher relative abundance of Enterobacteriaceae ($P = 0.001$) and unripe tomatoes demonstrated higher incidences of certain taxa including; Sphingomonas ($P = 0.036$), Pseudomonadaceae ($P = 0.036$), and Rhizobiaceae ($P = 0.036$).

Significance: These data will contribute to an improved understanding of food safety risks correlated with ripening dynamics and improved recommendations for agricultural practices associated with harvest of tomato at specific stages of maturity.

P1-188 Fate of *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes* on the Surface of Whole Cantaloupes and Watermelons during Storage

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Introduction: Recent outbreaks linked to whole melons highlight the importance of understanding the fate of pathogens on melon surfaces at various temperatures.

Purpose: The objective of this research was to quantify the fate of *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes* on the surface of whole cantaloupes and watermelons.

Methods: Rifampicin-resistant *Escherichia coli* O157:H7, *Salmonella,* or *L. monocytogenes* (ca. 10^3 CFU/3.1 cm^2) cocktails were spot inoculated on the sun-side of whole Athena cantaloupe or watermelons and allowed to dry for 1 h (n = 6). Melons were stored at 4, 10, 15, 20, or 25°C, and sampled up to 21 days. At each time point, the inoculated area (3.1 cm^2) was excised, stomached with 10 ml of buffer, and pathogen populations were enumerated on both selective and nonselective media supplemented with rifampicin.

Results: *Salmonella* on both melon surfaces, and *Escherichia coli* O157:H7 on watermelon, declined under all experimental conditions. *Escherichia coli* O157:H7 on cantaloupe held at 4 and 10°C decreased, but populations increased at 15, 20, and 25°C storage; maximum growth occurred when cantaloupe was stored at 25°C for 3 days (4.9 log CFU/3.1 cm^2). *L. monocytogenes* declined on watermelon at 4, 10 and 15°C, but grew on watermelon held at 20 and 25°C, reaching maximum populations at 7 days of storage (4.2 and 4.5 log CFU/3.1 cm^2, respectively). *L. monocytogenes* inoculated onto cantaloupe increased regardless of storage temperature; increases ranged from 0.4 (4°C) to 2.2 (25°C) log CFU/3.1 cm^2. Maximum populations occurred at 25°C at 7 days (5.3 log CFU/3.1 cm^2).

Significance: Food safety risks associated with contamination of whole melons vary depending on pathogen, melon, and postharvest handling. *Salmonella* did not increase on either watermelon or cantaloupe; *E. coli* O157:H7 did not increase on watermelon, but did increase above 15°C on cantaloupe; *L. monocytogenes* increased on watermelon above 20°C and at all temperatures on cantaloupe.

P1-189 Persistence of *Escherichia coli* O157:H7, *Salmonella*, and Poliovirus on Fresh and Pickled Bird Chili Peppers (*Capsicum frutescens* Linn.)

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Introduction: Fresh hot peppers such as chili, jalapeño, and serrano are commonly consumed raw or minimally processed. They may become contaminated with pathogens during production and post-harvest processing, posing a health risk to consumers. Pathogen survival during storage and processing varies by type.

Purpose: We investigated the survival of *Salmonella*, *Escherichia coli* O157:H7, and poliovirus on chili peppers at ambient temperatures, simulating some market conditions. Next, we investigated whether preserving by pickling or refrigeration affected pathogen survival rates.

Methods: Fresh bird chili peppers (*Capsicum frutescens* Linn.) were inoculated with a cocktail of *Salmonella*, *E. coli* O157:H7, and Poliovirus-1 and stored at 22 - 25°C. Separately, inoculated peppers were pickled (5% acetic acid vinegar, pH 2.54) and stored at 4°C for 8 weeks. Pathogens were enumerated from the fresh and pickled peppers (and the vinegar) initially and at days 1, 7, 28, and 56. Poliovirus was enumerated using the most probable number (MPN) infectivity assay. *Salmonella* and *E. coli* O157:H7 were enumerated on modified tryptic soy agar.

Results: Results validate the prolonged survival of pathogens on fresh and pickled bird chili peppers. Bacterial and viral pathogens showed differential trends in survival. On fresh peppers, *Salmonella* and *E. coli* O157:H7 remained relatively consistent (10^5 - 10^6 CFU/g) for 4 weeks at 25°C and significantly declined ($P < 0.05$) at week 8. Poliovirus numbers decreased significantly after 1 day and were undetectable at week 8. In contrast, no bacteria were recovered from pickled peppers and their vinegar following day one at 4°C. However, poliovirus persisted at significant levels on both pickled peppers and in their vinegar (10^3 MPN/g and 10^2 MPN/ml, respectively) for 8 weeks.

Significance: Results support the importance of preventing contamination of fresh produce during production and post-harvest. Additionally, results discredit the notion that common produce harvesting practices eliminate pathogens.

P1-190 Critical Control Point-linked Postharvest Risk Assessment of Angelicae Gigantis Radix

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Introduction: HACCP methodology was applied in the post-harvest processing and storage of domestic medicinal produces.

Purpose: Particularly in terms of mold and mycotoxin contamination, candidate critical control points (CCP) in the conventional practice in Korean farms were selected and monitored by comparing with on the standard guided processing and storage.

Methods: ELISA and HPLC/MS method were used to detect and quantify mycotoxins.

Results: When each processing of Angelicae Gigantis Radix were assessed for their safety, the drying steps such as the sun drying or the thermal drying depending on each farm made differences in mold contamination. Moreover, the storage conditions before or after the processing were another critical determinant in the fungal contamination. In other words, storage under 4oC rather than at room temperature was favorable for reducing mold growth in the harvested crops. Occurrence rate of Aflatoxin B1 (AFB1) in Angelicae Gigantis Radix were 12.8%, but amount of AFB1 in all the collected samples were below 10 ppb regulatory limit allowed in Korea. However, for a few samples of Angelicae Gigantis Radix, still relatively high levels of total amount of the major aflatoxins (aflatoxin B1+B2+G1+G2) were observed around 0.18~49.94 ppb, which is not regulated presently in Korea.

Significance: It thus can be suggested that post-harvest processing and storage of Korean medicinal crops need further investigation and monitoring to establish the Good Agricultural Practice (GAP), particularly to minimize microbial risk including mold and mycotoxin contamination under the changing climate. Additionally, it is also warranted for new enacting of regulatory limits for total aflatoxins in the medicinal crops (This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ01093206)" Rural Development Administration, Republic of Korea).

P1-191 Critical Control Point-based Reduction of Postharvest *Fusarium* Mycotoxins in Medicinal Grains of Job's Tears

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Introduction:Improperly practiced post-harvest procedures can pose mycotoxin-related risks in the production of medicinal herbs. As a health food with pharmacological supplements, cereal-based Job's tears has been broadly used in the oriental medical practice.

Purpose: Compared with the standard protocol, three provisional critical control points (CCPs) in the conventional procedure were identified and assessed for mycotoxin contamination in the Job's tears from small farms in Korea.

Methods:ELISA and HPLC/MS analysis were performed to detect and quantify myctoxins.

Results: Although various mycotoxins are present, the prevalence of deoxynivalenol (DON) or zearalenone (ZEN) was relatively high in the Job's tears. In terms of drying condition, field drying in the conventional pathway was associated with more exposure to DON than heated air drying. Moreover, DON or ZEN levels in chaff were higher than in inner grain, suggesting that the hulling process as another CCP would reduce the DON or ZEN exposure. In particular, DON or ZEN levels in Job's tears stored for protracted period without dehulling were very high, but a lower storage temperature of 12°C was not effective at reducing these mycotoxins significantly.

Significance: In this cases, inner grain were more contaminated with DON or ZEN than the chaff after protracted storage because surface fungi, which produce mycotoxin, can penetrate deep into grain with time. Heated air drying and non-protracted storage limited DON contamination in Job's tears. More importantly, early dehulling process should be adopted to reduce the risk of exposure to DON or ZEN in post-harvest as an easy preventive action. This is kept monitored as a central CCP for safer production of Job's tears before marketing from local farms (This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ01093206)" Rural Development Administration, Republic of Korea).

P1-192 Reduced Contamination of *Campylobacter* in Poultry Grown in Mixed Crop-livestock Farms with Cheap Byproducts of Berry Fruits

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Introduction: Farm animals serve as a reservoir for a variety of zoonotic pathogens in Mixed Crop-Livestock Farms (MCLFs). As a result, these farm environment and their products including chicken meats and eggs are at higher risk of cross-contamination with zoonotic bacterial pathogens including *Campylobacter*. Most of the MCLF farmers in the Mid-Atlantic region practice natural and/or pasture organic system and they are not allowed to use synthetic chemicals and antimicrobials. Natural bioactive extracts from cheap byproducts of berry fruits can serve as a potential antimicrobial alternative.

Purpose: The purpose of this study was to investigate the effects of bioactive components extracted from blackberry and blueberry pomace to control *Campylobacter* colonization in chicken as well as its applicability as animal feed supplement.

Methods: Minimum Bactericidal Concentration (MBC) was determined with broth microdilution method. Adhesion and invasiveness assay was carried out in chicken cell-culture model. Expression of inflammatory cytokine genes in host cells was determined with qRT-PCR.

Results: Our results indicated that MBC of blackberry and blueberry pomace extracts were 0.8 and 0.5 mg GAE/ml on *Campylobacter jejuni*. Physicochemical properties such as cell surface hydrophobicity and auto-aggregation of *C. jejuni* were altered and swimming motility was reduced by > 80% due to treatment with berry pomace extract. We also found that the pomace extracts influenced the expression of *C. jejuni* virulence genes, especially *flaA* and reduced attachment with chicken fibroblast (DF1) cells by > 0.5 log. Berry pomace extract showed anti-inflammatory effect on chicken macrophage (HD11) cell line by reducing the expression of pro-inflammatory cytokine genes, IL-1β and IL-6 when infected with *C. jejuni* for 24 h.

Significance: This study shows that bioactive extracts from blackberry and blueberry pomace can serve as a potential alternative to chemical antimicrobials and reduce *Campylobacter* colonization in farm animals specifically poultry, to improve product safety.

P1-193 Aflatoxin B_1 Detoxification Activities Produced by Fungi Isolated from *Meju*, a Korean Traditional Fermented Soybean Product

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❖ ***Developing Scientist Competitor***

Introduction: Aflatoxins (AFs) are toxic secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* on agricultural crops. Aflatoxin B_1 (AFB_1) is the most potent human carcinogen among major AFs. Since AFB_1 could pose a threat to human health, it is needed to find methods for controlling AFB_1 contamination in foods.

Purpose: The purpose of this study was to investigate AFB_1-biodegradation activities of fungal isolates from *meju*, a Korean traditional fermented soybean product for safe application to foods.

Methods: The AFB_1-biodegradation activities were investigated using cultures of *Aspergillus oryze*, *Aspergillus tubingensis*, and *Rhizopus oryzae*. The Ames test was used to confirm the AFB_1-biodegradation activities of the fungal isolates.

Results: All of the 3 fungal isolates showed significant AFB_1-biodegradation activities in liquid cultures; the amount of AFB_1 was reduced by more than 60% within 14 day cultures. Cell-free culture broth of the fungal isolates degraded more than 50% of AFB_1 within 7 day cultures. The Ames tests with *Salmonella* Typhimurium TA 98 mutants showed that the mutagenic effects of AFB_1 decreased to 5.6, 4.9, and 6.0% in frame-shift mutation when treated with the 3 fungal stains. The base-substituting mutagenicity of AFB_1 with the fungal isolates on *Salmonella* Typhimurium TA 100 also decreased to the same level as a control (52.3%).

Significance: The AFB_1-biodegradation activities produced by the 3 fungal isolates could be applied safely to foods.

P1-194 Identification and Characterization of Ochratoxin A Producing Mold from Grape Pomace

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❖ ***Developing Scientist Competitor***

Introduction: In recent times, grape pomace is gaining use as a food ingredient. Ochratoxin A (OTA), a toxic fungal contaminant of agricultural produce and their byproducts, including grape pomace is creating food safety concerns. Members of *Aspergillus* section Nigri (black *Aspergilli)* are mainly responsible for OTA buildup in grape pomace.

Purpose: The purpose of this study is to evaluate the safety of grape pomace as food ingredient by (i) determining the fungal population in the pomaces, and (ii) identifying and characterizing ochratoxigenic-producing *Aspergilli* from the pomaces and (iii) studying the ability of the pomaces to produce OTA.

Methods: Molds isolates were identified macroscopically according to Pitt and Hocking (1997) and OTA was detected using ELISA method.

Results: The population of yeast and mold ranged from 4.27 ± 0.05 to 5.35 ± 0.04 log CFU/ml. Out of an average of 37 mold counts, OTA producing mold *Aspergillus niger* (81.1%) and *Aspergillus carbonarius* (13.51%) were found to be the most dominant molds identified. Other molds detected were *Aspergillus fumigatus* (5.39%). Pomace derived from Chardonnay and Merlot grape cultivars were mostly populated with all

three *Aspergillus* strains detected. No mold was detected in pomace from Cabernet Franc, and Sangiovese grapes. The level of OTA in the pomace varied with grape cultivars used for the pomaces. High levels of ochratoxin A exceeding the 2 ng/g allowed level were detected in the pomace samples of Chardonnay (159.99 ± 36.99), Cabernet Sauvignon (133.46 ± 22.99), Sangiovese (134.00 ± 23.08) and Merlot (20.06 ± 3.87 ng/g).

Significance: The findings from this study indicates that pomaces obtained from some grape cultivars may not be safe to be used as a food ingredient due to the contamination of pathogenic molds and presence of high levels of ochratoxin A.

P1-195 Roles of NAG-1 in Epithelial Toxicity of Foodborne Carrageenan as IBD-Like Symptom Trigger

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Introduction: Carrageenan (CGN), a widely used food additive, has been shown to injure the epithelial barrier in animal models. This type of damage is a clinical feature of inflammatory bowel disease (IBD) in humans.

Purpose: In the present study, the effects of CGN on pro-apoptotic responses associated with NSAID-activated gene 1 (NAG-1) regulation in human enterocytes were evaluated.

Methods: Human and murine entrocytes were assessed using different tools of cell biology and immunology.

Results: CGN up-regulated the expression of NAG-1 that promoted epithelial cell apoptosis. Although NAG-1 induction was dependent on pro-apoptotic p53 protein, the pro-survival protein ATF3 was negatively regulated by p53 expression. However, NAG-1 enhanced the expression of the pro-survival protein ATF3 in enterocytes exposed to CGN. Functionally, NAG-1-mediated epithelial cell apoptosis was counteracted by the pro-survival action of ATF3 in response to CGN exposure.

Significance: These findings demonstrated that the counterbalance between NAG-1 and ATF3 is critical for deciding the fate of enterocytes under the food chemical stress (This study was supported by a grant of the Korean Health Technology R&D Project, Ministry of Health &Welfare, Republic of Korea (HI13C0259).

P1-196 Examination of Indicator Organism Levels in Open Surface Water Sources Used for Overhead Cooling of Apples in Washington

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Introduction: The currently proposed produce rule of the Food Safety Modernization Act provides numerical standards for generic *E. coli* associated with agricultural water.

Purpose: The study evaluated generic *E. coli* in open surface waters used for irrigation and overhead cooling of apples in Washington.

Methods: Variation in water quality was examined based on region, source, holding and delivery of water. Multiple sites from each location (ex. 2 canal, 3 pond) were evaluated for consistency. Samples were tested for *E. coli* using the FDA-BAM Most Probable Number method (2013, 2014) and IDEXX Colilert®-18, Quanti-Tray®/2000 system (2012); and for presence of *Salmonella* and *E. coli* O157:H7 (2013).

Results: For 319 samples evaluated in 2013 (24 locations) using the MPN method, 16 samples (5%) exceeded the originally proposed FDA standard for any single sample (235 MPN/100 ml generic *E. coli*). One site of 82 (1%) exceeded the originally proposed rolling geometric mean standard (126 MPN/100ml). IDEXX Colilert®-18 system results did not always align with FDA-BAM MPN values; biological variation likely contributed to this observation. For 523 samples evaluated using 2013 and 2014 MPN data (14 locations), 1 location (7%) exceeded the currently proposed FDA standard for statistical threshold value (410 MPN/100 ml generic *E. coli*). None of the locations exceeded the proposed geometric mean standard (126 MPN/100 ml). Fewer sources of water exceeded the supplemental proposed standards (1) compared to the originally proposed standards (16). *E. coli* O157:H7 and *Salmonella* were isolated from sites associated with a river (1), canal (1), waterbox (1) and ponds (3); however, these samples were not associated with locations that exceeded the currently proposed regulatory standards.

Significance: Evaluation of generic *E. coli* at multiple points within an open surface water delivery system can assist growers in evaluating risk associated with fecal contamination but may not align with risk of pathogen contamination.

P1-197 Improved Detection of *Staphylococcus aureus* with Medium Combined with Immunomagnetic Separation in Lettuce and Sprouts

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Introduction: *S. aureus* is a typical pathogenic bacterium and considered the most common cause of foodborne illness around the world. The immunomagnetic separation (IMS) method using immunomagnetic beads as capture reagents for the isolation of pathogens could effectively separate target bacteria from competitive microflora and the food samples.

Purpose: The purpose of this study was to develop a method using improved enrichment medium and immunomagnetic separation (IMS) with antibody-coated Dynabeads® protein G for detection of *S. aureus* in lettuce and sprouts.

Methods: The brain heart infusion (BHI) was used as basal medium and three supplement concentrations of manganese sulfate, sodium pyruvate and yeast extract was optimized with response surface methodology. The recovery of acid (pH 4.0), cold (4°C for 5 h) and heat (55°C15S) damaged *S. aureus* was also compared with the modified BHI (MBHI) and BHI. The artificially inoculated samples lettuce and sprout with low (0.54 log CFU/g) and high (1.35 log CFU/g) levels were enriched in MBHI and BHI followed with immunomagnetic separation.

Results: The best experimental parameter for the MBHI were manganese sulfate 0.023%, sodium pyruvate 0.762% and mannitol 1.986%. When incubated in MBHI for 6 h with the initial cell number 1.01 log CFU/ml, the mean of colony number could get to 5.43 ± 0.18 log CFU/ml. The recovery of acid, cold and heat-damaged *S. aureus* incubated in MBHI at 35°C for 4 h and 6 h was significantly higher than in BHIL ($P < 0.05$). After 4 h enrichment in MBHI with the low inoculum level, it was able to be detected by the immunomagnetic separation method.

Significance: This study suggests that with the low (0.54 log CFU/g) level spiked sample lettuce and sprouts could be detected using the immunomagnetic separation method after 4 h enrichment in the developed enrichment medium MBHI.

P1-198 Molecular Evaluation of Mold Growth and Aflatoxin Presence on Dry Aged Beef

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Introduction: Dry aging beef is commonly used to enhance quality. During dry aging there may be fungal growth. There is a lack of knowledge about the mold profile and possible toxins produced during dry aging. Strict control of environmental factors, including temperature and relative humidity, can minimize microbial growth.

Purpose: To evaluate the fungal characteristics of dry aged beef. Our objectives were to 1) identify molds present on beef trim samples, and 2) determine the change in concentration of aflatoxin during aging.

Methods: Beef trim samples (n = 8) were taken from subprimals prior to and after aging. The subprimals were dry aged for 21 days, with a relative humidity < 80%, at 1.7°C, approximately. Trim samples were swabbed using pre-hydrated spongesicles, plated on Potato Dextrose Agar, and incubated at 25°C for 72 - 144 h. Aflatoxin presence for each isolated colony was analyzed using a quantitative ELISA, and sequenced for identification by 18S rDNA sequencing. Sequences were assembled and proofread, and a BLAST search was performed in GenBank for identification.

Results: There was no mold or aflatoxin production for samples prior to aging ($P < 0.05$). Sequence data identified mold isolates on dry aged samples as *Cochliobolus sp.* (n = 2), *Cochliocolus sativus* (n = 1), and *Mucor racemosus* (n = 2). Mean aflatoxin concentration of aged samples was 0.1875 ppb ($P > 0.05$), which was not significantly different from non-aged samples.

Significance: Production of molds that may cause spoilage or be pathogenic can occur during dry aging. Aflatoxin production generated after dry aging was not significantly different from non-aged samples. The current aflatoxin action level of the United States federal government is 20 ppb, which is vastly higher than the mean values in these products. While molds were not isolated from each sample, the presence of mold and aflatoxin could still be a concern to public health.

P1-199 Heat Stability of Ochratoxin A in an Aqueous Buffered Model System

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❖ ***Developing Scientist Competitor***

Poster

Introduction: Ochratoxin A (OTA) represents one of the most widespread mycotoxins in agricultural commodities in the world and considered hazardous substances because OTA is a potent carcinogen. While OTA is rather stable under most food processing conditions, high temperatures may reduce OTA in foods. OTA not only exhibits a high thermal stability but also much more stable when present in water.

Purpose: Since OTA can be found in processed products destined for both human and animal consumption, factors affecting its stability or reduction during thermal processes are investigated.

Methods: Reduction of OTA was measured during variable heating times (up to 60 min) at different temperatures (100, 125, 150, 175, and 200°C) in de-ionized (DI) water and in aqueous buffer solutions at different pH values (pH 4, 7, and 10). Quantification of OTA was carried out with high performance liquid chromatography-fluorescence detection (HPLC-FLD).

Results: The results showed that the rate and extent of OTA reduction or decomposition were dependent on pH, processing time and temperature; greater than 90% of OTA reductions were achieved at 200°C for all the pH variations. After processing under alkaline conditions at 100°C for 60 min, about 50% of OTA was lost, while after 60 min under neutral and acidic conditions at 100°C did not show significant reduction of OTA. These results indicated that while thermal treatment in neutral and acidic conditions may not be enough to reduce OTA, thermal treatment under alkaline conditions yields a more significant reduction of OTA which is increased with increasing temperature.

Significance: This is the first systematic study of the thermal stability of OTA in the absence of a food matrix.

P1-200 Polyvinylpolypyrrolidone Reduces Cross-reactions between Antibodies and Phenolic Compounds in Enzyme-linked Immunosorbent Assay for the Detection of Ochratoxin A

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❖ ***Developing Scientist Competitor***

Introduction: Ochratoxin A (OTA) occurs in a variety of foods worldwide, and has been shown to be nephrotoxic and carcinogenic in animals. Enzyme-linked immunosorbent assay (ELISA) has emerged as a rapid test with its high sensitivity and low cost. However, presence of phenolic compounds, which have structural similarities to OTA, in foods may result in false positive or erroneous quantitative measurements due to the interference or cross-reaction between OTA and antibody in ELISA.

Purpose: Polyvinylpolypyrrolidone (PVPP), a fining reagent in wine, can reduce anthocyanin and other phenolic compounds. The objective of this project was to test the efficacy of PVPP in reducing cross-reaction between OTA and phenolic compounds in quantification of OTA.

Methods: The PVPP powder was mixed with 5 mg/ml of gallic acid and 100 ng/ml of OTA in 50% methanol; 1 g of pistachio skins and different PVPP amount were mixed with 4 ml of 50% methanol. The mixed sample was agitated with a wrist action shaker at high speed for 15 min, followed by centrifugation. The concentration of OTA and gallic acid were assayed using high performance liquid chromatography (HPLC), and cross-reactivity was assayed using commercial ELISA kits.

Results: HPLC analysis results of OTA and gallic acid concentrations demonstrated that 100 mg/ml PVPP could remove 95% of 5 mg/mL gallic acid while keeping OTA levels constant. Ten mg/ml, 25 mg/ml, and 50 mg/ml of PVPP reduced the cross-reactivity of pistachio skins (4.6 mg of total phenolic compounds/ml of extract) by 14%, 37%, and 80%, respectively. One hundred fifty mg/ml of PVPP was shown to completely remove the cross-reactivity of pistachio skins.

Significance: The results demonstrated the usefulness of PVPP addition extract method for decreasing cross-reactivity in OTA ELISA kits by binding with phenolic compounds.

P1-201 Development and Characterization Novel Avenin-specific Monoclonal Antibodies

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Introduction: Cereal grains contain a composite protein called gluten which consists of prolamins and glutenins. The prolamin fraction from several grains exhibits immunopathogenic potential, and consumption of these grains is associated with symptoms of celiac disease. While the majority of celiac subjects react to wheat, barley, and rye, a subset additionally respond to oats (avenins). For this reason, several countries and gluten organizations consider avenins in their gluten classification.

Purpose: In an effort to obtain novel serological reagents with specificity for avenins, we generated monoclonal antibodies in mice. Herein, we describe the immuno-reactive profile of candidate clones.

Methods: Avenins were isolated from R5 (-) oats using a modified Osbourne fractionation technique and subsequently used to immunize female Balb/C mice. Spleens from seroconverted mice were fused with SP2/0-Ag14 cells and cultured using HAT techniques. Colonies were screened and initially selected based on reactivity towards gliadin, hordein, secalin, and avenin using indirect ELISA. IgG+ clones that retained high activity against avenins alone were raised, subcloned, and IgG purified from ascites was further studied by ELISA and Western blot analysis.

Results: Subclones of 3G5, 3G7, 5B6, and 11E7 demonstrated very high activity against avenin with no cross-reactivity towards gliadin, hordein, and secalin, zein, orzenin, or soy as determined by indirect ELISA. Western blot analysis using these reagents established protein binding profile and was used to inform on the development of a sandwich ELISA for avenin that was capable of detecting avenin in food at levels lower than 10 ppm.

Significance: These 4 mAb reagents demonstrated potential for use in future avenin detection systems, including use in sandwich ELISA. The development of avenin detection systems should provide the celiac community with improved diagnostic tools to better assist in disease management.

P1-202 Development and Characterization of Novel R5-like Monoclonal Antibodies Directed against Gluten

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Introduction: Cereal grains contain a composite protein called gluten which consists of prolamins and glutenins. The prolamin fraction from wheat and related triticeae cereals exhibits immunopathogenic potential, and consumption of these grains is associated with symptoms of celiac disease. The current gold standard for detection of gluten residues used in the food industry relies on the use of immunodiagnostic tests based on the R5 monoclonal.

Purpose: In an effort to obtain novel serological reagents with improved specificity profiles relative to the R5 system, we generated monoclonal antibodies in mice. Herein, we describe the immuno-reactive profile of the candidate clones and compare their binding activity against that of the R5 mAb.

Methods: A combination of deamidated gliadin and synthetic R5-peptide (LQPQQPFPQQLQPQQPFPQQA) was used to immunize female Balb/C mice. Spleens from seroconverted mice were fused with SP2/0-Ag14 cells and cultured using HAT techniques. Colonies were screened and initially selected based on reactivity towards gliadin, hordein, secalin, and avenin using indirect ELISA. IgG+ clones that retained high activity were raised and IgG purified from ascites was further studied by ELISA and western blot analysis and used to develop a sandwich ELISA.

Results: Using a combined vaccine approach, two hybridoma clones were generated, 2B9 and 1A11, which demonstrated very high, and near equal activity against gliadin, hordein, and secalin, and no cross-reactivity against avenin, zein, orzenin, or soy as determined by indirect ELISA. Western blot analysis of these two clones demonstrated a pattern of reactivity that mirrored that of the R5 mAb. Both clones were conjugated to HRP and used to develop sandwich ELISAs.

Significance: Of the clones tested, 1A11 and 2B9 demonstrated the most potential for use in future gluten detection systems, including use in sandwich ELISA for detection of wheat, barley, and rye-derived gluten residues.

P1-203 Investigation of Heavy Metals and Oxidative Stress in *Sarotherodon melanotheron* Harvested at Abule-Ado Lagoon, Lagos, Nigeria

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Introduction: Contamination of aquatic life by heavy metals is common in petroleum polluted environment. Abule-Ado Lagoon (AAL) in Lagos State, Nigeria, houses a number of private petroleum depot and there is large scale oil spillage in the environment. Bioaccumulation of heavy metals in tissues of fish harvested in polluted areas may pose health risk to consumers

Purpose: Therefore this study was designed to assess the bioaccumulation of heavy metals and oxidative stress in *Sarotherodon melanotheron* harvested at AAL and compared with the control, harvested at Iyana-Iba Lagoon, Lagos.

Methods: Ten female fishes each were harvested from test and control sites and the concentrations of Iron (Fe), Lead (Pb), Cadmium (Cd), Chromium(Cr) and Nickel (Ni) were determined in the gills, liver and flesh using atomic absorption spectroscopy. The toxicity and oxidative stress in the fishes were investigated by monitoring aspartate amino transferase (AST), alanine amino transferase (ALT), malondialdehyde (MDA), reduced glutathione (GSH) and super oxide dismutase (SOD) activities in the liver and gills.

Results: Fishes harvested from AAL showed marked ($P < 0.05$) increase in the tissue concentration of the metals as compared with control. The decreasing trend of metals in the tissues of harvested fish was Fe> Pb>Cr >Ni>Cd. Fishes collected at AAL showed a significant ($P < 0.05$) increase in GSH, MDA, SOD and AST activities in the gills and liver when compared with those taken at the control site.

Significance: Our results suggest that Abule-Ado Lagoon is polluted with heavy metal and fishes inhabiting the lagoon may be vulnerable to stress and diseases, which may make them unsafe and unhealthy for human consumption.

P1-204 Risk-benefit Analysis of First Nations' Fish Smoking Practices

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Introduction: Smoke processing of fish is used for food preservation by First Nations communities. Unlike in commercial smoke processing where the control for temperature is well executed to allow consistent smoke exposure for RTE smoked fish products, little control is practiced by First Nations' methods.

Purpose: The purpose of our study was to determine the polyaromatic hydrocarbon (PAHs) residue content in smoked fish and relate this to the antimicrobial properties of partially and fully smoked fish, respectively.

Methods: Commercial smoked salmon were purchased from local grocers (cold-smoked; < 43°C). Half-smoked (2 - 3 days) and fully-smoked (5 - 6 days) salmon were obtained from Tl'azt'en and Lieidli T'enneh First Nations communities and previously smoked at temperatures greater than 85°C. PAH measurements were performed by GC-MS, using authentic standards with detection limits within 1ng/g. Fish fatty acids were measured by GC-FID. The lipid peroxides were measured using the ferrous oxidation-xylenol orange (FOX) assay. Antimicrobial property of PAHs against *Listeria innocua*

was tested in brain-heart infusion broth. A disk diffusion assay was also used to test the antimicrobial activity of hexane extracts recovered from smoked commercial and First Nations' fish samples.

Results: PAHs were not detected in commercial cold smoked fish; in contrast to First Nations' fully smoked salmon, which had significantly ($P < 0.05$) higher levels of both low and high molecular weight PAHs. PAHs recovered from half-smoked fish were low molecular weight. Salmon processed using First Nations' methods exhibited significantly reduced lipid peroxides and enhanced retention of EPA and DHA ($P < 0.05$). The generation of low molecular weight PAH's generated from partial and full smoking of fish significantly increased lag-phase and lowered maximum growth ($P < 0.05$) of *Listeria innocua*.

Significance: These data suggest that risks associated with PAH contamination of salmon, derived from First Nations' smoke preservation methods, also provide benefits of retained nutritional value and antimicrobial activity.

P1-205 Concentrations of Perfluoroalkyl Substances in Foods and Ingestion Exposure among Adults in Taiwan

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Introduction: Perfluoroalkyl substances (PFASs) are persistent, bioaccumulative, and ubiquitous in the environment and may contaminate foods; ingestion is a major route of human exposure.

Purpose: To evaluate human exposure to PFASs, this study quantified six perfluorocarboxylic acids (PFCAs) and two perfluorosulfonic acids (PFSAs) in fourteen majorly consumed foods (total 140 samples) in Taiwan using UPLC-MS/MS with isotope-dilution techniques.

Methods: One-gram homogenized wet samples were digested with 10 ml of 0.5 N potassium hydroxide (KOH) in methanol, and 5-ml supernatant of the samples after centrifugation were diluted with 500-ml Milli-Q water, adjusted to pH 3.5, and were extracted with Atlantic HLB disk by automated solid-phase extraction. Analytes were eluted with 20-ml methanol containing 0.1% ammonium hydroxide (v/v); the eluent were concentrated to 1 ml by a SpeedVac and were analyzed by ultra-high performance liquid chromatography/tandem mass spectrometry at negative electrospray ionization.

Results: The long-chained PFCAs with 10 - 12 carbons were detected in all of the samples with the geometric means ranged from 0.04 to 12.3 ng/g, which were higher than previous reports. Perfluorooctane sulfonate (PFOS) was not detected as frequently as demonstrated in other studies and the measured concentrations ranged from 0.11 ng/g (clam) to 9.91 ng/g (pork liver) in average. Rice and pork liver were rarely studied but some considerable concentrations, such as up to 283 ng/g of perfluorooctanoic acid (PFOA) in liver, were observed in this study.

Significance: Although the daily intake of PFOA (85.1 ng/kg b.w./day) and PFOS (0.46 ng/kg b.w./day) did not exceed the tolerable daily intake suggested by the European Union, Germany, and the U.K., people in Taiwan exposed to more perfluorohexanoic acid, PFOA, perfluorodecanoic acid, and perfluoroundecanoic acid (11.2, 85.1, 44.2, and 4.45 ng/kg b.w./day, respectively) than in western countries, demonstrating that the distribution of PFASs and the dietary habits are crucial to the exposure.

P1-206 Determination of the Toxigenic Profile of *Staphylococcus aureus* Strains Isolated from Clinical and Artisanal Cheese Samples Produced in Costa Rica

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Introduction: *S. aureus* is a pathogen with a wide capacity of producing disease in humans and some animals. This capacity is linked to the expression of toxins, its invasive capacity and the resistance to antibiotics. Toxins produced by these bacteria include enterotoxins (*SE*s), toxic shock syndrome (*TSST-1*) and eritrogenic toxins (*eta* and *etb*). Not all strains possess the genes that codify for these toxins but some might have two or more same time.

Purpose: The aim of this work was to determine which toxigenic genes are present in strains isolated from food and clinical samples.

Methods: Two hundred twenty-nine *S. aureus* strains coming from fresh cheese samples, and 210 clinical isolates from the same region were analyzed. *Sea, seb, sec, sed, see, eta, etb* and *tsst* genes were detected using PCR methodology described by Mehrotera *et* al. Extraction was done according to the methodology described by Brizzio *et a*l., with an additional incubation with ampicillin in order to help with wall rupture.

Results: In fresh cheese samples, enterotoxin B gene was the most frequent (19 positive samples), followed by D gene (6 positive samples). Enterotoxin A gene was not detected in these samples. For clinical isolates, B enterotoxin was also the most frequent one (42 positive samples) followed by *tsst* (38 positive samples). Eight samples were positive for enterotoxin A gene.

Significance: Both isolates from cheese and clinical samples present toxigenic genes, being these more frequent in isolates from hospital origin.

P1-207 A Multistate Outbreak of Niacin Toxicity in School Age Children Associated with an Infused Rice Product

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Introduction: Beginning in December 2013, the Food and Drug Administration (FDA), the Centers for Disease Control and Prevention (CDC), and state and local partners investigated an outbreak of gastrointestinal illness and rash in school age children caused by niacin toxicity linked to consumption of an infused rice product.

Purpose: The purpose of the investigation was to identify the vehicle responsible for the outbreak and take appropriate actions to protect public health.

Methods: The majority of cases reported headache, nausea, and a rash on ears, neck, arms, face or trunk. FDA reviewed records and production information from the manufacturing firm. Samples of leftover and uncooked infused rice product collected from school locations, a distributor, and manufacturer were tested for niacin (nicotinic acid) and iron levels.

Results: A total of 81 cases from 3 states were identified; 91% of cases were school age children. Both male and female students were affected equally; most spontaneously recovered within 90 to 120 minutes. Samples of leftover cooked rice and product from the same lot as rice consumed at one of the schools contained 1.4 – 4.4 mg/g nicotinic acid, approximately 1000 times higher than expected, and 2.8 – 2.9 mg/g iron, 100 times higher than expected. Samples of product obtained from other schools did not have elevated levels of niacin or iron, indicating a potential issue with the application of a vitamin mixture during processing of the infused rice product. Based on laboratory findings, niacin toxicity from consumption of the infused rice product was determined to be the cause of illnesses in case patients.

Significance: Laboratory findings, combined with epidemiologic information and findings from the manufacturing facility led the firm to voluntarily recall the infused rice product produced in 2013. Subsequently, the firm investigated and revised their vitamin mixture application process to prevent future issues.

P1-208 Synthesis and Characterization of Crosslinked Chitosan Beads for Removal of Nitrate Nitrogen from Drinking Water

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Introduction: Filtration has been an accepted technology to remove nitrate nitrogen from drinking water which is usually originated from fertilizers or composts. Interactions between positively charged chitosan and negatively charged nitrate nitrogen lead to the ionic removal of nitrate nitrogen. However, chitosan beads are needed to improve physical strength because they show low aqueous stability such as crosslinking of chitosan molecules formed as bead may be technically feasible to remove nitrate nitrogen from drinking water.

Purpose: The purpose of this research was to develop chitosan beads to remove nitrate nitrogen from drinking water by using chitosan. In detail, nitrate nitrogen was removed by ionic coupling with the chitosan beads. Sodium-tripolyphosphate (STPP) crosslinking was used to improve the physical strength and swelling capacity of the chitosan beads.

Methods: For crosslinked chitosan bead (CLCB) preparation, chitosan solution was prepared by dissolving 3.0 g of chitosan flakes in 60 ml of 5% acetic acid solution. Under stirring 0.5 M NaOH solution, the chitosan solution was added dropwise in the NaOH solution. Then, STPP was added to trigger crosslinking reaction at 30°C for 12 h. The adsorption kinetics of CLCB was investigated with respect to nitrate nitrogen concentration, temperature, and pH.

Results: The CLCB fabricated possessed porous surface (porosity 84.5%). The nitrate nitrogen adsorption capacity was 1.8 - 2.4 g nitrate nitrogen adsorption /kg CLCB at pH 5.5, 25°C and 100 rpm for 15 min. A diffusion model was used to interpret the kinetic data of nitrate nitrogen adsorption test and subsequently determined the removal rate constants of CLCB. The adsorption capacity increased largely with decreasing solution pH or with increasing collecting time.

Significance: The CLCB can be used as a positively charged filtering material for the drinking water filtration.

P1-209 Application of Low Wavelength UV-C Irradiation for Treating Mycotoxins in Turbid Fluids

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Introduction: Patulin, a mycotoxin produced by several genera of fungi, including *Aspergillus* and *Penicillium*, has been an important concern in apple-based products due to its toxicity. Ultraviolet (UV) irradiation is a sterilization method effective against a range of bacteria and viruses while being non-thermal and non-adulterating in its mechanism of action. This makes UV irradiation a unique and attractive method to control mycotoxins in fluids.

Purpose: The objective of this study was to evaluate the effect of UV irradiation in combination with a photosensitizer (ATZ1) on patulin stability.

Methods: Experiments were conducted using a collimated beam system operating at 254 nm wavelength. Appropriate fluence correction factors were accounted when quantifying the UV dose. Chemical profiling was conducted using HPLC. Kinetic modeling supplemented with two-way ANOVA was performed at 5 percent significance level to assess the effectiveness of UV irradiation along with the application of photosensitizer on patulin stability.

Results: Patulin reduction by UV irradiation followed the first-order kinetic model. It clearly indicated that patulin sensitivity to UV irradiation is relatively high. If UV irradiation is used alone, doses close to 4000 $mJcm^{-2}$ could only reduce patulin concentration by 45% ($P < 0.05$). Under this scenario, the doses delivered to patulin molecule were very high, higher than the dose required for the microbial inactivation. Using photosensitizer, ATZ1 in combination with UV irradiation caused a significant reduction in patulin concentration, 75% reduction in patulin concentration at 400 $mJcm^{-2}$ ($P < 0.05$).

Significance: The findings in this study only serve as a proof of concept of using UV technology for patulin degradation in turbid fluids. Application of UV irradiation along with photosensitizer (ATZ1) process can aid in reducing patulin concentration for highly turbid liquid foods.

P1-210 Evaluation and Comparison of the Sealing Performance of Three Major Types of Jar Lids Available for Home Canning

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Introduction: Significant increased activity in home canning has resulted in renewed interest in the research in this area. No published scientific evidence on the sealing performance for different jar and lid systems is available, yet research-based processing advice should include container recommendations.

Purpose: The objective of this study was to evaluate and compare vacuum levels, sealing rates and retention of food quality with three home canning lid systems (metal, plastic and glass) at 24 h and through 3 months storage.

Methods: Three foods (tomatoes, apples and carrots) were canned in three lid systems. Foods chosen represent two processing temperature requirements (100 and 115.6°C) and different stressors to lid performance. Lids were subjected to four application treatments (recommended, unwiped sealing surface, overfilled jars and a combination of unwiped and overfilled). Each lid, food and treatment combination was replicated 12 times resulting in 576 jars processed. A total of 192 of each lid type was used; 160 jars of each lid type were analyzed for this study at 24 h, 10 d, 1 mo or 3 mo.

Results: Through 3 mo storage, all three lid types had acceptable sealing rates and initial vacuum levels (13 - 18 in Hg for boiling water and 18 - 24 in Hg for pressure processed foods) in jars not overfilled. All jars sealed at 24 h. A total of 6 seal failures occurred during the storage period of this study; four were plastic lids and two were glass lids.

Significance: Food quality and successful thermal processing are influenced by the quality of the vacuum seal, as is protection of the economic investment in home food processing. Recommendations for home canning should be based on sound thermal processing advice well as scientific evidence for reliable containers and seals. Excessive air retention in jars can lead to sealing issues and possible microbial spoilage.

P1-211 Structural Transformations of Oil-in-Water Emulsions in the Presence of *Staphylococcus carnosus*

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Introduction: *Staphylococcus carnosus* is widely used as a starter culture for fermented meat products. Some meat products are emulsion-based systems in which hydrolytic degradation of proteins alter texture by inducing protein gel formation. As such, the microorganisms may play an important role as structure modifiers.

Purpose: To date though, limited research has been conducted with respect to the structure-altering capabilities of *S. carnosus* in non-meat model systems. The purpose of this study was therefore to evaluate if the presence of this microorganism may affect the microstructure of a model O/W emulsion.

Methods: A sterile base emulsion was carefully prepared by mixing 15% (v/v) Miglyol, with filter sterilized 5% (w/v) whey protein isolate (WPI), and 80% phosphate buffer (0.01M pH 7.0). *S. carnosus* LTH 4410 was added to the emulsion at a concentration of 10^5 CFU/ml. Bacterial growth was monitored for 48 h at 30°C by plate enumeration. To assess proteolytic activity, a modified Lowry assay was used to determine the concentration of tyrosine in the emulsion. Structural changes in the emulsion related to proteolytic activity were measured by rheology and optical microscopy.

Results: The initial concentration of tyrosine was 38.99 µg/ml. After 48 h the tyrosine concentration in the inoculated emulsions increased to 43.89 µg/ml. The rheological behavior of the inoculated and non-inoculated emulsion did not differ from each other ($P < 0.05$). On a structural level, microscopic observations did not reveal any flocculation or aggregation. The results suggest that there was insufficient proteolytic activity, possibly due to the concentration of bacteria being too low or the metabolic activity of bacteria being limited.

Significance: The study of structural transformations caused by starter cultures such as *S. carnosus* should in general be of interest to food manufacturers interested in using starter cultures to transform a food template structure to a texturally different product.

P1-212 Alpha-amylase and Chymotrypsin Inhibitor Activities, Hemagglutinating Activity, and Total Phenols in Select Dry Beans

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Poster

Introduction: Dry beans are a good source of proteins, carbohydrates, vitamins, minerals, and secondary metabolites. However, dry beans contain certain antinutrients that may interfere with nutrient utilization or cause deleterious effects.

Purpose: The objective of this study was to evaluate the α-amylase inhibitor activity (AIA), chymotrypsin inhibitor activity (CIA), hemagglutinating activity (HA), and total phenols (TP) in select dry bean seeds.

Methods: Twenty-two dry bean seeds were purchased from local grocery stores and ground to pass through 40-mesh sieve. Dry bean flours were extracted with water (1:10 w/v, 4°C, 12 h), 0.05 M HCl (1:15 w/v, 4°C, 12 h), 0.01 M phosphate buffered saline (1:5 w/v, 25°C, 1 h), and 70% ethanol (1:10 w/v, 25°C, 1 h) for AIA, CIA, HA, and TP, respectively. N-benzoyl-L-tyrosine ethyl ester (BTEE) and 1% potato starch were used as substrates for CIA and AIA, respectively. HA was determined using 2% (v/v) human blood erythrocytes (group O, A, B, and AB). TP were analyzed using Folin-Ciocalteu reagent.

Results: AIA (0.0-1.3 inhibitory unit/mg), CIA (19.9-484.9 inhibitory unit/g), HA (0-10254 hemagglutinating unit), and TP (0.4-2.9 mg gallic acid equivalent/g) exhibited significant ($P \leq 0.05$) differences between the samples. AIA was detected only in *Phaseolus vulgaris* beans. Val bean registered the highest CIA followed by the *Phaseolus spp*. *Phaseolus vulgaris*, tepary, and val beans contained abundant hemagglutinating activity while this activity was not detected in chickpea, cowpea, garbanzo, and moth beans. No blood group specificity was observed for hemagglutinating activity in the tested beans except for baby lima bean (blood group A specific). Soybean had the highest amount of TP.

Significance: A wide range of activity was detected for the tested analytes in the selected dry bean seeds. This information may be useful for optimizing processing conditions to remove the selected analyte.

P1-213 Thermal Inactivation of *Bacillus anthracis* Spores in Milk Using a Pilot-scale High-Temperature-Short-Time (HTST) Pasteurization System

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Introduction: Milk poses a high risk for contamination by *Bacillus anthracis*, a spore-former that causes anthrax. There are a number of studies, under well-controlled laboratory conditions, that characterizes the thermal inactivation of anthrax but there is no information if industrial milk processing could inactivate anthrax.

Purpose: This study evaluated whether previously laboratory derived thermal inactivation data and conditions could be scaled-up and if real world processing parameters could inactivate *B. anthracis* spores.

Methods: *Bacillus anthracis* spores (6 log CFU/ml) were inoculated into large quantities of reconstituted skim milk and processed by a MicroThermics Bantam 1S at 105, 110 and 115°C. Temperature measurements were recorded and treated samples were collected at sample exit port. Milk sample was allowed to flow out of the exit port for 1 min prior to sample collection and approximately 1liter of sample was collected for microbiological analysis.

Results: The targeted processing temperatures at the end of the hold tube within the HTST will require the HTST to ramp up heating above the targeted temperature by approximately 7°C at the start of the hold tube. This heating profile resulted in anthrax spores being exposed to different temperatures and not uniformly distributed inactivation rates throughout the hold tube. At 105°C, a 15 s hold time was required to achieve a 6-log inactivation and temperatures within the hold tube ranged from 112°C at the start to 105°C at the end of the hold tube. At 110 and 115°C, a hold time of 5 and 2 s, respectively, were required to achieve a 6-log inactivation of anthrax spores. In addition to the processing temperatures and hold times, the Re values of > 8,000 and turbulent flow type were other important parameters.

Significance: This study identified processing conditions that resulted in the inactivation of anthrax spores in milk during thermal processing using pilot-scale processing equipment.

P1-214 Thermal Stability of Ricin in Milk Using a Pilot-scale High-Temperature-Short-Time (HTST) Pasteurization System

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Introduction: Ricin, a potent cytotoxin, has potential for being used as a biological weapon since the toxin is relatively easy to isolate and purify. Lab-scale studies have shown that ricin is fairly heat stable in a variety of foods. However, little is known about the stability of ricin in foods that are thermally processed using pilot- or full-scale processing equipment.

Purpose: This study evaluated the effects of processing conditions on loss of ELISA detection of ricin in nonfat milk using a pilot-scale high-temperature-short-time (HTST) pasteurization unit. A secondary goal was to compare the thermal stabilities of purified ricin to those of crude ricin.

Methods: Purified or crude ricin extracts were added to reconstituted nonfat dry milk. The toxin-spiked milk (10 µg ricin/ml) was processed in a pilot-scale HTST at temperatures of 80 – 95°C. At each temperature, the milk was processed for 7.5 to 50 s by adjusting the flow rate of the HTST. A commercially available ELISA kit was used to estimate the amount of ricin remaining after thermal processing studies. Each processing study, which corresponded to processing ricin-spiked milk at each temperature/holding time combination, was done in duplicate, while ELISA analyses of each sample were done in triplicate. Half-life values for purified and crude ricin were calculated at each processing temperature.

Results: The apparent half-life values for purified ricin in milk processed at 80, 85, 90 and 95°C, as calculated for entire processing treatment, were 19.9 ± 2.35, 12.4 ± 1.56, 5.02 ± 1.25 and 2.00 ± 0.65 s, respectively, while the half-life values for crude ricin at 85 and 95°C were 8.36 ± 0.87 and 3.97 ± 0.58 s, respectively. The half-life values for purified and crude ricin at these processing temperatures were significantly ($P < 0.05$) different.

Significance: This study identified conditions that result in inactivation of ricin during thermal processing using pilot-scale processing equipment.

P1-215 Detection Method Evaluation for Abrin Toxin in Food Matrices

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Introduction: Abrin is an extremely potent bio-toxin produced from the seed of the tropical plant *Abrus precatorius,* commonly found in the southern U.S. and the Caribbean. Abrin toxin inhibits the synthesis of proteins in the cells of an exposed individual, causing severe illness and cell death. Due to the lethality and ease of accessibility, a screening method for the detection of abrin toxin in foods is necessary for biodefense purposes.

Purpose: This study's objective was to provide a method combining an optimized extraction and commercially available ELISA kit for the detection of abrin toxin in foods.

Methods: To determine the LOD, six brands each of hot dogs, liquid infant formula, and liquid eggs were fortified at five levels of abrin in quadruplicate and cold stressed overnight. Samples were homogenized with GBS and toxins were extracted via centrifugation. Following extraction, the aqueous layers were analyzed in accordance with the ELISA manufacturer's instructions. The full scale validation incorporated replicate sets in three fortification levels analyzed in duplicate.

Results: The limit of detection varied with each matrix tested. Liquid infant formula exhibited a 10 ng/g LOD, liquid eggs at 30 ng/g, and hot dogs at 50 ng/g. The sensitivity for the matrices evaluated ranged from 75% (18/24) to 91.7% (22/24). All matrices examined demonstrated 100% specificity (12/12).

Significance: The data from this study suggests that the optimized extraction procedure, combined with the ELISA detection assay, may offer a suitable method for the detection of abrin toxin in foods.

P1-216 Succession of the Raw Salmon Microbiome during Refrigerated Storage

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Introduction: Traditional culture-based shelf life experiments often underestimate richness and complexity of food microbiomes. Raw salmon is commonly examined for food safety pathogens with disregard to the diverse microbiota potentially contributing to its spoilage.

Purpose: The goals of this study were to analyze the microbial succession of raw salmon in a refrigerated shelf life model, identify potential spoilage microorganisms, and compare next generation sequencing with traditional microbiological methods.

Methods: Raw salmon fillets were stored aerobically at 4°C for a total of 14 days with duplicate sampling on days 0, 2, 4, 7 and 14. Aerobic plate count and lactic acid bacteria count were determined and representative isolates were genotyped by RS-RAPD. Microbial diversity was characterized by deep sequencing of 16S rRNA gene amplicons using the Illumina MiSeq platform and a QIIME 1.8 analysis pipeline.

Results: Aerobic plate counts were 6.03×10^5 CFU/g at day 0, increased rapidly to 3.24×10^8 CFU/g by day 4 before plateauing around 1.0×10^9 CFU/g. Lactic acid bacteria counts were 6.92×10^3 CFU/g at day 0, increased to 1.05×10^7 CFU/g by day 4, and slowly increased to 3.29×10^8 CFU/g by day 14. Genotypes from representative APC and LAB isolates clustered into 5 and 3 predominant clades, respectively. Based on number of unique sequences and Shannon diversity indices, samples at day 0 and 2 were least diverse with community complexity increasing over time. Deep sequencing indicated that proportions of taxa belonging to *Listeraceae* started to increase at day 4 with *Carnobacteriaceae* and *Flavobacteriaceae* becoming more predominant at day 7 and *Enterococcaceae* increasing at day 10. *Vibrionaceae*, *Pseudomonadaceae* and *Moraxellaceae* decreased in proportion of the total bacterial population over time, while increasing in abundance.

Significance: Next generation sequencing data corroborated traditional microbiological findings implicating lactic acid bacteria, specifically *Carnobacteriaceae* and *Enterococcaceae* as major spoilage microorganisms of raw salmon.

P1-217 Succession of the Hot Smoked Salmon Microbiome during Refrigerated Storage

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Introduction: Microbiological investigations of smoked salmon have traditionally focused on *Listeria monocytogenes*; however, few have examined total microbial spoilage communities over time. This is particularly true of hot smoked salmon, as curing reduces survival of heat-labile microorganisms.

Purpose: The objective of this study was to examine the microbiome of hot smoked salmon over a 5-week refrigerated shelf life using traditional microbiological and next-generation sequencing methodologies.

Methods: Hot smoked salmon fillets were stored aerobically at 4°C for 35 days with duplicate sampling on days 0, 4, 7, 10, 14, 17, 21, 27, and 35. Traditional culture-based methods were used to determine aerobic plate count and lactic acid bacteria count, and representative isolates were chosen for genotyping by RS-RAPD. Microbial diversity was characterized by deep sequencing of 16S rRNA gene amplicons using the Illumina MiSeq platform. Sequencing data were analyzed using QIIME 1.8.

Results: Microbiome analysis depicted a complex initial diversity that decreased from day 7 through 17 but rebounded at day 21. *Photobacterium*, *Pseudomonas* and *Acinetobacter* were initially predominant, followed by a transition towards a population dominated by *Moraxcellaceae*. Spoilage populations beyond 3 weeks include *Staphylococcus* and *Micrococcaceae*. *Brochothrix* were observed as major constituents throughout, except during an intermediate spoilage period. Total mesophilic APC reached 2.8 x 10^7 CFU/g after 5 weeks, from which isolates clustered into 8 major clades by genotyping. Total LABs steadily increased to 2.0 x 10^6 CFU/g and yeasts to 3.2 x 10^5 CFU/g over the same period. Enterococcal populations remained near detection limits until day 21, after which sporadic growth to 1.0 x 10^5 CFU/g was observed. Genotyping of 198 isolates revealed temporal relationships complementary to deep sequencing data.

Significance: Traditional microbiological culture and next-generation sequencing yielded complementary data that deepen understanding of microbial population dynamics during refrigerated storage of smoked salmon, and may enable targeted and more effective methods to increase shelf life.

P1-218 Study on Biochemical Indices of Albino Rats Supplemented with Oil Extracted from Groundnut, Roselle Seed and Their Validated Blend

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Introduction: Groundnut oil (GO) had been known as good source of oil if taken in desirable quantity, but roselle seed oil (RO) and its blends with GO has no documented literature on its toxicity and safe levels of consumption on any animal models. Roselle as adjunct in groundnut milling would aid oil extraction.

Purpose: To determine the effects of GO, RO and the validated oil extracted from blend of groundnut with roselle seed oil (GRO) on some biochemical and histology of albino rats to establish their toxicities.

Methods: Seven groups of seven albino rats weighing between 100 to 115 g were used the experiment. Group I was control and were fed 100% pelletized diet. Group II - IV and V - VII were fed with 7.5% and 15% substituted GO, RO and GRO, respectively, for 28 days along with water *ad libitum*. Serum total protein, albumin, aspartate aminotranferases (AST), alanine aminotransferases (ALT), and alkaline phosphatase (ALP) activities, liver, kidney, heart and body weight and feed intake were determined.

Results: The feed intake of all the groups were significantly different at $P < 0.05$ except for groups fed 7.5% substituted RO and 15% substituted GRO. Body weight change and growth rate were not different except groups fed 15% substituted RO and 15% substituted GRO were significantly lower and higher to that of control, respectively. Organs weight, total protein, albumin and AST were not significantly different from the control. ALP and ALT were significantly lower and higher from the control, respectively. Histopathological examination showed inflammation and fatty deposit in the liver of the groups feed with 15% substituted level of oil.

Significance: These results indicate that the oils are safe for consumption at lower levels of substitution but higher levels substitution (15%) shows compromise in the organs.

P1-219 Characterization and Control of *Mucor circinelloides* Spoilage in Yogurt

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Introduction: Consumer confidence in the food industry is severely affected by large-scale spoilage incidents. However, relatively little research exists on zygomycetes (e.g., *Mucor*), dimorphic spoilage molds which switch between a yeast and hyphal phase depending on environmental oxygen levels. The presence alone of *Mucor circinelloides* in yogurt will not cause spoilage, but outgrowth and subsequent changes in quality can cause spoilage if not controlled.

Purpose: The purpose of this study was to evaluate the effects of pasteurization regime, natamycin concentrations, and storage temperature in yogurt production on *M. circinelloides,* as measured by mold counts and gas production.

Methods: *M. circinelloides* was isolated from commercially spoiled yogurt. *D*-values and *z*-value were determined for hyphae/asexual spores in milk as an evaluation of the mold's ability to survive pasteurization. Natamycin was added to yogurt at 0, 5, 10, 15, and 20 ppm to inhibit *M. circinelloides* over the course of month-long studies at 4°C, 15°C, and 25°C. Survivors were recovered on acidified PDA and container bloating due to gas formation by *M. circinelloides* was recorded.

Results: The *D*-values at 54°C, 56°C, and 58°C were (in min) 38.31 ± 0.02, 10.17 ± 0.28, and 1.94 ± 0.53, respectively, which yielded a *z*-value of 3.09°C. This indicates that hyphae and asexual spores would not survive fluid milk pasteurization if contamination occurred prior to thermal treatment. Gas production was only observed when the mold was incubated under anaerobic conditions, and occurred faster at temperatures above 4°C. Addition of 10 ppm natamycin inhibited the growth and gas production of *M. circinelloides* when compared to the untreated control.

Significance: These data suggest that yogurt spoilage (container bloating) caused by anaerobic growth of *M. circinelloides* is due to post-pasteurization contamination. The addition of at least 10 ppm of natamycin was found to be effective at preventing *M. cicinelloides* growth and subsequent spoilage.

P1-220 *Alicyclobacillus* Contamination of Pasteurized Fruit Juices in West Africa

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❖ ***Developing Scientist Competitor***

Introduction: Consumer awareness and preference for safe food is improving in West Africa. There is also corresponding increase in the production and consumption of fruit juice in the sub-region. Endospore forming, thermo-acidophilic bacteria such as *Alicyclobacillus* species are spoilage agents that survive heat processing during fruit beverages production.

Purpose: In this study, *Alicyclobacillus* contamination of commercial fruit juices in W. Africa was investigated aimed at process improvement and safety quality of fruit juice.

Methods: Sixty-two juice samples from Ghana (n = 39) and Nigeria (n = 23) were heat shocked and enriched in acidified yeast starch glucose (YSG) broth followed by aerobic incubation at 45°C for 5 days. *Alicyclobacillus* was detected in eleven fruit juice samples (17. 5%); colonies obtained were screened with genus-specific polymerase chain reaction (PCR) and randomly amplified polymorphic DNA (RAPD- PCR) as well as sequencing of the 16S rRNA gene of representative strains of the RAPD clusters.

Results: The 16S rRNA gene sequencing identified the representative isolates as closest relatives of *Alicyclobacillus acidoterrestris, Alicyclobacillus acidocaldarius* and *Alicyclobacillus* genomic species 1. Strains source analysis indicated that *A. aciodcaldarius* was detected in eight juice samples while *Alicyclobacillus* genomic sp. 1 and *A. acidoterrestris* were detected in two and one samples, respectively.

Significance: This detection of *A. acidoterrestris* in heat processed fruit juices in W. Africa suggests economic risks for fruit juice manufacturers and retailers.

P1-221 Yeast Prevalence and Diversity in Frozen Concentrated Orange Juice and Resistance of Isolates to Different Concentrations of Peracetic Acid

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Introduction: Frozen concentrated orange juice (FCOJ) is among foods in which yeast play a significant role on spoilage. Assessing prevalence and diversity of yeasts in FCOJ as well as their resistance to sanitizers can provide useful information that can be used to further avoid the occurrence of episodes of spoilage or rejection of.

Purpose: The aim of this study was to assess the prevalence and diversity of yeasts isolated from FCOJ collected from an industry as well as to assess the resistance profile of the isolates against peracetic acid.

Methods: A total of 1,500 samples of FCOJ were collected between March - July, 2014, from an industry located in the state of Sao Paulo, Brazil. The juices were plated onto Wort Agar (WA), and plates were further incubated at 25°C for 5 days. The isolates were recovered and further subjected to identification through sequencing of D1/D2 major subunity of RNAr. The resistance of isolates to peracetic acid (0, 50, 80, 100, 150 and 200 ppm) was assessed through a microplate method with an exposure time of 3 min, initial level of yeasts of 10^{2-3} CFU/ml, and further plating onto WA, following incubation at 25°C/120 h.

Results: Yeasts were recovered from 17% of FCOJ samples, totaling 280 isolates. Until now, a total of 98 isolates have been identified, belonging to nine different genus, *Candida* spp., *Kluyveromyces* spp., *Saccharomyces* spp., *Toluraspora* spp., and *Zygosaccharomyces* spp., being the 5 major genera found. A total of 21 different species have also been identified. From the identified isolates, 36.7% (n = 36) were resistant to up to 200 ppm of peracetic acid.

Significance: Although the prevalence of yeasts in FCOJ could be considered low, the diversity of isolates and their high resistance to peracetic acid, potentially pose a challenge for their elimination of FCOJ processing environment.

P1-222 Isolation and Identification (SPME-GC-MS) of Potential Spoilage Compounds Produced by *Alicyclobacillus acidoterrestris* in Orange and Apple Juices

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Introduction: *Alicyclobacillus acidoterrestris* (AA) is cause of great concern for food industry due to its thermal and chemical resistances, as well as its spoilage potential. Despite this, not all AA strains are able to spoil fruit juices. In this way, identification of compounds produced by AA strains may assist on development of strategies to assess and control spoilage of fruit juices.

Purpose: The objective of this study was to identify and quantify the production of guaiacol, and other potential compounds associated with AA spoilage in orange and apple juice (11ºBrix).

Methods: Initially, we evaluated 497 strains of *A. acidoterrestris* isolated from fruit juices and tomato-based products collected in Argentina and Brazil. The ability of the isolates to produce guaiacol was assessed through the use of an enzymatic method. Positive strains for guaiacol production were then individually inoculated at level of 10^{2-3}CFU/ml in 10 ml of orange and apple juices, following storage at 45°C for 5 days. The techniques of micro-solid phase extraction by headspace (HS-SPME) and gas-chromatography with mass spectrometry (GC-MS) were used to identity and quantify volatile compounds produced by AA strains.

Results: From the 497 strains screened for guaiacol production, a total of 165 were confirmed by the enzymatic method to be able to produce guaiacol. Therefore, these strains were confirmed to be potentially deteriorigenic. CG-MS analysis was performed to further characterize and quantify the volatile compounds that can be associated with orange and apple juice spoilage by AA. A classification of AA strains in terms of their spoilage potential will be presented as well as the discrimination of volatile compounds produced by these strains.

Significance: This work will allow gaining insights on the incidence and spoilage potential of AA strains isolated from a variety of fruit-based products marketed in Argentina and Brazil.

P1-223 Incidence, Populations and Spoilage Potential (Rope) of Sporeforming Bacilli in Flour Used for Bread Production

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Introduction: Spoilage bacteria of the genus *Bacillus* are common contaminants of raw materials used for production of bakery products. These bacteria can cause spoilage of bakery products known as rope, characterized by an unpleasant fruity odor and enzymatic degradation of the crumb.

Purpose: The aim of this study was to assess the incidence, populations and spoilage ability of sporeforming bacilli (SB) in flour used for bread making.

Methods: Mesophilic and thermophilic aerobic sporeforming bacteria were enumerated in 100 samples of flours (wholemeal, n = 20, white bread flour, n = 40 and white flour cake, n = 40) after heat shock (80°C/30 min and 100°C/5 min, respectively) and plating in Tryptone Glucose Extract (TGE) agar and Dextrose Tryptone agar (DTA). SB isolates were evaluated for their ability to produce amylase and to cause rope spoilage in breads formulated with and without calcium propionate. The amylase positive strains were further identified through 16S rRNA sequencing.

Results: SB were isolated from all 100 flour samples; however, the highest counts were observed in wholemeal flour (3.1 log CFU/g). A total of 327 SB were isolated from flours, and 45 were shown to produce amylase. These isolates were identified as *B. licheniformis* (62%), *B. sonorensis* (20%) *B. cereus* (11%), *B. pumilus* (2%) and *Paenibacillus polymyxa* (2%). All 45 SB strains were able to cause bread spoilage by the ninety and third days of

storage when formulations with and without preservatives were used for bread making. The response of SB strains with the greater spoilage potential to bread baking and their ability to cause rope in bread will also be reported.

Significance: Most isolates recovered from flour belonged to *Bacillus* genus. Wholemeal flour was the most contaminated raw material used in bread making, highlighting its importance as a factor that can potentially reduce bread shelf life.

P1-224 Survey of Fungi in Raw Materials, Air and Final Products of a Multigrain Whole Meal Bread Production Line

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Introduction: The increase of consumption and production of foods with high fiber content has led the bakery industry to develop whole meal products. However, some raw materials can be important sources of fungi, which may result in environmental contamination, further bread contamination and reduction of shelf life.

Purpose: The aim of this study was to quantify fungi throughout a bakery factory located in the state of Sao Paulo, Brazil.

Methods: Raw materials (n = 120) from different lots, while sampling of equipment (n = 31), plastic film (n = 10) and handlers (n = 10) was performed by swab technique. Air samples (total n = 137) were collected in four different sampling days between December, 2014 and January, 2015 using *Sartorius* MD8 air sampler. Different points of processing were sampled (raw material and ingredients weighing, mixer, oven output, cooling, slicer and package). Quantification of fungi was performed on Dichloran Glycerol agar (DG-18) for all samples, while Dichloran rose bengal chloramphenicol (DRBC) was also used for enumeration of fungi in spoiled bread (n = 25). Fungi identification was performed according to appropriate identification keys. The statistical analyses were performed using ASSISTAT software.

Results: A total of 345 samples were analyzed and 12 different genera were isolated. *Penicillium* and *Aspergillus* were the most frequently isolated genera. Average fungi counts were: 3.09 log CFU/g (± 0.5) for whole wheat flour; 3.8 log CFU/g (± 1.8) for grains; 4.9 log CFU/g (± 2.9) for spoiled bread. The counts on environmental surfaces varied from 2.5 to 10 CFU/cm^2 (± 3.75), while in the air of processing environment levels were from 1.8 to 2.56 log CFU/m^3(± 0.38).

Significance: The knowledge of contamination sources and main species involved in bread spoiled samples comprise very useful data for adoption of measures and development of predictive models aiming to increase shelf life of breads.

Poster

P1-225 Isolation, Characterization and Spoilage Potential of Sporeforming Bacteria in Beers

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❖ ***Developing Scientist Competitor***

Introduction: Microbial spoilage of bottled beer has been restricted to few species due pasteurization process. Despite this, the potential occurrence of sporeforming bacteria able to survive beer pasteurization and further spoil this beverage is unclear.

Purpose: The aim of this study was to isolate, characterize and assess the spoilage potential of sporeforming bacteria recovered from Brazilian beers.

Methods: One hundred sixty-three samples of beer were acquired in Campinas, Brazil. The beer samples (100 ml, pale lager and 5 ml, dark lager) were previously subjected to a heat shock (80°C/30 min), filtered through 0.45 µm membranes and plated onto Tryptone Glucose Extract (TGE) agar at 37°C/48 h. The isolates were identified through 16S rRNA sequencing. PCR reactions were performed to assess the presence of *horC* gene in the isolates. The spoilage potential (change in optical density) of *horC* positive isolates was assessed through inoculation and storage of beers (with and without alcohol) from 10 - 40°C/2 months.

Results: Survey of beers for the presence of sporeforming bacteria resulted in the recovery of 260 isolates. Among these, 45.4% isolates (n = 118), 34.3% (n = 89), 15.4% (n = 40), 2.3% (n = 6) and 2.6% (n = 7) were found to belong to the genus *Bacillus*, *Paenibacillus*, *Brevibacillus*, *Lysinibacillus*, and others, respectively. The most prevalent species were *B. thuringiensis, B. megaterium*, *P. validus, P. humicus* and *Br. brevis*. Among all the isolates, only 2.3% (three *B. thuringiensis*, one *B. cereus*, *B. pumilus* and *Br. Invocatus*, each), presented *horC* gene, which is closely related to beer spoilage ability. The *horC* positive isolates were inoculated in pale lager beer (with and without alcohol) and their growth/spoilage potential will be reported.

Significance: The incidence of sporeforming bacteria able to spoil beer under commercialization conditions may significantly change quality control and hygienic practices in the brewery industry and represent new challenges for microbial stability of beers.

P1-226 Inhibition of *Bacillus cereus* Growth by Bacteriocin-producing *Bacillus subtilis* Strains Isolated from *Maari*, a Baobab Seeds Fermented Condiment, is Substrate Dependent

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Introduction: *Maari* is a spontaneously fermented alkaline fermented condiment made from baobab seeds. *Maari* fermentations are dominated by *Bacillus subtilis* contributing to desirable organoleptic properties. Due to the spontaneous fermentation, occasionally *Bacillus cereus* occurs in high numbers

Purpose: The aim is to select starter-cultures against *B. cereus.*

Methods: The Influence of substrate on antimicrobial activity was investigated by inoculating separately *B. subtilis* B3, B122 and B222 in baobab whole seeds, baobab ground seeds and in BHI incorporated with baobab ground seeds. For survival of *B. cereus,* BHI broth, ground baobab seeds broth and whole baobab seeds were inoculated with each B3, B122 and B222 as mono-cultures and in co-culture with *B. cereus* NVH391-98. Samples were collected for determination of pH, CFU/ml and antimicrobial activity.

Results: All three strains showed antimicrobial activity against *B. cereus* NVH391-98 in BHI-broth, whereas no antimicrobial activity was detected in cooked seeds and in ground baobab seeds broth. However, incorporation of ground baobab seeds with to 95-99.5% (w/w) BHI enhanced antimicrobial activity of *B. subtilis* in a strain dependent manner. Addition of $FeCl_3$, $MgSO_4$ and $MnSO_4$ to baobab ground seeds broth did not cause any antimicrobial activity. *B. cereus* NVH391-98 grew well in all the 3 substrates in mono-culture. Further, all the 3 *B. subtilis* strains were able to decrease *B. cereus* NVH391-98 to levels below detection limit in BHI, while outgrowth of *B. cereus* NVH391-98 was delayed in baobab ground seeds broth and during cooked baobab seeds fermentations by up to 40 h.

Significance: The present study contributes to the selection of *Bacillus* strains to be used as starter cultures for controlled production of *Maari.*

P1-227 Effect of Gamma Radiation on *Escherichia coli*, *Salmonella enterica* Typhimurium and *Aspergillus niger* in Peppers

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Introduction: Chili pepper (Capsicum frutescens L. var. conoides Bailey) and Sichuan pepper (Zanthoxylum bungeanum Maxim.) are important spices which extensively used as natural flavor in food, and they are especially in great demand in traditional Chinese cuisine. They were mixed with other spices, and added directly into many Ready-to-Eat foods. However, these spices are highly susceptible to microbial contamination in the process of growing, harvesting, processing and transporting, etc.

Purpose: The objective of this study was to examine the effect of gamma irradiation dose for inactivating pathogens including *E. coli*, *Salmonella* Typhimurium and *A. niger* inoculated in dried chili pepper and dried Sichuan pepper and to evaluate the sufficient dosages among different samples and pathogens.

Methods: Two widely used spices, Chili pepper and Sichuan pepper inoculated with *Escherichia coli*, *Salmonella enterica* Typhimurium and *Aspergillus niger* were irradiated with gamma ray. The efficacy of irradiation to inactivate pathogens was investigated.

Results: The results showed that irradiation at doses 4.00 kGy and 5.00 kGy was appropriate for eliminating almost all *E. coli* and *Salmonella* Typhimurium in two peppers, respectively. *A. niger* was undetectable in chili and Sichuan pepper at doses 1.50 kGy and 1.00 kGy radiation. The D_{10}-values of *E. coli*, *Salmonella* Typhimurium and *A. niger* in Sichuan pepper were 0.81, 0.93 and 0.20 kGy, and 0.82, 0.69 and 0.49kGy in chili pepper.

Significance: Low levels of radiation by gamma ray can assure the inactivation of *E. coli*, *Salmonella* and *A. niger* in peppers, which makes for a safer product for the consumer. The irradiation at appropriate doses is a promising approach for producing safe and pathogen-free peppers for consumers.

P1-228 Assessment of Microbial Contamination of Retail Beef in Korea and Pediction of the Shelf Life Using a Mathematical Quantitative Model

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Introduction: Beef, susceptible to microbial contamination, frequently causes foodborne illness via the consumption of microbially spoiled beef products. The growth of microbes present in beef is functional to the storage temperature and time, which determines its shelf life.

Purpose: This study was to assess the microbial contamination of retail beef in Seoul, Korea, and to predict its shelf life via the growth modeling of inert microbes present in retail beef using a mathematical quantitative model.

Methods: The quantity of beef specimens was 100. Total aerobic bacteria, coliform bacteria, *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, *E. coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* present in beef specimens were quantified using plate count methods. To investigate the growth of inert microbes present in retail beef, beef specimens were stored at 5, 10, 15, 20, and 25°C, and total aerobic bacteria in beef specimens were counted. The growth characteristics of total aerobic bacteria present in beef specimens were analyzed with a mathematical quantitative model (modified-Gompertz equation).

Results: The average value of total aerobic bacteria for 100 retail beefs was 4.57 log CFU/g. Coliform bacteria and *E. coli* were detected in 84 and 19 out of 100, respectively, but no other pathogens were detected. The analysis of the growth characteristics of inert aerobic bacteria in retail beef reveals that its microbial safety could not be guaranteed if beef, contaminated with bacteria at the level of 4 log CFU/g, was stored longer than 5 days at 5°C.

Significance: This study provided substantial information on the microbial safety of retail beef in Seoul, Korea, which enabled for the prediction of the shelf life of beef at refrigerated temperature.

P1-229 Influence of Vegetable Varieties on the Growth of *Listeria monocytogenes* and *Escherichia coli* O157:H7

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Introduction: Packaged fresh-cut vegetables are generally minimally processed without a processing step to eliminate contaminated microorganisms. The cut or damaged surfaces of vegetables release nutrients and moisture that may provide a favorable environment for microbial growth.

Purpose: The purpose of this study was to determine the influence of four varieties of fresh-cut vegetables on the growth rates of *Listeria monocytogenes* and *Escherichia coli* O157:H7 at refrigerated and abuse temperatures.

Methods: Iceberg lettuce, green pepper, carrot, and broccoli were washed with 0.1% chlorine and cut into pieces. A mixture of 5 strains of *L. monocytogenes* or *E. coli* O157:H7 was inoculated on the cut or damaged surfaces (0.5 cm x 0.5 cm) of vegetables pieces to achieve an initial level of 10^{2-3} CFU/g. Samples were placed in sterile containers and stored at 4 (*L. monocytogenes* only), 8, 12, 15, and 20°C. The populations of *L. monocytogenes* and *E. coli* O157:H7 in samples during storage were determined to estimate their growth rates (GR).

Results: At 4°C, the GR of *L. monocytogenes* were the lowest ($P < 0.05$) in carrot (no growth), followed by broccoli (0.12 ± 0.07 log/day), green pepper (0.13 ± 0.03 log/day), and iceberg lettuce (0.16 ± 0.04 log/day). At 8°C, the GR of *L. monocytogenes* was higher than *E. coli* O157:H7 in the vegetables. At temperatures ≥ 12°C, the GR of both pathogens on the vegetables were not significantly different ($P > 0.05$), and the GR were lower ($P < 0.05$) on carrot and broccoli than green pepper and iceberg lettuce.

Significance: The data suggest that *L. monocytogenes* is more of a concern than *E. coli* O157:H7 in fresh-cut vegetables and carrot is less supportive to growth of both pathogens than lettuce, green pepper and broccoli.

P1-230 Growth Behaviors of Stressed and Non-stressed Shiga Toxin-producing *Escherichia coli* in Ground Beef and Spinach

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Introduction: Meat products and produce contaminated with Shiga Toxin-producing *Escherichia coli* (STEC) were linked to several foodborne illness outbreaks. Among the STEC, O157 and non-O157 are the two major classes implicated in the outbreaks.

Purpose: The aim of this study was to compare the growth behaviors of O157 and non-O157 STEC and determine the effects of environmental stresses on their growth in ground beef and spinach.

Methods: Stressed cells of three O157:H7 and four non-O157 (O26:H11, O103:H1, O104:H4, and O145:NM) STEC were obtained by subjecting the cells to 2 ppm chlorine, a_w 0.97, pH 5, or 15-day starvation. A cocktail mix of stressed or non-stressed O157 or non-O157 was inoculated into 5 g irradiated beef or spinach and stored at 8, 12, or 16°C for 4 weeks. The cell populations during storage were compared to evaluate the growth behavior of O157 and non-O157 and the effects of stresses on their growth.

Results: At 8°C, the population of non-O157 (3.1 log CFU/g) was significantly ($P < 0.05$) higher than O157 (1.9 log CFU/g) in ground beef after one week, and the population difference (3.9 vs. 1.7 log CFU/g, $P < 0.05$) was also observed after 4 weeks. However, in spinach, the populations after 4 weeks at 8°C were not significant (3.9 vs. 3.3 log CFU/g). Starvation and chlorine stress induced better growth of non-O157 than O157 in ground beef (4.5 vs. 1.2 log CFU/g) and spinach (4.7 vs. 2.5 log CFU/g), respectively. Cell populations of stressed O157 or non-O157 STEC were not different from those of non-stressed O157 or non-O157 in beef and spinach stored at 12 and 16°C.

Significance: Results suggested that the growth behaviors of O157 and non-O157 in beef and spinach were different at 8°C, but not at 12 and 16°C. This information could also be helpful for designing temperature-abuse challenge studies concerning O157 or non-O157 STEC.

P1-231 Bacteriophage-resistant Mutants of *Lactobacillus casei* and *L. plantarum* and Their Interaction with Foodborne Bacterial Pathogens

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Introduction: *L. casei* (LC) and *L. plantarum* (LP) are widely applied in fermented food production and preparation. The antimicrobial effects of *Lactobacillus* may be able to prevent food spoilage. However, the activity and population density of *Lactobacillus* is seriously affected by bacteriophage. Replacing phage sensitive strains with phage-resistant starters offers a natural strategy to minimize phage dissemination in dairy production.

Purpose: The purpose of this study was to develop phage-resistant LC (φrLC) and LP (φrLP) strains, and to evaluate their roles in reducing colonization and infection of foodborne pathogens in gut.

Methods: The secondary culture method was used to generate *L. casei* phage (ATCC27139-B1)-resistant and *L. plantarum* phage (ATCC8014-B1)-resistant mutants. Effects of mutants against growth, interactions with human intestinal epithelium cells (INT-407), and virulent genes expression of enterohemorrhagic *E. coli* EDL933 (ATCC700927) (EHEC), *Salmonella* Typhimurium LT2 (ATCC19585) (ST), and *L. monocytogenes* LM2 (ATCC19115) (LM) were evaluated and analyzed by ANOVA.

Results: φrLC, φrLP and their cell-free culture supernatants (CFCSs) competitively excluded/eliminated (100%) EHEC and ST within 48 h. Further, φrLC and φrLP significantly ($P < 0.05$) inhibited INT407 cell-pathogen interactions including adherence, invasion and virulence gene expression. For instance, the adherence abilities of EHEC, ST, and LM were reduced by φrLC by 62.1%, 68.5%, and 58.5%, respectively; and 52.7%, 61.5%, 46.9%, respectively by φrLP. Furthermore, qPCR showed that CFCSs of φrLC and φrLP down-regulated the expression levels of multiple virulence genes including 6 for EHEC (*eaeA*, *espA*, *espD*, *fliC*, *hlyB*, and *ler*), 4 for ST (*fliC*, *fliD*, *hilA*, and *hilD*), and 4 for LM (*fbp*, *flaA*, *hlyA*, and *iap*).

Significance: Bacteriophage-resistant *Lactobacillus* offers dairy industry a good choice on solving phage contamination; their inhibitory effects on EHEC, ST, and LM may help in reducing and preventing foodborne pathogen colonization and infections.

P1-232 Spoilage-associated *Leuconostocaceae*; Genotypic and Phenotypic Diversity among Different Food Classes

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Introduction: *Leuconostocaceae* are a frequent cause of spoilage and gas formation in minimally processed refrigerated packaged foods. However, little is published on genetic relatedness of these organisms. It is not known whether subtypes of *Leuconostocaceae* species exist which more frequently cause spoilage.

Purpose: The purpose was to discern the genotypic/phenotypic structure of isolates from different spoiled finished food products. Information will be used to assess [a]distribution of genotypes, or existence of consistently occurring subtypes, and [b] patterns of occurrence or correlation to antimicrobial resistance or food processes.

Methods: Isolates (n = 82) were obtained from six different food types. DNA was isolated from each for 16S identification and RAPD subtyping. Sequence data was evaluated and patterns analyzed. Gas formation, dextran formation and nisin sensitivity were determined.

Results: Isolates were identified as either *Weissella* or *Leuconostoc* across 7 different species (*L. mesenteroides*, *citreum*, *carnosum*, *pseudomesenteroides*, *W. confusa*, *paramesenteroides*, *cibaria)* and segregated into subtypes (n = 35) distributed across food types. Five major RAPD subtypes composed 57% of observed diversity. Three major clades had members from across 3 major food types. The remaining clades represented < 2% of observed diversity. No correlation between tested phenotype and RAPD pattern was observed. Six isolates were tested, of which 4 were nisin sensitive and grouped in one major RAPD subtype; the remaining isolates (resistant to 100 ppm nisin) clustered in a minor clade.

Significance: Knowing predominant subtypes of *Leuconostocaceae* can focus antimicrobial research and intervention efforts on the more important spoilage organisms instead of less-significant or randomly occurring minor contaminants. This is perhaps the first investigation to ascertain the common *Leuconostocaceae* subtypes potentially impacting a large portion of food production and shelf life.

P1-233 Assessing Microbial Quality of Tofu in Korea, Identifying a Dominant Bacteria Contaminating Tofu, and Characterizing the Growth in Tofu

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Introduction: Tofu is susceptible to microbial spoilage as it contains carbohydrate, protein, fat, and water. In addition, microbial spoilage of tofu is highly related with the storage temperature and time.

Purpose: This study was to monitor the microbial safety of tofu in northern Seoul, to identify the dominant bacteria contaminating tofu, and to investigate the growth characteristics of identified bacteria.

Methods: The size of tofu specimens was 100. Total aerobic bacteria, *Staphylococcus aureus*, *Salmonella* spp., *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, coliform bacteria, *E. coli* O157:H7 in tofu were tested. A dominant colony with the typical shape and color was isolated and subjected for the 16S rRNA bacterial identification. The growth characteristics of isolated bacteria in tofu as temperature was investigated.

Results: Aerobic bacteria was detected in 32 out of 100 tofus. The dominant bacteria contaminating tofu was identified as *Bacillus cereus*. Gompertz modeling of the growth of the *Bacillus cereus* in fresh tofu reveals that the microbial safety of tofu cannot be guaranteed if tofu contaminated with such bacteria is stored at 10°C for 4 days.

Significance: This study identified a dominant bacteria contaminating tofu and informed of its growth characteristics in tofu.

P1-234 Antifungal Activity of Various Bacterial Species against *Botrytis cinerea*, *Fusarium pallidoroseum*, and *Fusarium moniliforme*

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Introduction: *Botrytis cinerea*, *Fusarium pallidoroseum* and *Fusarium moniliforme* are fungal plant pathogens that can cause significant produce loss, both pre- and post-harvest. Typical fungal control requires the application of chemical fungicides that may negatively impact the environment and human health. Alternatively, the use of biological control agents has shown potential for fungal control; however, commercial use is limited. Due to development of fungicide resistance in pathogen populations and the demand for more environmentally sustainable solutions, further research on the identification and development of biocontrol methods that could extend produce shelf life is merited.

Purpose: The purpose of this study was to screen 22 bacterial isolates for antifungal activity against *Botrytis cinerea*, *Fusarium pallidoroseum*, and *Fusarium moniliforme*.

Methods: Bacterial isolates were individually spot-inoculated onto Tryptic Soy Agar, Potato Dextrose Agar, or Lactobacillus MRS agar, depending on isolate growth requirements, and a 9 mm plug of fungal-colonized agar was placed onto the center of the isolate-inoculated plate. Plates were incubated at 24°C for 10 days. Fungal growth was evaluated daily, beginning on Day 3, by measuring the diameter of the fungal colony.

Results: Nine of the 22 isolates, including *Bacillus megaterium*, *Bacillus coagulans*, *Bacillus amyloliquefaciens*, and *Serratia plymuthica*, inhibited all three fungi; fungal inhibition ranged from 51 - 62% for *B. cinerea*, 60 - 68% for *F. pallidoroseum*, and 40 - 61% for *F. moniliforme*. Three Lactic Acid Bacteria species – *Lactobacillus plantarum*, *Pediococcus acidilactici*, and *Pediococcus pentosaceus* – and three *Bacillus* species – *Bacillus thiaminolyticus*, *Bacillus firmus*, and *Bacillus clausii* – inhibited *B. cinerea* only by 30 - 56%. Seven isolates showed no suppression of any of the three fungi.

Significance: This study identified nine bacterial isolates capable of suppressing the growth of *B. cinerea*, *F. pallidoroseum*, and *F. moniliforme in vitro*. Evaluation of antifungal efficacy on produce (*in planta*) is required to determine an isolate's potential use as a biocontrol agent.

P1-235 Characterization of Heat-resistant Fungus *Neosartorya pseudofischeri S*ubjected to Heat Treatments

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Introduction: Spoilage of pasteurized and canned fruit products caused by heat-resistant molds has been reported repeatedly. These heat-resistant organisms usually contaminate fruits on or near the ground and survive heat treatments used for fruit processing. They can grow and spoil the products during storage at room temperature, which results in great economic losses.

Purpose: The objectives of this study were to investigate the response of heat-resistant fungal spores of *Neosartorya pseudofischeri* isolated from contaminated blueberry juice to various heat treatments, establish heat treatment time and temperature combinations to inactivate this fungus, and elucidate the mechanism(s) of heat resistance.

Methods: The 15 and 30 days old heat-resistant fungal spores were collected and inoculated into the blueberry juice at 10^4 spores/ml. Blueberry juice was then subjected to heat treatments at 80, 85, 90, 93, 95, 96 and 99°C for different duration. Mold counts were conducted and survivor curves were plotted. D- and z-values were determined and compared using ANOVA. Proteomic approach was used to investigate protein changes of *N. pseudofischeri* subjected to heat treatment at 93°C for 0, 1, and 8 min.

Results: The 30 days old fungal spores had significantly higher thermal resistance than that of the 15 days old spores ($P < 0.05$). Practical heat inactivation data were generated for different heat time and temperature combination. The D-values of 30 days old spores of *N. pseudofischeri* at 85, 90, 93, 96 and 99°C were 53.8, 12.2, 2.03, 0.98 and 0.42 min, respectively. The z-value calculated from the thermal death time curves was 6.32°C. Protein profile of the fungal spores was determined using LC/MS/MS.

Significance: The results established by this study may be used by fruit (blueberry) processors to prevent losses due to spoilage caused by the heat-resistant microorganisms and establish appropriate thermal process schedules for fruit (blueberry) products.

P1-236 Determining the Shelf Life of Ready-to-Eat Deli Products Held at 40ºF, 45ºF and 50ºF Using Total Aerobic Plate Count as a Surrogate Growth Indicator of *Listeria monocytogenes*

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Introduction: Shelf Life Determination of Ready-to-Eat Deli products varies within the food industry. Food Safety Shelf Life, relying on product Total Aerobic Plate Count may not reflect the growth of pathogens such as *Listeria monocytogenes*. This study evaluated product Total Aerobic Plate Count in parallel with a product Challenge using a five-strain cocktail of *Listeria monocytogenes.*

Purpose: Determine if Total Aerobic Plate Count values correlate with *Listeria monocytogenes* growth values at 40°F, 45°F and 50°F and may be used to establish a Food Safety Shelf Life for Ready-to-Eat Deli Products.

Methods: Ready-to-Eat Deli Products (Salads and Meats) were separated into two groups and held at 40°F, 45°F, 50°F for the duration of the Shelf Life Study of 30 days. Group I was analyzed for Total Aerobic Plate Count (TAPC). Corresponding Group II was inoculated using a five-strain cocktail of *Listeria monocytogenes.* Samples were quantitatively evaluated at weekly intervals using Standard Methods agar for TAPC and MOX media for *Listeria monocytogenes*.

Results: Correlation of TAPC growth and *Listeria monocytogenes* growth is product dependent. Intrinsic product factors were more influential on growth than storage temperature. A challenge provides an accurate reproducible method to Determine Food Safety Shelf Life as opposed to quantitating Total Aerobic Plate Count.

Significance: Food Safety Shelf Life determination should be based on a reproducible quantifiable method such as an active challenge. Basing Shelf Life on a passive indicator test like TAPC, with product variability may not be an accurate predictor of Food Safety Shelf Life.

P1-237 Characterization of *Bacillus sporothermodurans* Isolated from Ultra High Temperature (UHT) Milk

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Introduction: Some strains of *Bacillus sporothermodurans* produce highly resistant spores during unfavorable conditions thereby having implications on the quality of ultra-high temperature (UHT) processed milk and other milk products. Identification at the strain level of vegetative cells is mainly through sequencing of the 16S rRNA gene; however, multiple copies of the 16S rRNA gene which differ in sequence are usually present in a bacterium leading to the identification of multiple ribotypes. Protein coding genes (including rpoB), single copy in nature may afford better discrimination especially at the species and sub-species level.

Purpose: To determine the genetic diversity of *B. sporothermodurans* strains through sequencing of the 16S rRNA gene and rpoB gene with the aim of improving molecular characterization to the strain level.

Methods: Genomic DNA was extracted from bacterial cells grown on brain heart infusion agar and amplified through PCR. Sequencing reactions were performed using the 16S rRNA and rpoB genes at sequencing read lengths of 750 bp and 1000 bp. Phylogenetic analysis were performed to determine genetic discrimination levels.

Results: Partial 16S rDNA sequencing showed a wide variation of B. sporothermodurans strains isolated from different locations. Eight clusters for the target organisms were identified at similarity level > 90%. Partial rpoB sequencing showed 10 clusters for the target organism at similarity level of > 90%. For a total of 10 clusters, the rpoB gene provided more phylogenetic resolution than the 16S rRNA gene in 6 clusters with observed differences, with equal resolution in 1 cluster and lower resolution in 3 clusters.

Significance: In the context of the growing number of studies focusing on sub-species diversity in the food and biomedical industries, this research would help develop rapid, efficient molecular detection and characterization protocols with higher resolution levels for food spoilage organisms and microbes of public health importance.

P2-01 Comparison of the FDA BAM *Salmonella* Method and a New PCR Method for Detection of *Salmonella* Species in Spices

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Introduction: Contamination of spices with *Salmonella* spp. can cause expensive and commercially damaging product recalls of batches of spices, semi-finished and finished food products. Many current detection methods for *Salmonella* are cumbersome and slow to provide results. The Thermo Scientific™ SureTect™ *Salmonella* species PCR Assay, a new method for the detection of *Salmonella*, enables results to be achieved in less than 24 h compared to 6-7 days for culture-based methods.

Purpose: To compare the FDA BAM and SureTect PCR methods to detect *Salmonella* from 375-g samples of powdered chili, onion and garlic.

Methods: Seven samples of chili consisting of 2 unspiked and 5 spiked (at 22.2 CFU), fourteen samples of onion; consisting of 3 unspiked and 11 spiked samples (ranging from 12.8 to 112 CFU) and 11 samples of garlic; consisting of 3 unspiked and 8 spiked samples (ranging from 3.4 CFU to 49 CFU) were prepared and incubated in Tryptone Soya Broth (TSB) at 35°C for 20 - 22 h according to the FDA BAM method (including adjustment of pH and addition of potassium sulphite, where required). Following incubation, samples were removed for analysis with the new PCR assay, then analyzed and confirmed as detailed in the kit instructions. TSB enrichments were also sub-cultured into selective broths and processed according to the FDA BAM method. Finally, presumptive positive results from all plating media were confirmed using a *Salmonella* latex kit.

Results: All unspiked and spiked samples analyzed returned results which were in complete agreement between the two methods compared in this study. No examples of false negative or false positive results were obtained by the PCR assay and PCR inhibition was not observed.

Significance: The results gained in this study demonstrated that the PCR method is an accurate and reliable alternative to the FDA BAM method for the detection of *Salmonella* from spices.

P2-02 Validation of a PCR Method for the Threshold Detection of *Salmonella* Species in Ground Poultry and Rinsates

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Introduction: An estimated 1.2 million cases of salmonellosis occur annually in the U.S. Based on the association of *Salmonella* with raw poultry products and the limitations of conventional quantitative methods, processors face a challenge in understanding overall process control and potential for high levels of contamination. A new approach was developed for assessing *Salmonella* contamination levels in poultry rinsates and ground products.

Purpose: The objective of this study was to evaluate the capability of the alternative method without enrichment for rinsates and short 5.5 h enrichment for ground product to detect *Salmonella* spp. at a threshold level of > 2.0 CFU/ml in rinsates and > 2 log CFU/325 g in ground product.

Methods: Rinsates from surface inoculated chicken thighs and ground turkey from a poultry processing plant were inoculated using standard method validation procedures. Five samples of each matrix served as uninoculated, negative controls. In addition, 231 production rinsates and 140 ground turkey samples were tested with the alternative method.

Results: The alternative method screened all 20 inoculated rinses and ground products as presumptive. The MPN estimate of *Salmonella* levels for the inoculated rinse samples ranged from 24 CFU/ml to > 110 CFU/ml and 58.5 CFU/325g to 17,875 CFU/325g for the ground product. All samples confirmed as positive for *Salmonella* following the reference method. Of the 231 side-by-side rinsate samples, 5 samples were presumptive with the alternative method but did not culturally confirm (2%). Of the 140 ground poultry samples, 10 samples were presumptive with the alternative method, of which 5 did not confirm (3.5%).

Significance: The Bio-Rad iQ-Check *Salmonella* II standard protocol with modifications of testing 1.5 ml of sample and shortened 5.5-h enrichment for ground product is as effective as the USDA-FSIS MLG 4.07, MLG 4C.05, and MLG Appendices 2.03 methods at detecting *Salmonella* in unenriched rinsates when recovered at a level of at least 2 log CFU/ml.

P2-03 Use of Graphene Oxide for Concentration of Human Norovirus

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Introduction: Graphene and its derivative graphene oxide (GO) are promising molecules for a variety of biotechnology uses, with features such as fluorescence quenching, ssDNA adsorption, ability to protect biomolecules from enzymatic cleavage, and antimicrobial properties. The impact of GO on noroviruses (NoV) has not yet been studied.

Purpose: The purpose of this work was to investigate the utility of GO for concentration/removal of NoV.

Methods: A representative GII.4 human NoV outbreak strain was inoculated into various concentrations of GO (50 - 1000 µg suspended in 1X PBS), incubated, and pelleted by centrifugation at 10,000 x g. Virus and viral RNA recovery from the GO precipitate and the associated supernatant was determined using RT-qPCR combined with various sample pre-treatments.

Results: Norovirus adsorbed to GO without virus or viral RNA degradation, as determined by preceding RT-qPCR with RNase treatment. The efficiency of virus capture with GO was concentration-dependent, with recovery efficiencies ranging from 60 to 70% at GO concentrations of 400 to 1000 µg, respectively. Both NoV and viral RNA could be efficiently released from GO by traditional elution methods (e.g., glycine-saline buffer, pH 9.0) with recovery efficiencies of 45 to 87% at GO concentrations in the range of 50 to 1000 µg. Norovirus RNA was protected from RNase treatment after desorption from GO, which suggests that the NoV capsid remained intact.

Significance: Both human NoV and NoV RNA adsorb effectively to GO without inactivation or degradation. This inexpensive compound shows promise for use in NoV concentration and purification as is necessary for food and environmental detection protocols.

P2-04 Loop-mediated Isothermal Amplification Assays for Detecting *Yersinia pseudotuberculosis* in Milk Powders

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Introduction: *Yersinia pseudotuberculosis* is a Gram-negative foodborne pathogen that causes several diseases, such as enteritis, septicemia, and reactive arthritis.

Purpose: Method for rapid detection of *Y. peudotuberculosis* in dairy products was developed.

Methods: Loop-mediated isothermal amplification (LAMP) assay targeting the 16S-23S rDNA internal transcribed spacer (ITS) region was developed to detect *Y. pseudotuberculosis* in milk powder.

Results: The DNA amplification could be completed in 1 h, and detected by produced white precipitate visible to naked eyes. The detection limit of LAMP assay was 100 fg/reaction for genomic DNA, and 100 CFU/100 g milk powder coupled with 12 h enrichment.
LAMP assay is 100 times more sensitive than conventional polymerase chain reaction method for detecting *Y. pseudotuberculosis*, and correctly identified 18 cases of *Y. pseudotuberculosis* contaminations from 236 commercial milk powder products.

Significance: The developed LAMP assay may facilitate rapid detection of *Y. pseudotuberculosis* contaminations in agricultural and food products.

P2-06 A Second Generation ANSR® *Listeria* Assay for Improved Ease of Use

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Introduction: ANSR® *Listeria* is an isothermal nucleic acid amplification assay for detection of *Listeria* spp. in food and environmental samples. The assay reagents have been reformulated for increased solubility, allowing an alternative mixing procedure by vortexing for improved ease of use and contamination control. Additionally, use of an improved enrichment medium, LESS Plus Broth, has been incorporated into the modified method.

Purpose: The purpose of the present study was to validate performance of the modified amplification method for detection of *Listeria* spp. in inoculated food and environmental samples.

Methods: Three foods (hot dogs, queso fresco, and cantaloupe) and one environmental surface (stainless steel) were inoculated with *Listeria* spp. using conditions intended to simulate natural contamination. Inoculation levels were sufficiently low to produce fractional positive data sets (5 - 15 of 20 test portions positive). Replicate test portions were tested by the amplification method (after 16 h and 24 h of enrichment at 36°C) and either the FDA/BAM or USDA/MLG reference culture procedures for *Listeria*. Test results were analyzed using a probability of detection (POD) model at $P < 0.05$ to determine if the numbers of positive results produced by the amplification and reference methods were statistically different.

Results: For the four sample types in total, by the amplification method there were 42 and 45 positive results after 16 h and 24 h of enrichment, respectively. There were 39 positive results by the reference methods. In no case was the difference in the number of positive results obtained by the amplification and reference methods statistically significant by POD analysis.

Significance: The amplification method provides the user with a simple, fast, and effective tool for detection of *Listeria* spp. in food and environmental samples. The modified version of the assay offers improved ease of use and minimizes risk of contamination.

P2-07 Design and Evaluation of Two-stage Multiplex Real-time PCR Method for Detecting O157:H7 and Non-O157 STEC Strains from Beef Samples

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Introduction: *E. coli* O157:H7 was first recognized as a human pathogen in 1982 and until recently was the only *E. coli* strain mandated for testing by the USDA. In June 2012, the USDA declared six additional Shiga Toxin-producing *E. coli* serogroups (O26, O45, O103, O111, O121, and O145) as adulterants in ground beef and beef trim, if they also contain virulence genes for Shiga Toxin 1 and/or 2 (*stx1*, *stx2*) and intimin (*eae*).

Purpose: To develop a complete workflow, including a two-stage real-time PCR method that meets USDA regulations to detect *E. coli* O157:H7 and the "big six" non-O157 STEC serogroups.

Methods: Using Applied Biosystems™ assay design software, TaqMan™ real-time PCR assays were designed against each of the 6 non-O157 STEC O-antigen genes and the virulence factors *stx1*, *stx2,* and *eae*. Each assay was tested against 132 STEC inclusion strains and 283 exclusion strains to determine assay sensitivity and specificity. Assays demonstrating 100% specificity and sensitivity were multiplexed with the MicroSEQ™ *E. coli* O157:H7 assay and optimized across two PCR reactions. The final optimized assays were tested against 375-g ground beef samples spiked with as low as 7 CFU

of representative *E. coli* isolates and enriched with TSB for 10 and 15 h. Real-time PCR was performed on the 7500 Fast real-time PCR system using RapidFinder™ Express software.

Results: All assays detected all inclusion strains and showed no cross-reactivity to any of the exclusion strains tested. The *stx* assays detected all known variants of *stx1* and *stx2*, including *stx2f* and *stx2g*. The optimized workflow showed equivalent detection to the USDA MLG reference method 5B.05.

Significance: The multiplex real-time PCR assays developed are part of a complete food testing solution for routine and rapid detection of *E. coli* O157:H7 and the "big six" non-O157 STEC strains in beef samples.

P2-08 Detection of *Salmonella* from Cloves by a Modified Culture Method and Three PCR Methods

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Introduction: Spices are typically added to Ready-to-Eat foods and present a unique challenge to food safety. Many spices, including cloves, have antimicrobial properties and current methods for detection of *Salmonella* from these food ingredients are not as sensitive as they are for foods that do not contain inhibitors.

Purpose: To improve the Bacteriological Analytical Manual (BAM) for detection of *Salmonella* from cloves by modifying the culture assay and integrating it with real-time PCR platforms.

Methods: A modified culture method was compared with the reference BAM method. For the modified method, 25 g of cloves were rinsed in 225 ml of tryptic-soy-broth for 60 s and the rinsate was pre-enriched. The BAM was followed for the remainder of the assay. Six trials with two clove cultivars and three *Salmonella* serotypes were conducted. Each trial was comprised of 40 dry-inoculated samples (10 - 810 CFU/g); 20 samples were processed by BAM method and 20 by the modified method. Pre-enrichment cultures from the modified method were used for comparison of the effectiveness of FDA-PCR, ABI-MicroSEQ® and GeneDisc® for the detection of *Salmonella*, with three DNA different extraction methods.

Results: With the modified method, for the six trials 100%, 30%, 100%, 90%, 95% and 80% of the 20 samples were positive for *Salmonella*. With the BAM method in the same trials, 75%, 0% 30%, 10%, 15%, and 25% of the samples were positive; respectively. There was 100% correlation among all the DNA extraction methods for the various PCR platforms examined. The molecular data matched the cultural data. In conclusion, the modified culture method was more sensitive than the BAM method for the detection of *Salmonella* in cloves. All three PCR methods were equally effective for detection of *Salmonella* from cloves.

Significance: This modified culture method will substantially improve the detection of *Salmonella* from cloves and can potentially be applied to other spices in the future.

P2-09 Identification of Five Shiga Toxin-producing *Escherichia coli* Genes by Luminex Microbead-based Suspension Array

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Introduction: Shiga Toxin-producing *Escherichia coli* (STEC) are among the leading causes of foodborne bacterial infections in the United States, O157:H7 being the predominant serotype.

Purpose: To optimize trace back and control of future outbreaks and to rapidly identify the presence of potentially virulent STEC, a PCR-based Luminex suspension array was developed to detect the genes coding for four virulence factors (*stx*1, *stx*2, *eae*, and *ehxA*) plus the O157:H7 specific +93 *uidA* single nucleotide polymorphism and an internal amplification control (IAC).

Methods: Multiplex PCR was performed in 25 µl reactions containing 1.2 pg IAC template using Qiagen® multiplex PCR plus kit under amplification conditions: 95°C for 15 min; 30 cycles, each cycle consisting of 95°C for 30 s, 60°C for 90 s and 72°C for 30 s; plus a final extension step at 68°C for 10 min. Reactions were analyzed in a Bio-Plex 200 instrument after oligonucleotide-microsphere conjugation and microsphere hybridization. Signal–to-background ratios were calculated from the Median Fluorescent Intensities (MFI) using the Bio-Plex Manager 6.0 software; ratios > 5.0 were considered as positive for each analyte.

Results: The inclusivity tests 100% accurately identified six *stx*2, 21 *eae*, six *ehxA* variant and the 45 STEC strains collected from various sources. An exclusivity panel consisting of 46 strains of non-STEC bacteria did not exhibit any false-positive signals. The Luminex suspension array identified STEC virulence genes in a 96-well plate format in less than 4 h. Addition of IAC excludes false-negative results due to PCR inhibitors in the reactions.

Significance: The rapid detection of these STEC genes will identify the presence of potentially virulent O157:H7 and non-O157 STEC. This assay can easily be expanded to include other STEC virulence genes or O serogroups of interest and is applicable to screening food enrichments.

P2-10 Specific Detection of Viable *Vibrio parahaemolyticus* Using Loop-mediated Isothermal Amplification Combined with Propidium Monoazide

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Introduction: *Vibrio parahaemolyticus*, a Gram-negative marine bacterium, is a major foodborne pathogen predominantly in raw or undercooked seafood. The viable but non-culturable (VBNC) state of *V. parahaemolyticus* cannot be detected by traditional culture methods while remains alive for long time and maintains the potential virulence.

Purpose: The objective of this study was to develop and optimize a method that combines propidium monoazide (PMA) staining with loop-mediated isothermal amplification (LAMP) to detect only viable cells of *V. parahaemolyticus*.

Methods: The viable or dead cells suspensions were treated with PMA in the dark for 10 min and were subsequently exposed to a 650 W halogen lamp for 5 min. The bacterial cells were harvested and DNA was extracted and amplified by LAMP. The primers targeted six distinct regions in the *tlh* gene of *V. parahaemolyticus* were designed for the PMA-LAMP method.

Results: The treatment with 3 µg/ml PMA and a 5min light exposure was suitable for PMA-LAMP to distinguish the viable cells from dead cells of *V. parahaemolyticus*. The optimized assay was specific and sensitive, it could detect as low as 12 CFU/ml viable *V. parahaemolyticus* in pure culture and artificially contaminated seafood samples (pomfret, shrimp, scallop and salted fish). The assay can detect VBNC state of *V. parahaemolyticus* without any interference of dead cells and other bacteria.

Significance: The results indicate that PMA-LAMP could effectively detect viable *V. parahaemolyticus* without being disturbed by other cells, it is a suitable technique for the detection of VBNC cells of foodborne pathogens in contaminated food.

P2-11 ANSR *Listeria monocytogenes*: An Isothermal Nucleic Acid Amplification Assay for Detection of *Listeria monocytogenes* in Food and Environmental Samples

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Introduction: ANSR® *Listeria monocytogenes* is an isothermal nucleic acid amplification method for specific detection of *Listeria monocytogenes* in food and environmental samples. Following a single-step sample enrichment of 24 h, results are available in 40 min.

Purpose: The purpose of the present study was to assess performance of the new amplification method for detection of *Listeria monocytogenes* in food and environmental samples.

Methods: Four foods (hot dogs, queso fresco, cantaloupe, and guacamole) and one environmental surface (stainless steel) were inoculated with *L. monocytogenes* under conditions intended to simulate natural contamination. Inoculation levels were chosen to produce fractional positive data sets (5 - 15 positives out of 20 replicate test portions). Samples were tested by the amplification method after 16 h and 24 h enrichment in LESS Plus Broth at 36°C, and by the appropriate reference culture method (FDA/BAM or USDA/MLG). Test results were analyzed using a probability of detection (POD) model to determine if differences in the number of positive results between methods were statistically significant.

Results: For the five sample types in total, there were 46 positive test portions by the reference methods. By the amplification method, there were 43 confirmed positives after 16 h enrichment and 62 confirmed positives after 24 h enrichment. There were more positives at 24 h for all sample types except cantaloupe, indicating that 24 h enrichment is required in most cases. At 24 h, the difference in the number of positives by the amplification and reference methods was significant ($P < 0.05$) only in the case of hot dogs (19 vs. 7).

Significance: The new isothermal amplification method for *Listeria monocytogenes* was shown to be an effective method across a variety of sample types and provides the user with a rapid, simple, and accurate tool for detection of *Listeria monocytogenes.*

P2-12 A Complete Solution for Virus Detection in Food in Accordance with ISO/TS15216-1 and 2

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Introduction: In July 2013, the international standardized method for virus detection in foods (ISO/TS15216-1 and 2) was published. To face the increase in virus detection demand, food analytical service laboratories need a complete standardized solution.

Purpose: The objective of this study was to develop and validate a global ceeramTools®/bioMérieux detection method from samples to results in accordance with ISO/TS15216.

Methods: The performance criteria (evaluation of inhibition impact, specificity, limit of detection) of the complete solution was compared with the ones of the ISO/TS 15216-1 and 2. Protocols described specifically for each type of matrices (shellfish, soft fruits, leafy greens, bottled water and surface) included in the ISO/TS15216 were used for virus elution and concentration. Mengo virus VMC0 (ceeramTools®) was used as a viral process control. RNA extractions were performed using the bioMérieux NucliSens® MiniMag® DNA/RNA extraction platform. ceeramTools® molecular detection kits were used for identification and quantification of Mengo virus, norovirus GI and GII and hepatitis A virus. A ring trial study for each type was conducted with different laboratories.

Results: Whatever the category of samples, evaluation of inhibition leads to exactly the same results using either an external positive control EPC as described in ISO/TS 15216 or internal positive control IPC included in ceeramTools® detection kits for NoV GI, NoV GII and HAV targets. The same limit of detection was achieved (500 genome copies with IC > 95%) for all type of food samples (shellfish, soft fruits, leafy greens, bottled water and surface) with both methods. All the participants in the inter-laboratory study were able to reach the same limit of detection whatever the matrices.

Significance: For all sample categories included in the reference method, the complete solution and the reference method ISO/TS 15216 were concluded to be equivalent.

P2-13 Validation Study of a Real-time PCR-based Method for the Detection of *Salmonella* in a Broad Range of Food Matrices and Environmental Surfaces

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Introduction: *Salmonella* is one of the most important microbiological criteria inspected routinely by food business operators and national authorities. Since reference methods usually rely on exhaustive and cumbersome procedures, lab operators use alternate methods offering shorter time to results and simpler enrichment and assay protocols. Nonetheless, such alternate analytical methods require official validation studies in order to demonstrate equivalency performances between alternate and reference methods.

Purpose: The objective of this study was to evaluate the Pall GeneDisc® Plate *Salmonella* spp., a PCR-based method for the detection of *Salmonella* in various test portions (25 g and 375 g) of a broad range of food and environmental surfaces in comparison to reference methods using the current AOAC-PTM guidelines.

Methods: The evaluation consisted of method comparison of 19 different food matrices (ground beef, beef trim, raw chicken, ground turkey, chicken carcass rinses, raw shrimp, lettuce, fresh cut cantaloupe, whole cantaloupe, peanut butter, beansprouts, spent irrigation water, dry milk, cheddar cheese, ice cream, milk chocolate, dry pet food, black pepper and shell eggs) and 2 environmental surfaces (rubber and stainless steel). The performances of the alternate assays were compared to USDA/FSIS MLG 4.05 and FDA/BAM Chapter 5.

Results: All samples enriched (N = 1,125) and assayed with the alternate *Salmonella* method were culturally confirmed by the various confirmation procedures (alternate and reference methods). For food matrices with high competing flora (poultry products, beansprouts, carcass rinsates) or with presence of inhibitory compounds (black pepper), some samples required a subculture of the BPW in selective enrichment broths (TT and RV) in order presumptive positive PCR results can be clearly confirmed. The statistical analysis with POD (Probability of Detection) showed the alternate method to be as good as the USDA and FDA reference methods.

Significance: This molecular method was shown reliable for the detection of *Salmonella* in various food matrices.

P2-14 Prevalence and Molecular Epidemiology of *Salmonella* in Poultry Carcasses

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Introduction: Salmonellosis is one of the most widespread infectious diseases in the world and is a common cause of gastrointestinal food poisonings. Raw and processed meat products, including poultry, beef, and pork, are the principal reservoir of *Salmonella*.

Purpose: The objective of this study was to identify and characterize the *Salmonella* isolated in poultry carcasses directly transported from poultry slaughter house in Korea. In addition, we evaluated the performance of automated repetitive sequence-based PCR system (DiversiLab™) for subtyping *Salmonella* isolated.

Methods: A total of 120 samples (60 of duck, 60 of chicken) were examined. Twenty-five grams of each sample were enriched in 225 ml of buffered peptone water and incubated for 24 h at 37°C. And then 0.1 ml of the enriched BPW was added to 9 ml of RV and incubated 24 h at 42°C followed by streaking onto XLD for *Salmonella* detection. After 24 h incubation at 37°C, presumptive colonies as *Salmonella* on XLD were confirmed by VITEK™ and with "O" antisera. In addition, an antibiotic resistance test was performed, and molecular subtypes of *Salmonella* isolates were ascertained using automated repetitive sequence-based PCR system (DiversiLab™, BioMerieux, France).

Results: A total of 23 of 120 (19.2 %) *Salmonella* strains were isolated, and 11 of 23 (47.8 %) *Salmonella* strains were identified as serogroup D. On antibiotic resistance test, most of *Salmonella* were resistant to erythromycin and the other antibiotics tested. Automated repetitive sequence-based PCR system for molecular subtypes represented weak differentiation among the same serovar of *Salmonella* isolates, but good differentiation among different serovars.

Significance: Poultry products contaminated by *Salmonella* have the possibility to give the serious risk for human health. DiversiLab™ differentiated similar serogroups of *Salmonella*. The DiversiLab™ would facilitate timely public health recognition and response to foodborne disease outbreaks.

P2-15 Evaluation of Viability of Human Norovirus Using Propidium Monoazide (PMA) and Ethidium Monoazide (EMA)

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Poster

Introduction: As in vitro culture systems have not been developed for the study of noroviruses (NoVs), it is not possible to determine the viability of norovirus by reverse transcription-polymerase chain reaction (RT-PCR) or real-time quantitative RT-PCR (qRT-PCR).

Purpose: The aims of this study were to examine the viability of human norovirus using PMA-combined qRT-PCR or EMA-combined qRT-PCR.

Methods: Human norovirus (hNoV) GII.4 stool was confirmed by qRT-PCR and sequence analysis. This virus stock was aliquoted into 500 µl with 10^6 copy number/µl concentration. Each virus was heat-treated at room temperature, 70°C, 75°C, 80°C, 85°C, and 90°C in water bath for 90 s. Each sample mixed with 250 µM PMA or 25 µM EMA was stored in a dark room for 10 min. Then, the PMA- or EMA-treated samples were exposed to 40 W LED light at 460 nm wavelength for 15 min. The negative control was not treated with PMA or EMA as well as LED light. hNoV RNA was measured using qRT-PCR, PMA-combined qRT-PCR, and EMA-combined qRT-PCR.

Results: Although the relative quantification of hNoV treated at 70°C, 75°C, 80°C, 85°C, and 90°C showed 0.87, 1.22, 1.64, 1.85, and 2.02 log reduction, qRT-PCR could detect all heat-treated hNoV without the treatment of EMA or PMA. In PMA-combined qRT-PCR, the hNoV treated at 70°C, 75°C, and 80°C reduced 0.86, 1.44, and 2.51 log, respectively. In EMA-combined qRT-PCR, virus aliquot treated at 70°C, 75°C, and 80°C decreased by 1.00, 1.38, and 2.33 log, respectively. Interestingly, hNoV RNA treated at 85°C and 90°C was not detected by both PMA- combined qRT-PCR and EMA-combined qRT-PCR.

Significance: PMA- or EMA-combined qRT-PCR could be useful molecular technique for determining the infectious and non-infectious human norovirus.

P2-16 Validation of a Real-time PCR Method for Detection of *Listeria monocytogenes* Compared to Health Canada Method MFHPB-30

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Introduction: *Listeria monocytogenes* is an important foodborne pathogen that has caused many outbreaks and recalls. It is an extremely important concern in food safety and public health because of its high mortality rate, especially in pregnant women, newborns, the elderly and immunocompromised individuals, with 90% of all reported cases of Listeriosis resulting in hospitalization.

Purpose: The iQ-Check real-time PCR method for detection of *L. monocytogenes* after a 24-h enrichment was evaluated compared to the MFHPB-30 Health Canada reference method. The method utilizes a double stranded DNA hybridization probe for increased sensitivity and specificity.

Methods: Five food categories were selected for evaluation: fish and seafood, dairy products, Ready-to-Eat meat and poultry, raw poultry and fruits and vegetables. A total of 1,350 samples were analyzed. A 25-g sample size was tested. In addition, a 125-g composite sample was also tested for one food type from each category. Limit of detection was evaluated for one food item per category. Probability of detection was used to evaluate significant difference between the test and reference method.

Results: The iQ-Check method yielded results of 100% relative sensitivity, 98.8% relative specificity and 99.6% efficacy across all samples tested. Limit of detection ranged from 0.294 - 3.188 MPN/25 g for all foods tested. Probability of detection showed no difference between the iQ-Check and reference method. The results support Health Canada approval of the method.

Significance: Reference methods for detection of *L. monocytogenes* take days to obtain results. The iQ-Check method combines a nutritive 24-h enrichment with specific DNA probe technology for rapid detection of *L. monocytogenes* in 24 h. The method presented greatly shortens the time to an *L. monocytogenes* result so faster food safety decisions can be made.

P2-17 Loop-mediated Isothermal Amplification for the Rapid Detection of Genogroup I and Genogroup II Noroviruses

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Introduction: Human norovirus (NoV) was identified as one of the major foodborne viruses causing acute viral gastroenteritis. Highly specific and sensitive LAMP technique for NoVs should be developed because RT-PCR or real-time RT-PCR performed in lab is limited to apply for on-site detection.

Purpose: This study aimed to develop the rapid and reliable LAMP for detecting NoV genogroup I (GI) and genogroup II (GII).

Methods: Twenty set of LAMP primer for NoV GI and 24 set for NoV GII were designed in highly conserved region that obtained from aligned sequence of each 30 NoV GI and GII NoVs strains. The sensitivity and sensitivity of LAMP primers for NoV GI and NoV GII were compared with semi-nested RT-PCR and real-time RT-PCR reported in previous study.

Results: For NoV GI, both LAMP and real-time RT-PCR could detect as low as 1000 copy of NoV GI RNA. The sensitivity of semi-nested RT-PCR for NoV GI was 10 times higher than those of LAMP and real-time RT-PCR. For NoV GII, semi-nested RT-PCR, real-time RT-PCR, and LAMP could detect as low as 100 copy of NoV GII RNA. In specificity test, LAMP detected only NoV GI and GII but not hepatitis A virus, Hepatitis E virus, rotavirus, adenovirus.

Significance: As one-step LAMP in this study detected NoV GI and NoV GII reliably, this technique could be used in field-testing.

P2-18 An Improved Method to Simultaneously Detect *Salmonella* Enteritidis, *Escherichia coli* O157 and *Listeria monocytogenes* in Ground Black Pepper Using Multiplex Real-time PCR

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Introduction: The three common foodborne pathogens implicated in foodborne outbreaks are *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes.* Hence, it is important to identify these pathogens in contaminated foods so that they can be eliminated from the marketplace. At present, there is no good method available for the simultaneous detection of these organisms in foods regulated by FDA. In general, detecting pathogens in spices poses challenges due to the anti-microbial activity of these foods.

Purpose: To optimize, and adapt a multiplex real-time PCR method, originally developed for meat samples at the USDA, to the simultaneous detection of *Salmonella* spp., *E. coli* O157 and *L. monocytogenes* in ground black pepper.

Methods: Ground black pepper was spiked with 5-5000 CFU/2 g of *Salmonella* Enteritidis, *E. coli* O157:H7 and *L. monocytogenes*, stomached and incubated for 2 hours at 37°C in BLEB broth, followed by the addition of four antibiotics and grown overnight. The enriched culture was subjected to the extraction of genomic DNA (Qiagen) to carry out the real-time PCR with primers and TaqMan probes specifically targeting *invA* (*Salmonella*), *rfbE* (*E. coli* O157), *hlyA* (*L. monocytogenes),* and an internal amplification control.

Results: All three gene targets of the tested pathogens were detected in the spiked ground black pepper with a sensitivity of 15-25 CFU/2 g in the presence of 2% corn oil. In the absence of corn oil, the sensitivity of detection was 15-25 CFU/2 g for *Salmonella* spp., and *E. coli* O157:H7 whereas it was ~900 CFU/2 g for *Listeria monocytogenes*.

Significance: This method can be applied to the effective detection of *Salmonella* Enteritidis, *E. coli* O157 and *Listeria monocytogenes* in the outbreaks associated with ground black pepper or other foods. Therefore, it would allow FDA to take rapid action and prevent the spread of the outbreak.

P2-19 Detection of Shiga Toxin-producing *Escherichia coli*, Seven *stx* Subtypes and *Salmonella* via a Two-tiered Multiplex Real-time PCR

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❖ ***Developing Scientist Competitor***

Introduction: Shiga Toxin-producing *Escherichia coli* (STEC) are strains producing Shiga toxin type 1, type 2 or both, leading to hemorrhagic colitis and hemolytic uremic syndromes. *E. coli* O157:H7 and non-O157 serotypes O26, O45, O103, O111, O121 and O145 are currently considered as adulterants in non-intact beef. Further, *Salmonella* leads to the second highest number of foodborne infections in the United States. These two pathogens continue to be a major food safety challenge worldwide.

Purpose: The aim of this study was to design a two-tiered multiplex real-time PCR assay for the detection of seven STEC serotypes, stx_1, stx_2 genes and virulent strains of *Salmonella*.

Methods: PCR primers were designed for the specific amplification of each target. Two multiplex real-time PCR melt curve assays with an internal amplification control (IAC) were standardized for the detection of seven STEC serotypes and *Salmonella*. The applicability of the assays was tested using ground beef and beef trims.

Results: The first multiplex assay detected *E. coli* O121, *E. coli* O145, *E. coli* O157, stx_1, stx_2 and IAC; while the second set targeted *E. coli* O26, *E. coli* O111, *E. coli* O103, *E. coli* O45, *Salmonella*, and IAC. Following an enrichment period of 8 h, all targets of the multiplex assays could be detected in 325 g of food samples inoculated with 10-20 CFU of each pathogen. The assay showed a reproducible result for beef products with different fat content.

Significance: These assays, which do not rely on the use of fluorescent-labeled probes or immunomagnetic beads, can detect seven STEC serotypes, seven of the most common *stx* gene subtypes and *Salmonella*, and can be completed in less than 11 h, making them highly suitable for industrial application.

P2-20 Evaluation of FDA Developed Real-time Quantitative PCR (qPCR) Using Two Different PCR Master Mixes for the Detection of *Salmonella* in Leafy Greens Using Five Different Pre-enrichment Media

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Introduction: *Salmonella* outbreaks traced to leafy greens show the need for rapid and reliable detection methods for fresh produce. The *Bacteriological Analytical Manual* (BAM) culture method for *Salmonella* uses lactose broth (LB) for pre-enrichment, which has not reliably supported qPCR assays as shown in our former studies.

Purpose: The performances of FDA qPCR assay to detect *Salmonella* in lettuce, parsley, cabbage, and spinach was evaluated using two different PCR master mixes (VeriQuest™ and illustra™ PCR beads) and ABI 7500Fast real-time PCR detection system in LB and four other pre-enrichment media: buffered peptone water (BPW), modified BPW (mBPW), Universal Pre-enrichment broth (UPB), and BAX®MP media.

Methods: Produce, equivalent to at least 100 test portions, was inoculated with a single *Salmonella* serovar and stored at 2 - 8°C for 3 d prior to analysis. On the day of analysis, twenty test portions (25 g) from the bulk inoculated produce were prepared for each of the five pre-enrichment media, and then 225 ml pre-enrichment media was added to respective test portions. The BAM culture method was followed thereafter. qPCR was performed from 24 h pre-enriched cultures.

Results: No significant differences ($P > 0.05$) were found among the five media for leafy greens by culture results, but LB was the least effective broth. qPCR using both PCR master mixes produced significantly ($P < 0.05$) higher false negatives in 24 h pre-enriched LB than the other four media. The VeriQuest mix provided more sensitivity than PCR beads and culture methods in all five media.

Significance: This study addressed a need to improve current BAM *Salmonella* culture method for the detection of *Salmonella* from leafy greens when using real-time PCR as screening method.

P2-21 Murine Norovirus Detection in Fresh and Frozen Raspberries and Fresh Strawberries by Real-time Reverse Transcription-PCR (rtRT-PCR)

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Introduction: Human noroviruses are a human health concern globally, being responsible for a majority of foodborne illness outbreaks. Strawberries and raspberries have been implicated as vehicles of human norovirus-related gastroenteritis outbreaks in the United States and Europe. The challenge remains in detecting the low number of viral particles in berries that can cause outbreaks.

Purpose: The aim of this work was to determine the suitability of using murine norovirus (MNV-1) as an extraction process control for the improved molecular detection of enteric viruses in fresh strawberries and raspberries and frozen raspberries by real-time RT-PCR.

Methods: Fresh strawberries and raspberries or frozen raspberries from local grocery stores were washed with 10% trisodium phosphate, allowed to air-dry under ultraviolet light and aseptically surface-spiked with 5 log PFU/ml MNV-1 (as an extraction process control) or with phosphate buffered saline (PBS, negative control). Viruses were first eluted with PBS containing pectinase, followed with TRIzol™ for RNA extraction, and passed through a QIAshredder. Ten-fold diluted RNA extracts from MNV-1 stock, and spiked and control berries were used for detection by SYBR green I real-time RT-PCR. Agarose gel electrophoresis and Tm analysis were used to confirm product size. All experiments were replicated thrice.

Results: TRIzol RNA extraction followed by RT-PCR could detect MNV-1 up to -7 log diluted RNA (corresponding to <1 PFU/ml), indicating 7 log RT-PCR units in the stock. Fresh strawberries and raspberries spiked with MNV-1 showed detection to -6 log diluted RNA extracts (corresponding detection up to 1 log RT-PCR units) by both RT-PCR and gel electrophoresis. Frozen raspberries showed detection to -8 log dilution (corresponding to < 0.1 PFU/ml or -1 log RT-PCR units) by both RT-PCR and gel electrophoresis.

Significance: The simple pectinase-TRIzol extraction method showed that MNV-1 was a suitable extraction process control to rapidly detect human enteric viruses from berries by RT-PCR to prevent berry-related outbreaks worldwide.

P2-22 Accelerated Detection of *Listeria* and *Salmonella* in Environmental and Food Samples Using PCR Technology

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Introduction: Testing for *Listeria* and *Salmonella* in foods is an essential aspect of any quality control and food safety program to minimize the risk of infection for the consumer. The availability of reliable, accurate and rapid methods to detect these pathogens is increasingly important for the food industry.

Purpose: The aim was to develop and to validate a rapid screening method that significantly minimizes the complexity and length of enrichment for PCR-based detection of *Listeria* and *Salmonella* in environmental and food samples.

Methods: Actero™ *Listeria* Enrichment Media and Actero™ *Salmonella* Enrichment Media, optimized for rapid recovery of *Listeria* and *Salmonella*, were used for enriching these pathogens from environmental and food samples. Enriched samples were tested using BAX® System real-time PCR assays. For *Listeria*, seven internal studies and one external validation study were conducted that included 240 environmental (stainless steel, plastic and sealed concrete) and 240 food (hot dog, bagged fresh spinach, frozen cooked shrimp and soft Mexican-style cheese) samples. For *Salmonella*, five internal and two external validation studies were completed and included 240 environmental (stainless steel and plastic) and 240 food (dry pet food, chocolate liquor and milk chocolate) samples. The alternative detection methods were compared to the appropriate reference methods. The results were evaluated using the AOAC Probability of Detection statistical model.

Results: No false negative or false positive results were observed after enriching at 35°C for 20 - 26 hours for *Listeria* spp. and 14 - 22 hours for *Salmonella* spp. The detection of *Listeria* and *Salmonella* in environmental and food samples enriched with the proposed media using the real-time PCR assays showed equal or better performance as compared to the gold standard methods.

Significance: These alternative methods were determined to be reliable, accurate, and faster than other methods for detection of *Listeria* and *Salmonella.*

P2-23 Real-time PCR for *Vibrio anguillarum* Detection in Maintaining Seawater Quality of Aquaculture

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Introduction: *Vibrio anguillarum* is a well-known opportunistic bacterial pathogen that causes vibriosis in a range of marine organisms around the world. Vibriosis infection of aquaculture or wild fish has been reported as a detrimental factor of fishery yields and financial stability to the industry. As such, measures of *V. anguillarum*-control via vaccination, phage therapy, and chemical sterilization of aquaculture waters are exhibited to maintain healthy seafood yields and commercial sales. Alongside growing consumer awareness of the ethics involved in mass food production and related practices are an increased demand for natural, organic methods of agricultural commerce.

Purpose: In order to contribute to this growing industrial trend, the present study served to develop a rapid method of pathogenic detection that will allow technicians the opportunity to better evaluate the quality of aquaculture waters based on virulent *V. anguillarum* levels therein.

Methods: Four conserved virulence genes in *V. anguillarum* were screened for amplification efficiency via PCR in real-time and conditions such as cycle repetition, reagent concentration, reaction duration, and temperature of amplification were optimized.

Results: Conventional methods of pathogenic detection can take several hours, require additional enrichment strategies for low bacterial levels, and do not produce quantifiable data. By simultaneously targeting three known virulence genes in the *V. anguillarum* genome, the real-time PCR set forth in this study allows for the quantification of pathogenic-specific *V. anguillarum* populations in less than 70 minutes and is able to detect as little as 3 CFU ml^{-1} *V. anguillarum* in seawater; without the need for additional enrichment or electrophoresis steps for data analysis.

Significance: This protocol can be used to enhance standard food safety operations in the aquaculture industry by offering technicians quantifiable proof that some aquaculture tanks can be exempt from vaccination procedures that will promote natural cultivation of fish for consumer safety and satisfaction.

P2-24 GENE-UP® PCR Method for the Detection of *Listeria* Species in Food and Environmental Samples

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Introduction: Detection of *Listeria* spp. can take at least five days using traditional method. Alternative rapid screening methods have been developed to obtain quicker results for food and environmental samples. bioMérieux has developed a universal PCR method on the GENE-UP® thermocycler for the detection of foodborne pathogen, including *Listeria* spp.

Purpose: The study compares this alternative method to the ISO 11290-1/A1:2004 Reference method for the detection of *Listeria* spp. in food and environmental samples.

Methods: A unique protocol has been developed for next day detection. For each of five food categories, 60 samples of 25 g were tested by both methods. For larger sample size (125 g, Mexican Soft Cheese), 30 samples were tested. This new method consists in a single enrichment in LPT broth incubated 22 h at 37°C. Then, DNA is extracted using mechanical lysis in a dedicated lysis tube: 15µl are introduced through a specifically designed cap that does not require any tube or cap handling. Extracted DNA is used directly with freeze dried PCR reagents. The PCR method is based on dual probe detection (Fluorescence Resonance Energy Transfer) allowing real time detection and melting curve analysis. The call is positive when it combines an amplification curve and a melting peak allowing a strong specificity of the test. Co-detection of *Listeria monocytogenes* is possible using same extracted DNA with GENE-UP® *Listeria monocytogenes* PCR kit.

Results: Methods were compared following ISO16140 guidelines. The comparison did not show any statistical difference on the 90 samples tested.

Significance: GENE-UP® *Listeria* spp. method enables a reliable and rapid detection of *Listeria* spp. in food and environmental samples including a user-friendly workflow.

P2-25 GENE-UP® ECO PCR Method for the Detection of *E. coli* O157:H7 in Raw Meat and Produce

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Introduction: *E. coli* O157:H7 is the most commonly *E. coli* strain associated with foodborne illness in beef and produce. Early detection of *E. coli* O157:H7 is essential in such foods with limited shelf life, this is why alternative rapid methods have been developed. Most PCR methods target *E. coli* O157 without any discrimination between non-pathogenic O157 and pathogenic O157:H7 strains. bioMérieux has developed GENE-UP® ECO for the specific detection of *E. coli* O157:H7.

Purpose: The study compares this alternative method to the ISO 16654:2001 Reference method for the detection of *E. coli* O157:H7 in raw meat and produce.

Methods: Ninety raw meat samples of 25 g and 375 g and 30 portions of 200 g produce were tested by both methods. The new method consists in a single BPW enrichment incubated at 42°C during 8 to 24 h depending on the food type and sample size. Then, DNA is extracted using mechanical lysis in a dedicated lysis tube: 15 µl are introduced through a cap that does not require any tube or cap handling. Extracted DNA is used directly with freeze dried PCR reagents. The PCR method is based on dual probe detection (Fluorescence Resonance Energy Transfer) allowing real time detection and melting curve analysis. The call is positive when it combines an amplification curve and a melting peak corresponding to the temperature defined exclusively for *E. coli* O157:H7, allowing a strong specificity of the test.

Results: Methods were compared following ISO16140 guidelines. The comparison did not show any statistical difference on the 120 samples tested.

Significance: Rapid (8 to 24 h enrichment) and specific detection of *E. coli* O157:H7 is made possible with GENE-UP® ECO in raw meat and produce for both 25 g and 375 g samples including a user-friendly workflow.

P2-26 GENE-UP® PCR Method for the Detection of *Salmonella* Species in Food and Environmental Samples

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Introduction: Detection of *Salmonella* spp. can take at least three days using traditional method. Alternative rapid screening methods have been developed to obtain quicker results for food and environmental samples. bioMérieux has developed a universal PCR method on the GENE-UP® thermocycler for the detection of foodborne pathogens, including *Salmonella* spp.

Purpose: The study compares this alternative method to the ISO 6579 Reference method for the detection of *Salmonella* spp. in food and environmental samples.

Methods: Two protocols have been developed for same day and next day detection. For each of five food categories, 60 samples of 25 g were tested by both methods. For larger sample size (raw meat, produce), 60 samples were also tested by both methods. This new method consists of a single enrichment in BPW incubated 8 to 24 h (depending on food) at 42°C. Then, DNA is extracted using mechanical lysis in a dedicated lysis tube: 15 µl are introduced through a cap that does not require any tube or cap handling. Extracted DNA is used directly with freeze dried PCR reagents. The PCR method is based on dual probe detection (Fluorescence Resonance Energy Transfer) allowing real time detection and melting curve analysis. The call is positive when it combines an amplification curve and a melting peak allowing a strong specificity of the test.

Results: Methods were compared following ISO16140 guidelines. The comparison did not show any statistical difference on the 360 samples tested.

Significance: GENE-UP® *Salmonella* method enables a reliable and rapid detection of *Salmonella* spp. in food and environmental samples: 8 to 24 h enrichment depending of food category and size, including a user-friendly workflow.

P2-27 Rapid qPCR Detection and Cultural Isolation of *Yersinia pestis* in Artificially Contaminated Meat Products

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Introduction: Rapid detection of high priority agents such as *Yersinia pestis* from difficult food matrices is essential in preventing foodborne outbreak of disease. Meat products, especially, can be a challenging matrix for sensitive detection and isolation of pathogens at low levels.

Purpose: The objective of the present study was to validate a new multiplex qPCR screening assay for *Y. pestis* in meat products compared to cultural isolation procedures.

Methods: Hot dog and meat-based baby food were artificially contaminated with *Y. pestis* at un-inoculated, low (~1 CFU/g) and high (~10 CFU/g) levels (n = 6/level). Ground beef patties were inoculated at 10-fold greater concentrations based on earlier experimental data. After 24 h enrichment, samples were screened by two different qPCR assays and plated for cultural isolation and recovery. Four additional food matrices (ground beef, ground turkey, raw chicken, and deli meats, 20 sources each) were surveyed by qPCR and culture at one inoculum level (10–400 CFU/g).

Results: Fractional qPCR detection and cultural recovery rates were observed at the low level inoculum for hot dog and baby food (4/6 and 2/6, respectively). Screening of beef patties by qPCR at low levels was positive in all samples and 4/6 by cultural methods. All samples were positive by all methods at the higher level. There was no difference ($P < 0.05$) between detection rates using either qPCR method or culture. However, in the survey portion of the study, average qPCR detection rates of *Y. pestis* were 80%, 50%, 33% and 100% from different sources of beef, turkey, chicken and deli meat, respectively, while cultural recovery rates were 0%, 0%, 5% and 55%, respectively.

Significance: Rapid screening of food enrichments by qPCR can provide vital information on potential presence of *Y. pestis*, especially if background microflora adversely affects cultural isolation and recovery.

P2-28 Preliminary Identification of *Salmonella* Serovars by Multiplex High-resolution Melting (HRM) Analysis

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Introduction: Identification of *Salmonella* serovars in food and environmental samples requires expensive and/or lengthy methods. There is a need for rapid and cost effective methods for comparing *Salmonella* isolates at the subspecies level.

Purpose: To evaluate multiplex high-resolution melting analysis assays as a simple method for preliminary identification of *Salmonella* serovars.

Methods: *Salmonella* cultures (95 unique strains; 62 unique serovars) were grown in tryptic soy broth (37°C, 24 h) prior to DNA extraction (wash, spin, boil method). Two multiplex HRM assays (HRM-STM, HRM-STY) were developed targeting loci previously used for differentiating *Salmonella* serovars. Together, the two HRM assays target ten unique sequences present in some combination in many *Salmonella* spp. The HRM assays were prepared using MeltDoctor HRM Master Mix, 200 nm of each primer, and 2 µl of template DNA. PCR was performed on the ABI7500 Fast using the SDS software (v1.4) with an initial denaturation of 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. Melt curve data was collected using standard dissociation stage settings and analyzed using HRM software (v2.0).

Results: The HRM-STM assay effectively grouped the *Salmonella* strains into 12 broad groups and easily differentiated Typhimurium, Enteritidis, and Montevideo (among others). Typhimurium and Saintpaul could be differentiated when HRM-STM and HRM-STY were compared in combination. Similarly, Enteritidis could be differentiated from Hadar, Cubana, Mbdanka, and Thompson using both assays. Enteritidis/Dublin and Kentucky/Thompson strains could not be differentiated with the combination assays. Strains representing Montevideo, Tennessee, Senftenberg, and Kentucky serovars showed large variability in HRM results.

Significance: Multiplex high-resolution melting analysis is useful for preliminary identification of *Salmonella* serovars. The assay(s) may provide a greater service as a screening tool to differentiate unique isolates from environmental or food sources prior to more expensive and time-consuming methods (e.g., serotyping/fingerprinting).

P2-29 Inoculation and Recovery Methods for Internal and External Inoculation of Sliced Beef Sirloin with *Salmonella* Species

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Introduction: USDA process validation requires demonstration of a 5-log reduction in *Salmonella* spp. for dehydrated beef products. Simple and consistent inoculation and recovery procedures are necessary to ensure accurate process validation.

Purpose: Evaluate methods for external and internal inoculation of *Salmonella* spp. in sliced whole-muscle beef for reproducibility and subsequent recovery.

Methods: Unique *Salmonella* serovars (n = 11) were lawn harvested from tryptic soy agar (10 log CFU/ml) and equally mixed prior to inoculation onto thawed sliced beef sirloin (25.1 cm x 14.2 cm x 0.5 cm). Three inoculation methods were evaluated: spread (external), dip-puncture (internal), and spread-puncture (internal). For Spread inoculations, 100 - 600 µl of inoculum was applied to each side of beef. For the spread-puncture, plastic separated beef from inoculum prior to puncturing. Recovery was performed at three time intervals: immediately after inoculation, after drying for 30 min per side (22°C biological safety cabinet), and after storage at 4°C for 18 h. Beef samples were aseptically cut (2 cm squares) or remained whole and stomached with 0.1% peptone water (1:1). Dilutions were plated on selective agar (HE/XLD), incubated at 37°C for 24 h, and enumerated.

Results: Immediate recovery after inoculations produced highest recovery for external inoculation (6.92 ± 0.27 log CFU/g), followed by spread-puncture (6.66 ± 0.17), and dip-puncture (6.04 ± 0.12). *Salmonella* levels were lower after drying: 6.54 ± 0.11, 4.95 ± 0.34, and 5.52 ± 0.53 log CFU/g (identical order). Extended storage at 4°C resulted in higher levels of *Salmonella:* 7.14 ± 0.70, 6.88 ± 0.15, and 5.65 ± 0.10 log CFU/g. Chopping did not affect recovery for methods tested.

Significance: Dip-puncture is a simple and effective method to internally inoculate whole muscle beef strips for process validation studies. Time interval between inoculation and recovery is critical; extended storage had a positive impact on recovery and reduced variability.

P2-30 Evaluation of a New Selective Enrichment Protocol to Improve the Detection of Shiga Toxin-Producing *E. coli* in Dairy Products

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Introduction: STEC has emerged as important public health threats. Most human infections caused by STEC have been acquired by the consumption of contaminated foods especially those of bovine origin. According the ISO/TS 13136 standardized method for the detection of STEC, dairy testing requires the addition of acriflavin in the enrichment broth in order to decrease the high level of competitive flora. However, the subsequent PCR assay which should reveal the presence of STEC can be affected by the fluorescence of acriflavin, leading to non-interpretable results. In this context, a new selective enrichment protocol using Buffered Peptone Water supplemented with the STEC supplement (BPW+STEC sup.) has been developed.

Purpose: The objective of this study was to compare the ISO/TS 13136 reference method for the detection of STEC in dairy products to the new method combining the enrichment in BPW+STEC supplement and a final detection with the iQ-Check VirX PCR kit. The STEC supplement consists in a mixture of 3 selective agents inhibiting the background flora, thus enhancing the growth of STEC strains.

Methods: Twenty-five g samples of raw milk and raw milk cheese were spiked with a STEC strain at a concentration below 10 CFU/sample. They were then enriched according to the ISO/TS 13136 reference method and in BPW+STEC sup. (dilution ratio 1:9). After 16 - 20 hours of incubation, the culture obtained was analyzed either with the ISO/TS 13136 reference PCR assay or with the iQ-Check VirX PCR kit.

Results: All spiked samples were detected with the new protocol. The reference method failed to detect all spiked samples. This new method showed also lower Cqs in the final PCR tests due to higher selectivity during the bacterial growth.

Significance: Results demonstrated that the selective protocol with Buffered Peptone Water + STEC supplement is an effective and convenient method for the enrichment of STEC in dairy products.

P2-31 Evaluation of the 3M™ Molecular Detection Assay (MDA) *Listeria* (LS) and *Listeria monocytogenes* (LM) for the Detection of *Listeria* in a Variety of Foods: Collaborative Study

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Introduction: The 3M Molecular Detection Assay (MDA) *Listeria* (LS) and *Listeria monocytogenes* (LM) methods use loop-mediated isothermal amplification of nucleic acid sequences to detect *Listeria* in enriched food and environmental samples. The isothermal amplification is a polymerase chain reaction conducted at a constant temperature, eliminating the need for temperature cycling and decreasing the time-to-results. The new methods employ unique DNA target sequences with high specificity and efficiency to detect target pathogens in 24 - 30 hours of enrichment.

Purpose: The purpose of this AOAC®OMA℠ Collaborative Study was to compare the new methods to the USDA-FSIS/MLG for deli turkey (125 g) (MDA LM only) and the AOAC OMA 993.12 for full fat cottage cheese (25 g) (MDA LS and MDA LM).

Methods: The two rapid methods were compared in a multi-laboratory collaborative study to the USDA-FSIS MLG Chapter 8.09 and to the AOAC OMA 993.12 reference methods. A total of 17 laboratories, representing government and industry, throughout the United States and Canada participated. Each laboratory evaluated 12 replicates at 3 levels of inoculation; an un-inoculated control level (0 CFU/test portion), a low inoculum level (0.2 - 2 CFU/test portion) and a high inoculum level (2 - 5 CFU/test portion). All test portions were confirmed using the appropriate reference method.

Results: Statistical analysis was conducted according to the Probability of Detection (POD) statistical model. No statistically significant difference was observed between the new and reference methods. The new *Listeria* method correctly identified whether a test portion was positive or negative more than 99.2% of the time. The new *L. monocytogenes* method correctly identified whether a test portion was positive or negative more than 98.1% of the time.

Significance: The collaborative study demonstrated the reliability and sensitivity of these new methods for the rapid detection of *Listeria* in food products using both 125 g and 25 g sample test portion sizes.

P2-32 Development of Novel Patulin Aptamer Using GO-SELEX and Detection of Patulin in Apple Juices

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Introduction: Patulin (PAT) is one of the mycotoxins produced by some fungi, such as *Penicillium*, *Aspergillus* and *Byssochlamys.* PAT is known as carcinogenic, mutagenic, and teratogenic and mainly found in rotten apples and apple juices. In order to prevent exposure to the PAT, many countries have set regulation limits on PAT. However, due to its simple structures, there is no commercialized bio-receptor against PAT.

Purpose: We developed novel PAT binding aptamers and applied the aptamers to detect the PAT in apple juices by lateral flow assay (LFA) and localized surface plasmonic assay (LSPR).

Methods: Aptamers specific to PAT were developed through a systematic evolution of ligands by exponential enrichment (SELEX) based on graphene-oxide (GO-SELEX) using 30, 40, 50, and 60 mer single strand DNAs (ssDNAs) library. Unbound ssDNAs are absorbed on the GO, but ssDNAs bound with PAT are not. In addition, we used the aptamers to develop a lateral flow assay (LFA) and localized surface plasmonic assay (LSPR) for the detection of the PAT in apple juices.

Results: Eight aptamers (Papta No. 1 -8) specific to PAT were successfully developed by GO-SELEX. Among the 8 aptamers, Papta No. 6 showed the highest affinity to PAT. Thus, we used the aptamer to develop and optimize LFA and LSPR assays. The detection limits of both methods are all 5 μg/ml in buffer and apple juice.

Significance: Recently, since aptamers have been considered as a good candidate to replace antibodies which are used in other immunoassays, the aptamer-based dipstick assay developed in this study is superior to other immunoassays with respect to its setting speed and stability. In this study, we firstly reported PAT binding aptamers and aptamer-assays. The results provide great opportunities to apply the aptamer to the development of aptamer assays for PAT analysis.

P2-33 Evaluation of a Rapid Isothermal Amplification Method for the Direct Detection of Human Norovirus in Complex Samples

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Introduction: Rapid, in-field or point-of-care testing for human noroviruses (HuNoV) is not very feasible using reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) due to reliance on thermocycling. Recombinase polymerase amplification (RPA), a novel isothermal method that is potentially less sensitive to inhibitors, utilizes multiple DNA repair enzymes to anneal primers and rapidly amplify target sequences.

Purpose: To evaluate the performance of a reverse transcription (RT)-qRPA assay for rapid detection of HuNoV in multiple samples.

Methods: A previously developed primer-probe set (G2F5/G2R11/G2P1) used in RT-qRPA (TwistDx, Cambridge, UK) developed for detection of HuNoV GII.4 was applied to multiple serially diluted, heated stool samples – or purified RNA thereof – derived from foodborne outbreaks.

Results: The G2F5/G2R11/G2P1 primer-probe set was successfully used to detect HuNoV GII.4 New Orleans by RT-qRPA in all six patient stool samples (2 - 20% suspended in PBS) and RNA extracted from these samples. The average rates of detection [time in min of signal development per log genomic copy (LGC) of template in the reaction] for RNA and stool samples were 1.44 ± 0.29 min/LGC and 1.45 ± 0.38 min/LGC, respectively. There was no significant difference in rate of signal development when comparing RT-qRPA done using heated stool suspension dilutions with purified RNA ($P > 0.05$). Direct detection of HuNoV in dilutions of all heated HuNoV stools was achieved between 7.2 and 16.7 min. The limit of detection (LOD) of the assay was 5.5 ± 1.6 LGC for stool and 4.7 ± 0.5 LGC for purified RNA.

Significance: HuNoV could be detected using the RT-qRPA method in 20% fecal suspensions of the majority of patients and 2% suspensions of all of the patients screened – both being relatively dirty matrices – in less than 20 min with reasonable detection limits. This rapid, portable, isothermal method has potential for use in direct detection of HuNoV in food, food handler and environmental samples.

P2-34 Viability rt-PCR: A tool for Discrimination of Viable *Listeria* and Non-viable *Listeria* Phage DNA in Cheese Culture

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Introduction: Viability rt-PCR (BLU-V™, QIAGEN) uses the DNA masking compound propidium monoazide (PMA), to discriminate between DNA from living and dead cells. PMA renders the dead cell DNA undetectable by rt-PCR. *Listeria* phage is used in cheese manufacture to inhibit the growth of *Listeria.* However, *Listeria* phages may be contaminated with non-viable *Listeria* DNA, with the possible detection of contaminating rt-PCR signals..

Purpose: To demonstrate the use of viability rt-PCR to discriminate between living pathogenic *Listeria* and non-viable *Listeria* phage/ Listeria DNA in a cheese production setting.

Methods: Mozzarella cheese was obtained from a cheese manufacturer. To test for effect of PMA treatment on the detection of *Listeria* spp. in cheese treated with *Listeria* phage, cultures were grown in Listeria ONE Broth, 26 h at 30°C. QIAGEN QIAsymphony workflows were applied. Two studies were performed: One assessed the detection of *Listeria* spp. on uninoculated cheese cultures with and without PMA treatment. The second was performed with low level *L. monocytogenes* inoculation, with and without PMA treatment.

Results: In the uninoculated study, *Listeria* spp. was detected in 4 cultures. PMA treatment completely masked the *Listeria* spp. signal. This suggests that there was dead *Listeria* DNA present in these cheese cultures, most likely derived from the *Listeria* phage solution applied to the cheese during manufacture. In the spiking study, *Listeria* spp. was detected in 19 culture replicates. PMA treatment completely masked the rt-PCR signal in 6 of these and resulted in significant Ct shifts toward higher values in the other 12. PMA (viability rt-PCR) masks the signal from the *Listeria* phage DNA but does not decrease the detection rate of living *Listeria monocytogenes* cells.

Significance: Viability rt-PCR can be used to increase the power of rt-PCR in live-dead differentiation of pathogens in a number of settings of interest to the food industry.

Poster

P2-35 Development of Polymerase Chain Reaction for Rapid Identification of *Paenibacillus* and *Cohnella* with High Peracetic Acid Tolerance

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Introduction: Peracetic acid is one of the bactericides used for sterilization of container in soft drink manufacturing process. Certain species of genera *Paenibacillus* and *Cohnella* were reported to have peracetic acid tolerance. Therefore it's important to detect these species rapidly in manufacturing environment for quality control of soft drink.

Purpose: The purpose of this study was to develop a PCR assay for rapid identification of species of genera *Paenibacillus* and *Cohnella* resistant to peracetic acid.

Methods: A primer set specific to genera *Paenibacillus* and *Cohnella* and a primer set specific to *P. favisporus, P. cineris,* and *P. chibensis* with high peracetic acid tolerance were designated on the basis of 16S rDNA sequences and literatures. To test the specificity of the primers, 26 *Paenibacillus* species, 6 *Cohnella* species, 13 *Bacillus* species and 4 *Brevibacillus* species were used.

Results: DNAs from 49 strains of spore-forming bacteria were analyzed by the developed PCR method. PCR products were generated from templates of 26 *Paenibacillus* and 6 *Cohnella* species by the PCR using the primer set specific to genera *Paenibacillus* and *Cohnella.* The template DNAs only from *P. favisporus, P. cineris,* and *P. chibensis* generated PCR products by the PCR using the primer set specific to these high peracetic acid tolerant species. Ten strains isolated from manufacturing environment, 3 *Paenibacillus* species including 2 *P. favisporus* and 7 other genera, were correctly identified by the present PCR methods.

Significance: The developed PCR methods are specific to detect peracetic acid tolerant genera and the species with peracetic acid tolerance and easy to use at the manufacturing environment on site in the beverage manufacturing.

P2-36 Evaluation of 3M™ Molecular Detection Assay and a Variety of Pre-enrichment Media for the Recovery of *Salmonella* from Serrano Peppers

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Introduction: Serrano peppers have been linked to at least one documented outbreak of *Salmonella* in the US. Fast and accurate results on pathogen determination are key in food safety management. Reactions in DNA-based detection systems can potentially be inhibited by compounds typically found in peppers (e.g., capsaicin) or enrichment broth ingredients (peptones and surfactants).

Purpose: To evaluate the performance of the 3M™ Molecular Detection Assay (MDA) and a variety of pre-enrichment media for effective recovery of *Salmonella* from serrano peppers.

Methods: *Salmonella* serotypes *Salmonella* Typhimurium*, Salmonella* Agona, *Salmonella* Poona and *Salmonella* Newport isolated from farm environments were used to artificially inoculate serrano peppers (0.89 - 1.06 CFU/g). Twelve inoculated media-only, non-inoculated peppers or inoculated peppers samples were analyzed by both the MDA and the FDA-BAM method, using in each case 5 different pre-enrichment media (D/E Broth, UPB, Lactose Broth, BPW and BPW-ISO). Sample size for peppers was standardized to 60 g (4 peppers), and combined with 200 ml of broth (FDA BAM protocol). Results for detection methods were compared using McNemar's test for paired data, while media results were compared using the Chi-square test for unpaired data (α=0.05).

Results: A total of 180 samples were analyzed. For inoculated samples, following the MDA and FDA-BAM protocols yielded 114 and 120 positive results, respectively, reflecting no significant difference ($P > 0.05$) between detection methods. When using the recommended media for pre-enrichment (BPW-ISO), no false positive or false negative results were found by MDA, while 10 false negative and 3 false positive results were obtained when the other broths were used. No significant difference ($P > 0.05$) was observed among pre-enrichment media results.

Significance: For detection of *Salmonella* in serrano peppers, the MDA method provides fast and accurate results that are equivalent to those obtained by the FDA-BAM reference method. Using BPW-ISO as pre-enrichment media ensures its best performance.

P2-37 Validation of the Detection Limit of One Colony Forming Unit (CFU) of *Salmonella* per Gram of Wet-pooled Ground Beef and Raw Beef Trim Samples with BioControl Assurance® GDS for *Salmonella*

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Introduction: A critical requirement for beef processors to implement a new 1 CFU/g limit of detection testing approach is the availability of an economical method able to very quickly detect *Salmonella* at this level. This study determines the minimum length of enrichment time required for 375 g raw fresh beef samples with 1 CFU/g *Salmonella* to be detectable with Assurance GDS® for *Salmonella*.

Purpose: To determine the minimum enrichment time required to detect *Salmonella* at inoculation target level of 0.3 to 0.55 CFU/g in post-enrichment, wet pooled samples of ground beef and beef trim with Assurance GDS for *Salmonella* (GDS) which will ensure the desired limit of detection of 1CFU/g of *Salmonella*.

Methods: A five-strain cocktail of *Salmonella* spp. was used for this study. Seventy-four samples of 375 g ground beef were inoculated at 0.55 CFU/g. Seventy-three samples of 75 g beef trim were inoculated at 0.3 CFU/g. Four uninoculated ground beef and four beef trim samples were enriched for wet pool analysis. All ground beef samples were incubated for 5 hours and all beef trim samples were incubated for 4.5 hours. All individual samples were tested individually and as wet pooled samples (1:5).

Results: In summary, a total of 147 inoculated samples were tested. All 147 individual inoculated samples were positive and all uninoculated samples were found negative with GDS. All 147 wet pooled samples, created by combining 4 uninoculated samples with 1 inoculated sample, also were positive by GDS. All individual samples were culturally confirmed.

Significance: This validation study demonstrates that five hours incubation for fresh raw ground beef and 4.5 hours of incubation for fresh raw beef trim, with an average inoculation level of 0.55 and 0.3 CFU/g, respectively, which is less than the *Salmonella* limit of detection of 1 CFU/g met the USDA sensitivity requirements of 97%.

P2-38 Validation Study of a Novel Secondary Screening Assay for the Identification of Individual Top 6 Shiga Toxigenic *E. coli* (STEC) Serogroups in Various Meat Products

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Introduction: Meat products, such as beef trim and ground beef have been identified as a source of past non-O157 Shiga toxigenic *E. coli* (STEC) outbreaks. This study proposed a novel secondary screening method, Assurance GDS®MPX ID for Top STEC, utilizing Immunomagnetic Separation (IMS) and Polymerase Chain Reaction (PCR) to identify the individual top 6 STEC non-O157 serogroups in 14 h or less.

Purpose: To demonstrate the equivalence of Assurance GDS MPX ID for Top STEC to the reference culture method for the identification of the Top 6 non-O157 STEC serogroups in STEC positive meat samples.

Methods: Various meat matrices including beef trim, ground beef and pre-formed beef patties were included in the study. One hundred eighty-five samples, ranging in size from 25 g to 375 g, were inoculated with low levels of each of the Top 6 non-O157 STEC and enriched in mEHEC® for 10 – 18 h at 42°C. After initial analysis with Assurance GDS MPX for Top 7 STEC all positive samples were then analyzed using Assurance GDS MPX ID for Top STEC and the reference culture method, USDA FSIS MLG 5.06. Inclusivity and exclusivity of Assurance GDS was determined by analyzing 128 strains of Top 6 STEC and 69 strains of potential cross-reacting organisms, including non-*E. coli* and *E. coli* non-STEC serogroups.

Results: Assurance GDS identified the following Top STEC serogroups: O103 (10 samples), O121 (41 samples), O145 (25 samples), O26 (9 samples), O111 (34 samples) and O45 (66 samples) from the original 185 samples. There was a 100% agreement between Assurance GDS MPX ID for Top STEC and the USDA MLG reference confirmation method.

Significance: This new secondary screening method provides the industry with a faster option for identifying Top 6 STEC serogroups than culture methods, while retaining the necessary accuracy.

P2-39 Next-day Assay for the Detection of *Salmonella* in Various Foods with Assurance VIP *Salmonella* Gold and Transia Plate *Salmonella* Gold

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Introduction: *Salmonella* is a significant pathogen and the causative factor of many foodborne illnesses and hospitalizations. Fast and accurate methods are needed to detect *Salmonella* in foods before they reach the consumer. BioControl Systems, Inc. has developed a protocol using a single proprietary enrichment media that allows detection of *Salmonella* in 24 h using the Assurance VIP *Salmonella* Gold lateral flow device or the Transia Plate *Salmonella* Gold (TPSG) ELISA.

Purpose: To determine the how well the Assurance *Salmonella* VIP and TPSG can detect *Salmonella* in various foods, including spinach, chicken rinses, dry pet food, ground chicken, beef trim, almonds, ice cream and raw eggs; using a proprietary enrichment media (mEHEC, BioControl Systems) with novobiocin incubated for 20 - 24 h.

Methods: Various 25-g food samples were inoculated with low levels (1 - 5 CFU/sample) of *Salmonella* cultures that had been grown in BHI overnight, then diluted to 100 CFU/ml in BPBD and stored at 2 - 8°C for 24 h prior to sample inoculation. The samples were enriched in 225 ml of the mEHEC + novobiocin for 20 - 24 hours at 42°C. The samples were tested using the Assurance *Salmonella* Gold VIP and Transia Plate *Salmonella* Gold ELISA. The enrichment broths were transferred to RVS and incubated an additional 20 h at 42°C. These samples were streaked onto XLD plates for confirmation.

Results: Positive results were obtained from 81/82 inoculated samples with both VIP and TPSG assays. The one negative sample was also confirmed as negative from the XLD plate. All uninoculated control samples yielded negative test results.

Significance: This study demonstrates that food samples inoculated with low levels of *Salmonella* and enriched in mEHEC + novobiocin for 20 - 24 h at 42°C are reliably detected with VIP and TPSG, yielding results equivalent to plate confirmation.

P2-40 Developing Methods for Efficient Norovirus Recovery from Carpets

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❖ ***Developing Scientist Competitor***

Introduction: Human Noroviruses (HuNoV) are a leading cause of acute gastroenteritis worldwide. HuNoV can persist in the environment making preventing HuNoV infections and controlling outbreaks extremely challenging. Epidemiological evidence suggests that soft surfaces, such as carpets, may be a relevant source of HuNoV due to ineffective and inadequate soft surface disinfection strategies.

Purpose: The aim of this study was to develop a viral recovery method in order to adequately assess disinfectants against HuNoV on carpets.

Methods: Shaved wool carpet fibers were packed into a cylindrical column and the electrokinetic potential (zeta potential) was measured with an Anton Paar SurPASS machine. Wool carpet fibers (0.1 g) were inoculated with HuNoV surrogates, feline calicivirus (FCV) strain F9 and murine norovirus (MNV) strain 1, and sampled at 0, 6, 12, 24, and 48 h. The recovery of MNV and FCV was assessed with 4 different elution buffers (DI water, PBS+Tween 80, PBS+Tween 20, Butterfield buffer) using a mini spin column method. The elution buffers were assessed based on percent recovery of HuNoV surrogates.

Results: Electrokinetic potential measurements indicated wool fibers become increasingly negative when saturated with higher pH solutions (- 56 mV at pH 7.7). The recovery efficiency of MNV from wool fibers was ca. 100% (5.8 log) at 0 h and 13.69% (4.9 log) at 48 h. FCV demonstrated a similar recovery 12.0% (5.1 log) at 48 h. Among the elution buffers, viral recovery rates were not significantly different ($P > 0.05$).

Significance: This was the first study to attempt to develop a method for recovering viruses from carpet surfaces. Recovery with a mini spin column, although not sensitive enough to assess elution buffer effectiveness, demonstrates superior viral recovery compared to previous soft surface recovery methods, which may lead to more accurate inactivation studies.

P2-41 Comparison of PickPen® and OctoMACS™ Immunomagnetic Separation Procedures for Confirmation of *E. coli* O157:H7 and Non-O157 STEC Positive Samples

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Introduction: As part of the USDA MLG isolation and confirmation procedures for *E. coli* O157:H7 and non-O157, presumptive positive samples are plated following immunomagnetic separation (IMS) using the OctoMACS™ paramagnetic columns and sample acid treatment. The Assurance GDS® PickPen® device provides a rapid and simpler alternative to isolate the immunobead-captured organisms

Purpose: To compare the equivalence of immunomagnetic separation using the Assurance GDS PickPen®and the OctoMACS™ columns for the isolation of *E. coli* O157:H7 and non-O157 STEC in raw beef matrices

Methods: Eighty inoculated raw ground beef, 60 beef trim and 10 finely textured beef (FTB) samples were analyzed. IMS was performed on an aliquot of all samples by the PickPen device and the OctoMACS. Concentrated samples were plated according to MLG 5.06 (*E. coli* O157:H7) and MLG 5B.05 (non-O157 STEC).

Results: A total of 150 inoculated samples were tested. One hundred twenty-five samples were confirmed positive with both the PickPen and OctoMACS columns. For the USDA MLG 5.06 reference method, 30 out of 30 samples confirmed positive for *E. coli* O157:H7 using both PickPen and OctoMACS methods. For the Assurance GDS MPX method, 13 out of 25 Ground Beef samples and 17 out of 25 Beef Trim samples confirmed positive for non-O157 STEC using both PickPen and OctoMACS IMS methods. For the USDA MLG 5B.05 reference method, 12 out of 25 Ground Beef samples and 14 out of 25 Beef Trim samples confirmed positive for non-O157 STEC using both PickPen and OctoMACS IMS methods.

Significance: This validation study demonstrates the equivalence of the IMS concentration when either the Assurance GDS PickPen device or the OctoMACS paramagnetic columns is used for immunomagnetic separation of *E. coli* O157:H7 and non-O157 STEC from enriched samples.

P2-42 Rapid Detection of *Salmonella* Species Using an Isothermal Amplification-based System, DNAble

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Introduction: The need for improved testing solutions continues to grow with growing intricacies of pet food and livestock production logistics. While PCR has filled the niche for highly sensitive detection, PCR inherently burdens the end user with cumbersome sample preparation and complex instrumentation with long time-to-result (TTR) (1.5 – 3 h post-enrichment, excluding sample preparation).

Purpose: The purpose of this study was to evaluate the use of DNAble for *Salmonella* spp. detection in dry pet food and poultry environmental swabs. DNAble is an isothermal DNA amplification system with significantly reduced TTR (15 min amplification time) compared to PCR, and requires minimal sample preparation.

Methods: The DNAble *Salmonella* spp. detection kit was validated (1) on dry pet food and poultry environmental swabs. Instrument variation was tested across two instruments. For ruggedness studies, a combination of eight different assay parameters were varied, while for product consistency tests, two different lots of test kits were evaluated. At least eight technical replicates were tested per study. A total of 50 *Salmonella* serovars and 20 non-*Salmonella* strains were tested for inclusivity and exclusivity, respectively. At least two biological and eight technical replicates were tested for reproducibility trials. Statistical evaluation near the limit of detection per AOAC was used for data analysis (1).

Results: The assay exhibited analytical sensitivity of < 50 genome copies/reaction and a reproducible limit of detection of < 10 CFU/ml post-enrichment. Inclusivity and exclusivity data indicate 100% accuracy. Statistically equivalent performance (95% CI) was observed during the instrument variation and product consistency studies, and in seven of eight combinations of non-standard assay user interface parameters tested during ruggedness studies.

Significance: These data support the capacity of DNAble for *Salmonella* spp. detection in dry pet food and poultry environmental swabs.

Reference: (1) http://www.aoac.org/imis15_prod/AOAC_Docs/StandardsDevelopment/AOAC_Validation_Guidelines_for_Food_Microbiology-Prepub_version.pdf

P2-43 Polony Detection and Genotyping of Hepatitis A Virus from Frozen Raspberries Possibly Implicated in an Acute Case of Hepatitis A

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Introduction: Hepatitis A virus (HAV) is one of the leading causes of foodborne infections. HAV can be transmitted by ingestion of contaminated food or water as well as direct contact with HAV-infected individuals through the fecal-oral route. It is highly critical to develop rapid and sensitive detection methods for HAV in food samples in order to prevent and control HAV-related foodborne diseases.

Purpose: In this study, we described a polymerase colony (polony) method for simultaneous detection and genotyping of HAV from frozen raspberries that possibly implicated in an acute case of hepatitis A.

Methods: RNA extraction was performed on frozen raspberries (collected in New Hampshire outbreak, July 2013) by ultracentrifugation concentration and QiaAMP viral RNA mini kit. Polony assay was used to detect and genotype the virus. Additional analysis using nested reverse transcription PCR (RT-PCR) was completed to confirm the presence of HAV, and obtain the amplicon sequence information for phylogenetic analysis and confirmation of virus genotype.

Results: HAV was detected and identified as sub-genotype IB in the frozen raspberries using polony approach. This approach amplifies multiple individual cDNA molecules reverse transcribed from viral RNA to produce immobilized colonies within a thin acrylamide gel on a microscope slide. The resulting polonies were then genotyped by single base extensions with fluorescent dye-labeled nucleotides. This sample was also tested positive for HAV by nested RT-PCR. Phylogenetic sequence analysis of the nested RT-PCR amplicon from the sub-genomic region of VP1/P2A confirmed that the virus belonged to sub-genotype IB.

Significance: The polony method was designed to be an effective tool with the ability to simultaneously detect and genotype HAV embedded in the food matrix in a single assay.

P2-44 Simultaneous Multi-pathogen Enrichment of *Salmonella* Species, *E. coli* O157:H7 and *Listeria monocytogenes* in Tomatoes

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Introduction: For successful prevention of foodborne illness, rapid and reliable methods are needed for pathogen detection including *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes*, the most significant pathogens in FDA regulated food products. As per the Bacteriological Analytical Manual (BAM), depending on the food matrix and target pathogen, different pre-enrichment broths are used for sample preparation prior to conventional culturing detection methods. The use of multiple broths is labor intensive and a roadblock for multi-pathogen detection screening methods in foods or environmental swabs.

Purpose: The objective of this study is to identify the best broth to use for simultaneous enrichment of *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes* in tomatoes followed by detection using multiplex qPCR.

Methods: *Salmonella* Typhimurium, *E. coli* O157:H7, *Listeria monocytogenes* were co-inoculated into tomato homogenate in candidate enrichment broths (1:3 ratio), and incubated at 37°C. Candidate broths included Universal Pre-Enrichment Broth (UPB), Tryptone Soy Broth (TSB), published research broth *Salmonella*, *Staphylococcus* and *Listeria* (SSL), and a FDA research broth (BMW). After 24 h incubation, DNA was extracted for multiplex qPCR analysis.

Results: All three pathogens were able to grow simultaneously and be detected by multiplex qPCR after 24 h enrichment in all candidate broths without food matrices. For enrichment in tomato homogenate, BMW broth showed the greatest potential for a universal enrichment broth as Ct values for *Salmonella* Typhimurium, *E. coli* O157:H7 and *Listeria monocytogenes* were 24.74 ± 4.3, 21.44 ± 0.18 and 29.43 ± 4.5 after 24 h, respectively. The Ct value for *Listeria monocytogenes* enrichment in BMW broth is significantly lower ($P < 0.05$), indicating greater growth, than the TSB (36.66 ± 1.12), UPB (35.02 ± 3.25) and SSL (33.05 ± 2.71).

Significance: The results will improve current regulatory microbiological methods encompassing the recovery, enrichment, and detection of microbial pathogens from foods to provide a less labor intensive means to sample preparation and pathogen screening.

P2-45 Adhesive Tape-based Sampling and *invA*-Specific Recombinase Polymerase Amplification for Detection of *Salmonella* on a Model Surface

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Introduction: Previously, we have shown that adhesive tape-based sampling can be combined with fluorescence *in situ* hybridization (FISH) for rapid detection of *Salmonella* on produce surfaces. However, the limited sensitivity of FISH does not enable detection of low levels of *Salmonella* without enrichment. The combination of adhesive tape-based sampling with a method for pathogen-specific nucleic acid amplification could provide a rapid and sensitive solution for screening of food processing surfaces for the presence of *Salmonella*.

Purpose: Others have combined tape-based sampling of environmental surfaces with PCR for forensic analysis of short tandem repeats (STRs) from low numbers of human cells. Inspired by this work, we sought to extend our previous method for tape-based sampling for *Salmonella* to include specific detection of this pathogen using Recombinase Polymerase Amplification (RPA), a rapid isothermal method for nucleic acid amplification.

Methods: Different levels of *Salmonella enterica* ser. Typhimurium ATCC 14028 were inoculated onto a model sample surface (a Tryptic Soy Agar plate) and allowed to adhere. FungiTape (Scientific Device Laboratory, Inc.) was used to remove *Salmonella* cells from this surface, followed by on-tape lysis and DNA extraction using the PrepMan Ultra reagent (Life Technologies). A 10-min isothermal RPA assay (TwistDx, Ltd.) was used to amplify the *invA* gene, with lateral flow-based detection of the product (Ustar Biotechnologies, Ltd.). Limit of detection was determined using parallel RPA analysis of *Salmonella* genomic DNA of known concentration.

Results: Our approach enabled detection of *Salmonella* at levels as low as 10^1 CFU/µl initial inoculum within 30 min. No inhibition of the RPA assay from co-extraction of tape-based chemicals was detected.

Significance: Our results suggest this approach may enable simple and rapid sampling and molecular detection of *Salmonella* in support of environmental testing efforts. Further evaluation on surfaces typical of food processing environments (stainless steel, tile, plastic) is warranted.

P2-46 Novel Use of PVPP in a Modified QuEChERS Extraction for HPLC-MS/MS Analysis of Neonicotinoid Insecticides in Tea

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Introduction: Neonicotinoids are a new class of insecticides with a distinct mode of action. To ensure consumer health and safety, many countries and international organizations have defined temporary maximum residue levels (MRLs) for seven neonicotinoids in tea. In 2014, the temporary MRL for imidaclothiz (3 mg/kg) has been implemented in China.

Purpose: The aim of the present study was to develop a simple, selective and reliable method - based on the QuEChERS extraction approach - for the determination of eight neonicotinoids using UPLC-MS/MS.

Methods: In order to eliminate the matrix effect and obtain satisfactory recoveries, an inexpensive and excellent absorbent material, polyvinylpolypyrrolidone (PVPP), was used to diminish the tea polyphenols. Further, combinations of PVPP and the commonly used sorbents PSA and GCB were investigated in this study. The optimized 'quick, easy, cheap, effective, rugged and safe' protocol briefly follows. Tea sample was soaked with water and extracted with acetonitrile. Sample extracts were treated with 400 mg PVPP to remove tea polyphenols, and then cleaned up with a combination of PSA (25 mg), GCB (100 mg) and C18 (50 mg). Finally, the dried extract was dissolved with acetonitrile / water (15:85, v/v) and analyzed by UPLC-MS/MS.

Results: The recovery ratios from tea for eight neonicotinoid insecticides ranged from 70-109% at 0.01~0.5 mg kg^{-1} spiked levels. Relative standard deviations were < 15% for all of the recovery tests. The limit of quantification ranged from 0.001 to 0.004 mg kg^{-1}.

Significance: This method should prove to be highly useful for monitoring neonicotinoid insecticides in commercial tea products.

P2-47 A Loop-mediated Isothermal Amplification Assay for the Rapid Detection of *Salmonella* in Animal Feed and Pet Food

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Introduction: *Salmonella* is a significant contaminant in animal feed and pet food. Recent outbreaks and an updated FDA guidance underscore the need for rapid and reliable detection methods in these diverse commodities. Loop-mediated isothermal amplification (LAMP) is a promising molecular method that gained wide application in food testing recently and could be applied in detecting *Salmonella* in animal feed and pet food.

Purpose: A modified LAMP assay was developed and evaluated for the rapid detection of *Salmonella* in various animal feed and pet food matrices.

Methods: A previously published *Salmonella* LAMP assay was used with slightly modified primers. The assay specificity and sensitivity was determined using 186 bacterial strains (151 *Salmonella* and 35 non-*Salmonella*) and serially diluted *Salmonella* reference strains, respectively. Cattle feed, chicken feed, swine feed, dry cat food, and dry dog food were inoculated with serially diluted cultures (10^8 to 10^0 CFU per 25 g feed) of *Salmonella* serovars Newport, Enteritidis, Choleraesuis, Typhimurium, and Infantis, respectively, and tested directly or after overnight cultural enrichment. A well-established qPCR assay was run as a comparison.

Results: The *Salmonella* LAMP assay was 100% specific among the 186 strains tested, without false positive or false negative results. The detection limits were *ca.* 3.6 cells per reaction in pure culture and 10^5 CFU per 25 g in spiked feed samples when tested directly. After overnight enrichment, all feed samples tested positive by LAMP at 10^0 CFU per test portion except for cattle feed, which was positive at 10^1 CFU. Comparable results were obtained with qPCR.

Significance: Animal feed and pet food contaminated with *Salmonella* is a growing public health concern. The *Salmonella* LAMP assay represents a rapid, specific, sensitive, and robust alternative to qPCR for routine screening of *Salmonella* in these commodities.

P2-48 Detection of Gluten by a Reliable ELISA Method

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Introduction: In August 2013, gluten-free labeling regulations were enacted and gluten limits for gluten-free foods were established by the Food and Drug Administration (FDA) of the United States. These gluten limits, which were based on Codex standards, allow items with gluten content of less than 20 ppm to be labeled as "gluten-free." The establishment of these evaluation standards made it possible to measure the gluten content of foods and other products and label them accordingly.

Purpose: To improve usability, Morinaga Wheat/Gluten (Gliadin) ELISA Kit which has been validated as Japanese official allergen analysis method and announced as a gluten determination method of US-FDA Gluten Free Food Labeling Rule was developed two additional application, "Short Time Extraction Method" and "High Range Assay." "Overnight Extraction Method" which is original extraction method needs over 12 h for extraction, but "Short Time Extraction Method" finishes extraction by heating only 10 min in boiling water. "Recommended Assay" which is original assay is designed assay range between 0.26 to 17 ppm gluten, but "High Range Assay" is expanded assay range between 1.05 to 68 ppm gluten to directly detect 20 ppm gluten. Because gluten-free food generally defined that it contains less than 20 ppm gluten.

Methods: "Short Time Extraction Method" was evaluated by comparing with "Overnight Extraction Method," and "High Range Assay" was evaluated by comparing with "Recommended Assay."

Results: "Short Time Extraction Method" showed high extraction ability (96 - 104%) in incurred food analysis and a good correlation with "Overnight Extraction Method" (92 - 105%). "High Range Assay" showed a good correlation with "Recommended Assay" (98 - 126%).

Significance: Two new applications of Morinaga Wheat/Gluten (Gliadin) ELISA Kit provide reliable and more user-friendly gluten analysis method to food industry.

P2-49 Evaluation of a Bead-based Molecular Serotyping Tool for *Salmonella*

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Introduction: The Kauffman-White (KW) classification scheme is the standard for determining > 2,500 *Salmonella* serotypes through the use of antisera, however, reagent lot variability, quality, and shortages limit this method. Molecular serotyping addresses these limitations. One method, developed by the CDC, is the *Salmonella* Serotyping Assay (SSA) which utilizes xMap technology from Luminex. Luminex microspheres are coated with reagents specific to particular DNA biomarkers and are used to capture analytes. Three separate PCR assays: O-group Assay (8 targets), H-group Assay (35 targets) and Additional Target Assay (3 targets) are multiplexed, which allows for the simultaneous amplification of two or more targets in one PCR tube. The dye-labeled amplification product is detected on the Luminex analyzer and converted to digital data. The technician classifies and interprets the data to determine serotype based on the KW scheme.

Purpose: The purpose of this work was to assess SSA from Luminex as a *Salmonella* serotype identification tool in comparison to traditional serotyping using antisera.

Methods: One hundred twenty-four *Salmonella* isolates were used in this study. All samples were prepared for analysis following the manufacturer's protocol and screened with the Luminex assay. Data was analyzed and entered into the Luminex software tool to determine the serotype or candidate serotypes. The identities of these isolates were also determined by traditional serotyping.

Results: SSA results agreed with the antisera result for 60 isolates (48%). An additional 51 isolates (41%) were partially matched but required further analysis of single factors (due in part to several factors not being included in assay). Thirteen isolates (11%) results did not match antisera result.

Significance: The SSA can fully serotype 80 of the top 100 clinically-associated serotypes. However, SSA capability of partial serotyping, thus lowering the number of agglutination assays required to determine identity, could make it an option for molecular-based serotyping of *Salmonella*.

P2-50 Validation of a Real-time PCR Assay for Detecting *Listeria monocytogenes* from Foods and Environmental Surfaces

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Introduction: There are significant costs associated with holding finished food products after manufacture, however releasing product before microbiological tests are complete increases the risk of a product recall. Therefore, rapid, well-validated methods to detect *L. monocytogenes* are needed by industry. The DuPont™ BAX® System Real-Time PCR assay for *L. monocytogenes* is one such assay.

Purpose: This study evaluated inclusivity, exclusivity, and effectiveness of a PCR assay for screening *L. monocytogenes* in foods (frankfurters, bagged spinach, queso fresco, and cooked shrimp) and from environmental surfaces (stainless steel, plastic, and concrete).

Methods: Inclusivity (n = 53 strains) testing was performed at ~10^5 CFU/ml, while exclusivity testing (n = 61 strains) was performed at ~10^8 CFU/ml. For method effectiveness, foods were inoculated with *Listeria* at target levels likely to yield fractional positive results then evaluated using the appropriate culture-based method with twenty fractionally inoculated, five high level inoculated, and five uninoculated samples per matrix, per method. Alternative enrichments were performed in Oxoid 24 LEB Complete media for food and environmental matrices and in FoodChek Actero™ media for environmental matrices. One food type, queso fresco, and one environmental sample type, stainless steel, were tested in an external independent laboratory. PCR testing was conducted from both reference method and alternative method enrichments.

Results: Inclusivity and exclusivity of the assay were evaluated and shown to be 100% consistent with expected results. For effectiveness testing, 900 PCR tests were performed across all matrix/media/enrichment/time conditions with a > 99% concordance to culture confirmation. Statistical analysis using the AOAC POD model indicated PCR and culture results were indistinguishable.

Significance: These data suggest that the PCR method is an acceptable alternative to the reference methods when tested from the reference culture enrichments, or using either of the two proprietary enrichment media for more rapid target detection.

P2-51 Proficiency Test on Determination of Melamine and Cyanuric Acid in Fish Tissues

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Introduction: Melamine can be deliberately added to foods or animal feeds to increase the apparent protein content by increasing nitrogen content. Melamine and cyanuric acid can crystallize in kidneys and cause serious illness or death of animals. Therefore, validated methods for quantitation of melamine and cyanuric acid in feed are important.

Purpose: To evaluate laboratory performance through an inter-laboratory study for quantitation of melamine and cyanuric acid in fish tissue samples to meet U.S. action level of 2.5 mg/kg in animal feed.

Methods: Incurred fish tissues were prepared by feeding catfish melamine (10, 20 mg/kg) and/or cyanuric acid (10, 20, or 40 mg/kg) followed by euthanizing 1 or 3 days after feeding. The concentrations of melamine and cyanuric acid in these fish tissue samples were determined using LCMS. One control fish and five dosed fish fillets containing melamine/cyanuric acid at concentrations of (<0.1/1.2, 0.29/<0.1, 1.6/<0.1, 2.5/0.1 and 3.6/<0.1 mg/kg) were used to prepare the proficiency test (PT) samples. Fish fillets were processed by cutting into slices and then homogenizing together with dry ice in a blender. The resulting powder was subdivided and samples were stored frozen prior to the shipment to six participating laboratories. A total of twelve blind coded test samples (15 g each) were shipped to each participating laboratory. The results from five laboratories that used suitable methods were statistically analyzed according to the internationally harmonized protocol ISO 13528:2005.

Results: The consensus values (n = 10) of melamine/cyanuric acid obtained for the dosed samples were <0.1/1.5, 0.28/<0.1, 1.9/<0.1, 2.8/0.1 and 4.2/<0.1 mg/kg with corresponding relative standard deviation of na/18.7, 51/na, 17.5/na, 14.6/76.7 and 22.4/na %. The melamine/cyanuric acid concentrations obtained were in agreement with the pre-determined values.

Significance: These data showed that the participating laboratories were able to accurately measure melamine and cyanuric acid in fish tissues at action level.

P2-52 Development of a Reliable Gluten Detection Lateral Flow Device

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Introduction: Gluten-free labeling regulations were enacted and gluten limits for gluten-free foods were established in United States by the Food and Drug Administration (FDA) in August 2013. These gluten limits, which were based on Codex standards, allow items with gluten content of less than 20 ppm to be labeled as "gluten-free."

Purpose: Growing need of determination of gluten, easier test method was requested. We developed the new extraction method without heating step for Morinaga Gluten (Gliadin) Lateral Flow device to improve usability although the original extraction method was necessary to heating step for extraction.

Methods: The new extraction method was evaluated by comparing with the original extraction method, Morinaga Wheat/Gluten (Gliadin) ELISA Kit announced as a gluten determination method of US-FDA Gluten Free Food Labeling Rule. In addition, it was compared the both methods with commercially available gluten lateral flow devices to show usefulness of gluten detection.

Results: The new extraction method showed to detect gluten in most of commercially available gluten labeled foods and incurred foods as well as the original extraction method. Both of original and the new extraction method showed the good consistency with Morinaga Wheat/Gluten (Gliadin) ELISA Kit in soft processed and liquid food analysis. It showed the original method detected gluten in processed foods which are not detected by other commercially available lateral flow devices.

Significance: The new extraction method of Morinaga Gluten (Gliadin) Lateral Flow device provides reliable, quick, and user-friendly quantitative gluten analysis in foods, including unprocessed foodstuffs and processed products.

P2-53 Monitoring of Indicator Organisms and Foodborne Pathogens in Various Food Categories for Establishment of Novel Microbial Criteria

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Introduction: Food safety regulations and standards vary from country to country and could cause issues in trading unless the regulation is grounded by scientific knowledge.

Purpose: In this research, we monitored the microbial population and analyzed the results to determine the actual level of microbial contamination in various foods using relatively new statistical analysis (Microbiological Sampling Plan, International Commission on Microbiological Specification for Foods). Our goal is building entirely new standards for various food categories addressed in the Korean "Food Code."

Methods: Indicator organisms (i.e., total aerobic count, *E. coli* and coliform) and foodborne pathogens (i.e., *Bacillus cereus*, *Vibrio parahaemolyticus*, *Staphylococcus aureus* and *Clostridium perfringens*) have been monitored in forty-four food categories.

Results: As the result of the monitoring, total aerobic count, *E. coli* and coliform in noodles were found at the range of 0~5.70 and 0~3.80 log CFU/g, respectively. Long shelf life foods such as coffee and soft drink did not show any contaminations of those indicators except total aerobic count with contamination range of 0~1.30 log CFU/g and 0~0.30 log CFU/g, respectively. Fermented foods such as Kimchi and Korean traditional sauces showed *B. cereus* contamination of 0~2.10 log CFU/g and 0~3.30 log CFU/g, respectively. With the monitoring, we proposed a revised version of statistic standard for microbial contaminations in 6 different food categories. We also proposed new regulations for 2 types of foods including Kimchi and alcohol.

Significance: The proposed standards here will be great additions to the "Food Code" after evaluation of social and economical impact as well as professional advisory committee discussion.

P2-54 Development and Validation of a Rapid Qualitative Test Kit for Detection of Cooked Pork Meat and Gelatin Residues

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Introduction: Incidental contamination of meat with pork residues is relatively common, despite the health and religious concerns that are raised by this practice. Pi Bioscientific Inc. has previously reported on the development of a Lateral Flow Device (LFD) for detection of raw pork meat residues. In an effort to broaden the intended application of the device, we developed an enhanced extraction buffer system than enables recovery and detection of cooked pork meat residues and pork gelatin.

Purpose: To develop a highly specific detection kit that can rapidly detect raw and cooked pork meat as well as pork gelatin residues in meat and gelatin-containing foods.

Methods: Pi Bioscientific Inc. raised and purified goat antibodies against pork serum albumin (PSA) for use in a lateral flow immunochromatographic assay. Sample extraction buffers and running buffers were developed to enable detection of raw meat, cooked meat, and gelatin from samples. The ensuing kits and test methods were validated for sensitivity, specificity and dynamic range. Method concordance was assessed using a PCR-based meat authentication method (IEH).

Results: The Pi Bioscientific Inc. pork meat lateral flow test demonstrated a limit of detection (LOD) of 0.01% spiked raw pork meat (into beef meat), 1 - 5% spiked cooked meat (into cooked beef meat), and 0.1 - 2.5% spiked gelatin (depending on the food matrix). Specificity analysis revealed no cross-reactivity with meats derived from chicken, turkey, horse, beef, lamb, and goat. The assay was able to detect gelatin residues where PCR methods failed to due to the inability to recover DNA.

Significance: The development of a highly specific test method capable of detecting trace amounts of both raw and cooked pork meat as well as gelatin in ~40 min should aid food safety authorities in their continued efforts to monitor adulteration or contamination with pork residues.

P2-55 Development and Validation of a Lateral Flow Test Kit for Detection of Hydrolyzed Gluten Residues in Food

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Introduction: Gluten intolerance disorders such as celiac disease require complete avoidance of gluten in the diet. However, strict elimination of gluten is problematic due to contamination that can occur during food processing. Accordingly, numerous countries have established a regulatory threshold limit for gluten content in foods labeled "gluten-free." Observance of the limit requires the use of antibody-based immunoassays that can detect gluten from a variety of foods. This objective is somewhat problematic for foods containing hydrolyzed proteins.

Purpose: To develop a rapid test kit capable of reporting hydrolyzed gluten residues from swabs down to 2.0 µg/swab and 20 µg/g (or µg/ml or ppm) for foods in under 20 min.

Methods: Prolamins were prepared by Osborn and quantified by Kjeldahl method. To generate monoclonal antibodies, BALB/c mice were repeatedly immunized with rye prolamins, splenocytes were fused with a mouse myeloma cell line, and ensuing colonies were screened against triticeae-derived prolamins. An IgG-secreting hybridoma, 25A5, was identified and raised in ascites. The mAb was purified by FPLC on a protein G column, characterized, and then used to develop a lateral flow device (LFD) for the detection of gluten. Concordance was assessed using a commercial ELISA kit.

Results: The MEI/IEH competitive gluten lateral flow test method demonstrated a sensitivity of 2.0 µg/swab and 20.0 µg/mL for foods. Specificity analysis revealed no cross-reactivity with common commodities except for teff. The assay was equally sensitive as the commercial R5-based ELISA kit in its ability to report gluten residues from hydrolyzed foods, and required less time (< 20 min) to perform.

Significance: The development of a highly sensitive and rapid test method capable of detecting trace amounts of hydrolyzed gluten residues in under 20 min should aid food manufacturers' and regulatory entities in monitoring for gluten residues, particularly in testing of fermented foods.

P2-56 Development and Validation of a Lateral-flow Device for Detecting Total Milk Residues

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Introduction: IgE-dependent and IgE-independent immuno-toxicities to cow's milk constitute the most common causes of food "allergies," affecting roughly 3% of the population. The most clinically significant allergens in milk are casein and beta-lactoglobulin (β-LG). Due to current food manufacturing practices, casein and β-LG can be present in food samples at very different levels, necessitating the use of dual casein and β-LG screening measures to correctly identify milk allergen contamination.

Purpose: To develop and validate a rapid detection kit that can specifically detect total milk residues from both swabs (at 0.01 µg/swab) and foods (0.1 ppm) in under 20 min.

Methods: Polyclonal antibodies (pAbs) against casein and β-LG were raised in goats and individually purified on affinity columns. The pAbs were then used to develop a lateral flow immunochromatographic assay configured in sandwich format. Sample extraction and running buffers were developed to enable rapid and highly sensitive operation of the lateral flow device (LFD). The ensuing Total Milk LFD kit was then validated.

Results: The Pi Bioscientific Total Milk LFD test method demonstrated a sensitivity of 0.01 µg/swab and 0.1 ppm milk protein for foods. The test was specific for bovine residues, and did not appreciably report caprine or ovine residues. Selectivity testing showed all spiked food matrices tested retarded LOD by 2-4 folds whereas real food testing showed highly detectable levels in all cow's milk-based foods tested with the exception of goat or sheep's milk-based foods. Specificity analysis revealed no cross-reactivity with common food commodities.

Significance: The development of a highly sensitive and rapid test method capable of detecting trace amounts of casein and/or β-LG in under 20 min should aid food manufacturers and regulatory entities in monitoring for milk allergens in environmental and food testing.

P2-57 An Innovative Lateral Flow Device for Dual Detection of Triticeae-derived Prolamins and Avenins

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Introduction: Cereal grains contain a composite protein called gluten which consists of prolamins and glutenins. The prolamin fraction from several grains exhibits immunopathogenic potential leading to the manifestation of celiac disease. While the majority of celiac subjects react to wheat, barley, and rye (triticeae), a subset additionally respond to prolamins derived from oats so called avenins. There are differences in the approach to regulating avenins as part of Gluten-Free Labeling. In the USA, oats/avenins are not regarded under labeling legislation, though the celiac community maintains advisory guidelines for oat-containing food products.

Purpose: To develop and validate a highly specific detection kit that can dually and independently detect triticeae- and oat-derived prolamins in under 20 min.

Methods: Monoclonal antibodies (mAbs) against avenin and gliadin were raised in mice using vaccine prepared using a modified Osborne fractionation method to derive pure prolamins from hard red winter wheat and R5(-) oats. An avenin-specific and R5-like clone were characterized and then developed into a rapid assay using lateral flow format. The ensuing kit and test methods were validated.

Results: The Pi Bioscientific Celiac Aide Lateral-flow test method demonstrated a sensitivity for R5(-) avenins and triticeae prolamins (gliadin, hordein, and secalin at 0.5 µg/swab and 5.0 µg/ml each for foods. Specificity analysis revealed no cross-reactivity with common commodities or extracts prepared from soy, lupins, sorghum, corn, rice or millet. Selectivity analysis using a panel of problematic food matrices revealed that the assay was sufficiently robust to enable detection of analyte at levels between 5 - 10 ppm.

Significance: The development of a highly sensitive and rapid test method capable of detecting trace amounts of gliadin, secalin, hordein, and avenin in under 20 min should aid the celiac community and food industry in achieving gluten outcomes that are recommended by the medical community.

P2-58 Development and Validation of Lateral-Flow Devices for Detection of Tree Nuts and Peanuts

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Introduction: Allergies to tree nuts and peanuts are relatively common, with a combined prevalence of 1.5 - 2.5%. The symptoms of allergies to nuts can range in intensity but frequently manifest as a severe, life-threatening reaction termed anaphylaxis. Accordingly, regulatory agencies in Canada and the USA require food labels to clearly indicate tree nut and peanut content so as to alert consumers of potential allergy risks. Observance and enforcement of these labeling laws necessitate the use of antibody-based immunoassays that can detect nut residues in a variety of foods.

Purpose: To develop and validate a panel of LFDs that can rapidly detect trace tree nut and peanut residues in under 20 min.

Methods: Total protein extracts from raw and roasted (12 min at 180°C) nuts were prepared using a TRIS buffered, 200 mM NaCl, pH 7.2 extraction solution and then used to raise pAbs in goats. IgG was purified from plasma using protein G columns/AKTA Prime FPLC and then applied to lateral flow immuno-chromatographic assay development. In parallel, a proprietary sample extraction buffer was developed to enable rapid and highly sensitive operation of each LFD test.

Results: LFDs were developed for detection of residues derived from peanut, hazelnut, pine nut, brazil nut, cashew nut, pistachio nut, coconut, almond, macadamia nut, and walnut/pecan. Each nut LFD demonstrated a sensitivity of 0.1 µg/swab and 1.0 µg/ml for foods. Specificity analysis revealed no cross-reactivity with common commodities, and minimal cross-reactivity between different nut types, with the exception of cashew and pistachio which cross-reacted with each other.

Significance: A complete testing panel of LFDs that can rapidly detect tree nut and peanut residues in under 20 min should aid food manufacturers and regulatory entities in monitoring for nut residues.

P2-59 Development and Validation of a Lateral Flow Test Kit for Detection of Deamidated Gliadin Residues

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Introduction: Gluten intolerance disorders including celiac disease are relatively common and necessitate strict limits in dietary intake. Consequently, gluten content in foods labeled "gluten-free" is regulated under Federal Law. However, accurate determination is hindered by the fact that wheat protein isolate (deamidated gluten) is difficult to detect due to chemical alterations at glutamine (Q) residues located at epitopes of diagnostic interest.

Purpose: To improve upon current gluten-detection capabilities, PiBio Scientific has developed and validated a highly rapid diagnostic tool specifically designed to detect deamidated gliadin derivatives in foods, environmental surfaces, and personal hygiene products.

Methods: Monoclonal antibodies against deamidated gliadin were raised in mice, screened against a panel of prolamins, purified on a protein G using FPLC, then one of the candidate clones was used to develop a lateral flow immunochromatographic assay configured in sandwich format.

Sample extraction buffers and running buffers were developed to enable rapid and highly sensitive operation of the LFD. The ensuing kit and test method were validated.

Results: The PiBio deamidated gluten lateral flow test method demonstrated a sensitivity of 0.1 µg/swab and 10 µg/ml food for both deamidated and native gluten in under 20 min (sample preparation and LFD operation). Specificity analysis demonstrated cross-reactivity with teff grains and selectivity analysis using a panel of problematic matrices revealed no alterations to LOD.

Significance: Wheat protein isolate is increasingly used in the food industry for improving food textures and fortifying protein content. The development of a highly sensitive and rapid testing method capable of accurately detecting trace amounts of deamidated gliadin residues in under 20 min should aid food manufacturers and regulatory entities in monitoring for gluten derivatives that are challenging to detect using existing diagnostic tools.

P2-60 Rapid Detection of *Bacillus cereus* by Immunomagnetic Separation and Real-time PCR

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Introduction: *Bacillus cereus* is a rod-shaped and Gram-positive food pathogen, and has been described to cause diarrhea and emetic types of food poisoning. The immunomagnetic separation (IMS) method followed by real-time PCR has been used to capture cells and decreased the assay time which could help to minimize the possibility of exposing consumers to foodborne pathogen.

Purpose: The purpose of this study was to develop a method rapid, specific and quantitative method to detect *B. cereus* in lettuce and spinach.

Methods: Immunoaffinity magnetic beads were prepared by coating anti-*B. cereus* antibody to Dynabeads® protein G. The capture kinetics and capacity of the anti-*B. cereus* antibody against *B. cereus* were studied between anti-*B. cereus* antibody and Dynabeads® protein G for concentrations (4, 5, 6, 8 and 10 µg/20 µl), five immunoreactions times (10, 20, 30, 40, 50 min) and a serial of concentrations (102 was carried out using two *B. cereus* strains and 6 non-target bacteria. The standard curve was constructed using the DNA isolated from the serial dilutions of *B. cereus*.

Results: The IMBs prepared by 5 µg antibody per 20 µl Dynabeads® were optimized and with the immunoreactions time of 20 min the colony number in the suspension reached the highest. For the specificity assay, *B. cereus* (2.35 and 2.63 log CFU/100 ml) could be recovered by the IMBs, but for non-target bacteria, less than 1.5 log CFU/100 µl were recovered except for the *B. subtilis* (2.34 log CFU/100 µl) which showed similar recovery with *B. cereus*. When combined with the real-time PCR, *B. cereus* was detected in lettuce and spinach samples with *B. cereus* > 103 CFU/ml.

Significance: The developed method using IMS combined real-time PCR was rapid, specific and quantitative for detection of *B. cereus*.

P2-61 Determination of Flunixin in Culled Dairy Cows Using Screening Assays

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Introduction: Flunixin is a non-steroidal anti-inflammatory agent used to treat serious inflammatory conditions in livestock; it is also a commonly detected violative residue in dairy cattle. Ante-mortem matrices such as milk, saliva, or urine could prove valuable for predicting possible violative tissue residues.

Purpose: Using ELISA and lateral flow immunoassays to determine flunixin residues in tissues and ante-mortem matrices

Methods: After IACUC approval, 20 cows were given the labeled dose of flunixin by IV or IM administration (10 each) for 3 consecutive days. One-half of the cows were challenged with IV LPS. Milk, saliva, and urine were collected at timed intervals and cows were slaughtered with a 4-day withdrawal period (WP). USDA, FSIS methods (CLG-FLX3.01 and Bulletin 4246) were used to screen tissues for flunixin residues. Saliva flunixin was determined using a matrix matched calibration standards on a 96-well ELISA format. Lateral flow analyses were utilized to determine flunixin in milk, saliva, and urine. Milk was assayed directly while saliva and urine samples were diluted.

Results: At a WP of 96 hours, no animals exceeded the minimum applicable levels for muscle (≥ 10 ppb) while livers of two animals exceeded minimum applicable levels (≥ 50 ppb). Saliva did not produce a predictable flunixin elimination pattern using ELISA determination. Using lateral flow analysis, 16 of 20 urine samples were flunixin positive; 6 of 20 saliva samples were positive at 96-h tissue WP. Milk of 12 cows were positive at the 36-h milk WP.

Significance: The FSIS' ELISA screening method detected liver flunixin violative residues (confirmed by FSIS' LC-MS method) with one false-positive result. Based on lateral flow screening assay results, urine and saliva are not good ante-mortem predictors for tissue flunixin violations; rather, they potentially can be used to test flunixin exposure in off-label species.

P2-62 Development of Metallic Contaminant Detector for Food Inspection Using Ultra-sensitive Superconducting Magnetic Sensor

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Introduction: A practical magnetic metallic contaminant detector using three SQUIDs (Superconducting Quantum Interference Device) magnetic sensor for food inspection was developed. Finding small metallic contaminants is important for food safety. There is a possibility that individuals ingest contaminants that have been accidentally mixed with food. When the contamination occurs, the manufacturer of the product suffers a great loss to recall the defective products.

Purpose: Therefore a detection method of small contaminants in food is important. We developed a practical food contaminant detection system based on ultra-sensitive SQUID magnetic sensors.

Methods: The method is based on the detection of the remnant magnetic field from small metallic contaminant in food. After magnetization, the remnant magnetic field from any metallic contaminants is detected by the ultra-sensitive SQUID sensors when the food passes below the sensor. The SQUIDs are cooled at 77 K by liquid nitrogen, which can be supplied automatically from a reservoir by a pump. The system is covered with waterproof stainless steel plates and acceptable to HACCP (Hazard Analysis Critical Control Point) program. The acceptable object size is W150 mm x H100 mm, which is large enough for practical inspection. All the system is controlled by PC. This system employs tri-layered permeable metal magnetic shield box with thickness of 1 mm and an aluminum electromagnetic shield box for preventing from magnetic and radio frequency interferences. The shielding factor of the magnetic shield is -60 dB at 0.01Hz, which is good enough to operate the system in a factory. A digital filtering technique has been newly introduced to reduce noise.

Results: The signal-to-noise ratio (SNR) was dramatically improved, and we were able to robustly detect a steel ball as small as 0.3 mm in diameter.

Significance: This system is the first product using rf-SQUID sensor in the world.

P2-63 Rapid Testing of Food Matrices for *Bacillus cereus* Enterotoxins

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Introduction: A variety of food products frequently associated with *Bacillus cereus* outbreaks were chosen as representative matrices to be evaluated with end-point PCR, ELISA, LFD, and mass spectrometry for detection of enterotoxins associated with human illness.

Purpose: The study aim was to decrease the turn-around time for enterotoxin detection in food matrices.

Methods: Food products obtained locally included dehydrated potato flakes, rice, pancake batter, gravy mix, protein powder and a dietary supplement as well as liquid milk and canned soup. Food portions were inoculated with Butterfield's phosphate-buffered dilution water as the matrix control or one of five bacterial strains representative of either inclusivity or exclusivity test strains. Testing was performed on inoculated food portions following 5-h and 24-h incubation periods. An end-point multiplex PCR was used as a screen for four enterotoxin gene targets to include the emetic toxin gene and three diarrheal toxins, Hbl, Nhe and Cytk. Commercially available ELISA and LFD kits were used to determine the presence/absence of Nhe and Hbl. Finally, a quantitative analysis using mass spectrometry was performed for the detection of the emetic toxin.

Results: Inclusivity strains included three *B. cereus* strains that were all positive for Nhe, two were also positive for Hbl, one was positive for CytK, and one was positive for the emetic toxin gene. The PCR results of several DNA food product extracts revealed natural *B. cereus* contamination which was validated with plate enumeration, additional PCR testing and serological testing.

Significance: Definitive results were available after the five hour pre-enrichment for the emetic toxin testing in five food products and for the diarrheal enterotoxins for six of the food products demonstrating the availability of rapid turn-around times when compared to traditional methods. Definitive results were available for all food matrices following 24-h incubation.

P2-64 Development of Analytical Methods for Determination and Monitoring of Levels of Aflatoxin M_1 in Dairy Products Collected from Markets in South Korea

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❖ ***Developing Scientist Competitor***

Introduction: Aflatoxin M_1 (AFM_1), a 4-hydroxy derivative of aflatoxin B_1, is formed from aflatoxin B_1 by cytochrome P450 in liver and excreted into milk of animals that have been fed with aflatoxin B_1-contaminated feeds. Since AFM_1, a Group1 human carcinogen, is stable during food processing, it could pose a health risk to public.

Purpose: The purpose of this study was to develop sensitive and reproducible analytical methods for determination of AFM_1 in milk, yoghurt, and cheese by using high performance liquid chromatography-fluorescence detection (HPLC-FLD) after immunoaffinity column clean-up and to monitor the levels of AFM_1in the dairy samples collected from markets in South Korea.

Methods: The linearity, recovery, repeatability, and reproducibility of the analytical methods were assessed for AFM_1 analysis using HPLC-FLD after toxin purification by immunoaffinity columns. The levels of AFM_1 in a total of 224 dairy samples including milk, yoghurt, and cheese were determined using the established analytical methods. The AFM_1 in the dairy samples was confirmed by liquid chromatography-mass spectrometry (LC-MS/MS).

Results: The analytical method showed high linearity ($r^2 \geq 0.999$) for AFM_1 in the range of 0.05–10.00 µg/l. The limits of detection of the methods for AFM_1 were 0.001 µg/l in milk, 0.02 µg/l in yoghurt, and 0.015 g/kg in cheese, respectively. The recovery of AFM_1 in the dairy products was 83-108% along with 2.1-12.8% of repeatability and 0.0-13.1% of reproducibility. The occurrence of AFM_1 was 55% in the total of 224 dairy samples collected from South Korea. Low levels of AFM_1 (0.001-0.136 µg/l) below 0.5 µg/l of the legal limit of the level of AFM_1 in milk were detected in the dairy samples.

Significance: The levels of AFM_1 in dairy products did not pose a significant health risk to public in South Korea.

P2-65 Simultaneous Determination of Multi-mycotoxins in Cereal Grains by LC-MS/MS

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❖ ***Developing Scientist Competitor***

Introduction: Aflatoxins (B_1, B_2, G_1, and G_2), ochratoxin A, fumonisins (B_1 and B_2), nivalenol, deoxynivalenol, 3-acetyldeoxynivalenol, zearalenone, T-2 toxin, and HT-2 toxin are major mycotoxins found in agricultural crops. Since these mycotoxins can occur simultaneously in cereal grains, it is needed to determine the levels of the multi-mycotoxins in the grains.

Purpose: The purpose of this study was to establish a highly sensitive and reliable analytical method for simultaneous determination of the 13 mycotoxins in cereal grains by liquid chromatography-mass spectrometry (LC-MS/MS) after immunoaffinity column clean-up and to monitor the levels of the mycotoxins in the samples collected from retail markets in South Korea by using the validated analytical method.

Methods: We evaluated the linearity, sensitivity, specificity, and accuracy of the analytical methods for the mycotoxins in cereal grains by LC-MS/MS after improved toxin extraction and purification using Myco6in1$^+$ immunoaffinity columns. The methods were used to determine the levels of the multi-mycotoxins in a total of 257 cereal grains (brown rice, maize, millet, sorghum, and mixed cereal) collected from South Korea.

Results: The calibration curves of the mycotoxins showed higher r^2 than 0.999 by using LC-MS/MS. The recovery rates of the mycotoxins in rice were 73.9-133.0% along with RSD_r of 1.65 - 14.31% at 1.24 - 100 ng/g of the spiked levels. The limits of quantification of the method for the mycotoxins were in the range of 0.6 - 6.0 µg/kg. The occurrence of the mycotoxins ranged from 1.2% to 78.6%. The co-incidence of more than two mycotoxins was 65% in brown rice, 37% in maize, 85% in millet, 94% in sorghum, and 72% in mixed cereal samples, respectively.

Significance: The established method was proven to be suitable for assessment of the levels of multi-mycotoxins in cereal grains.

P2-66 Multiplex Detection of Food Allergens and Antigen Profiling of Cumin

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Introduction: In late 2014, several cumin-containing products were recalled for the presence of undeclared peanuts and almonds detected using ELISA methods. The detection of multiple undeclared food allergens is difficult, especially when there is the possibility that homologous proteins may be present that can cross-react with the antibodies used in the ELISAs. Currently the only methods that can simultaneously detect multiple allergens entail the use of mass spectrometry, a time-consuming and expensive process.

Purpose: To develop an inexpensive, rapid, high-throughput, multiplex assay for the simultaneous detection and quantification of food allergens and gluten. The assay should also be able to distinguish between known food allergens and novel cross-reactive proteins.

Methods: Established antibodies, used in the detection of food allergens, were conjugated to paramagnetic, color-coded beads to generate two multi-analyte profiling (xMAP®) assays. A 29-plex and a nine-plex were designed to detect non-denatured and denatured food allergen proteins, respectively. Included in each mixture were AssayCheX™Process Control microspheres to confirm instrument and reagent optimal performance.

Results: The multiplex assays were able to detect all food allergens at concentrations less than 50 ppb (e.g., 50 ng/g or ng/ml). By comparing the intensities of the responses generated by cumin with the 30 antibodies included in the assays, it was shown that the cumin samples contain either a mixture of many undeclared allergens (i.e., peanut, almond, Brazil nut, cashew, coconut, hazelnut, macadamia, and pistachio) or a novel cross-reactive protein(s).

Significance: The development of a multiplex assay for food allergens makes it possible to analyze 48 samples in one day for the presence of 14 food allergens and gluten, a process that would otherwise take over one month using standard ELISA technology. Further, without the use of expensive equipment, it is now possible to identify the presence of novel cross-reactive proteins. This should enhance efforts to identify potentially allergenic foods.

P2-67 Evaluation of an *E. coli* and Coliform Method in Ground Meats, Liquid Eggs and Carcass Rinses

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Introduction: Meat production is monitored for generic *E. coli* as a process control under HACCP regulations. Peel Plate EC is a simplified *E.coli* and coliform method using dual indicators to detect *E. coli*, as blue/purple, and coliform, as red, colonies at 35°C in 24 h.

Purpose: The purpose of this evaluation was to perform inclusivity/exclusivity and an inter-laboratory comparison of the new method in comparison to the reference methods, VRB agar with MUG overlay and alternatively AOAC 991.14 in ground meat, liquid eggs and carcass rinses.

Methods: Methods Peel Plate EC and AOAC 991.14 were evaluated at 35°C for inclusivity using 56 coliform strains. In the exclusivity study, 31 non-coliform strains were studied. In sample comparisons ground-beef, ground-turkey, liquid-egg, chicken rinse and hog-carcass- rinse were fortified at low, medium and high concentrations and split into 5 replicates. Extractions of meats 50 g/450 ml and eggs 100 g/900 ml and rinses were tested in duplicate and compared to reference methods BAM (VBRA/MUG-overlay) and USDA-MLG3.01(AOAC 991.1).

Results: The method detected 55 of 56 coliform strains including all 15 *E. coli* strains, compared to 41 of 56 coliform, and 14 of 15 *E. coli* strains, by OMA 991.14. In exclusivity, the new method excluded 28, compared to 26 by the reference, of 31 strains. *E. coli* strains not producing GLU, mainly hemorrhagic type, were excluded as *E .coli* but detected as coliform by both methods. For the method comparison, results were evaluated for repeatability (S_r) and by paired *t*-test for statistical difference, > 0.5 log. The method results were not significantly different from AOAC 991.1 in detecting *E. coli* and/or coliform. The new method was not significantly different from BAM except with chicken rinse in which *E.coli* were non-fluorescent because of background flora acidification of plates.

Significance: The method demonstrates high selectivity with comparable *E. coli* /coliform detection to reference methods for meat and egg matrices.

P2-68 Characterization of Shiga Toxin-producing *Escherichia coli* Recovered from a Beef Processing Facility within Southern Ontario and Comparative Performance of Molecular Diagnostic Platforms

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❖ ***Developing Scientist Competitor***

Introduction: There has been an increased incidence of non-O157 Shiga Toxin *Escherichia coli* (STEC) with six serotypes (Top 6) being implicated in causing hemolytic uremic syndrome (HUS). Beef has been suggested to be a significant vehicle for non-O157 STEC although conclusive evidence has yet to be obtained.

Purpose: The following aimed to determine the prevalence of the Top 6 non-O157 STEC in beef processing using three different diagnostic platforms then characterize the recovered isolates.

Methods: Hide, carcass and environmental swab samples (n = 60) were collected from a beef processing facility over a 12-month period. Enriched samples were screened using Biocontrol GDS, BAX or PALLgene molecular diagnostic tests. Presumptive non-O157 STEC positive samples were confirmed using conventional PCR and serology.

Results: STEC was detected by GDS (55% positive), BAX (85% positive), and PALLgene (93%). However, during confirmation testing only 8 of the 60 samples (13%) were found to harbor STEC. Interestingly, the presence of virulence factors in the recovered isolates were unstable and readily lost during subsequent sub-culturing.

Significance: There is a low prevalence of Top 6 non-O157 STEC associated with beef although other serotypes are encountered. Yet, the instability of the virulence factors in recovered strains would question their clinical relevance.

P2-69 Molecular Characterization of Extended Spectrum β-lactamase (ESBL)-producing *E. coli* Isolated from Imported Meat in Korea

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Introduction: The prevalence of extended-spectrum β-lactamase (ESBL)–producing bacteria is increasing rapidly worldwide. A connection between ESBL-producing *E. coli* in retail meat and humans has been suggested, and therefore the bacteria could pose a possible public health risk through consumption of improperly prepared meat.

Purpose: The objective of this study was to characterize antimicrobial resistance, genotypes, integrons, virulence genes, and plasmids in ESBL-producing *E. coli* isolates that were obtained from imported meat in Korea.

Methods: Antimicrobial susceptibility test, multilocus sequence typing (MLST), detection of integrons, β-lactamase, and virulence factors by PCR, plasmid replicon typing, and repetitive sequence-based PCR (rep-PCR) DNA fingerprinting were carried out.

Results: A total of twenty ESBL-producing *E. coli* strains were isolated from imported pork and chicken in Korea and they were resistant to ampicillin, cefazolin, cefepime, cefpodoxime, ceftriaxone, cephalothin, and gentamicin. Fifteen different MLST types and four CTX-M groups (1, 2, 8, and 9) were identified. PCR was carried out for the detection of integrase genes *Int1*, *Int2* and *Int3*, integron-associated *aadA1*, *aadA5*, *sulI*, and *qacEΔ1*

genes, and β-lactamase genes bla_{OXA} and bla_{CMY}. Only three genes, *int1*, *qacEΔ1* and bla_{OXA-1}, were found in CC22 and EC12-5 strains. Among 31 tested virulence factor genes, P-fimbriae assembly (*papC*), P fimbria tip pilins (*papEF*), P fimbria adhesin (*papG*) allele II, type 1 fimbriae (*fimH*), aerobactin (*lutA*), O antigen polymerase (*rfc*), and serum resistance associated (*traT*) were detected in CC21, CC22, ES92, EC12-5, and EC12-6 isolates. The IncFIB plasmid replicons were present in all isolates, followed by IncI1 (80%), IncFIA (65%), IncHI1 (40%), and IncB/O (35%). IncA/C, IncFIC, IncFIIA, IncHI2, IncK/B, IncL/M, IncN, IncT, IncW, and IncX were not detected. The dendogram analysis of rep-PCR showed 4 major clusters for 80% similarity cut-off.

Significance: The presence of ESBL-producing *E. coli* harboring virulence factors in imported meat suggests that it could be a great public health concern.

P2-70 *Bdellovibrio bacteriovorus* Can Effectively Predate on *Salmonella* in an *in vitro* Cattle Model but Lacks Efficacy against *Escherichia coli* O157:H7

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Introduction: *Escherichia coli* O157:H7 and *Salmonella* spp. are known for their ability to induce severe gastroenteritis and life-threatening illness in humans. Cattle hides and digestive tracts serve as reservoirs for these pathogens and provide a means for carcass contamination at harvest. Thus, interventions are necessary to control these pathogens in the pre-harvest environment in order to reduce contamination of the food supply.

Purpose: This study was conducted to gain knowledge of *Bdellovibrio bacteriovorus* efficacy as a pre-harvest intervention to control *Salmonella* and *Escherichia coli* O157:H7 in cattle using an *in vitro* model to simulate the digestive tract of the animal host.

Methods: Cattle rumen fluid and feces were inoculated with antimicrobial susceptible or resistant strains of *Salmonella* or *Escherichia coli* O157:H7. HEPES was added to control samples while experimental samples were supplemented with HEPES containing *Bdellovibrio bacteriovorus* and all samples were incubated anaerobically at 38.6°C and 50 rpm. At 0, 24, 48, and 72 hours, samples were plated onto Sorbitol MacConkey Agar with Cefixime and Tellurite (CT-SMAC) to enumerate *Escherichia coli* O157:H7 and subjected to Most Probable Number (MPN) before streaking to Xylose Lysine Tergitol-4 (XLT-4) to enumerate *Salmonella*.

Results: *Bdellovibrio bacteriovorus* did not significantly reduce antimicrobial susceptible and resistant populations of *Escherichia coli* O157:H7 in either rumen or fecal environments. Significant reductions in *Salmonella* populations in rumen fluid were also not observed. In comparison to control fecal samples, *Bdellovibrio bacteriovorus* decreased resistant *Salmonella* by 3.79 (P = < 0.0001) and 2.24 (P = 0.0013) log MPN/g following 24 and 48 hour incubations, respectively, and susceptible *Salmonella* by 2.02 log MPN/g (P = 0.0005) when time was held constant.

Significance: The significant decrease in *Salmonella* populations achieved in cattle feces *in vitro* suggests that *Bdellovibrio bacteriovorus* may be able to predate within the gastrointestinal tract of cattle. These data suggest that future *in vivo* research is warranted.

P2-71 Prevalence and Antibiotic Susceptibility of Pathogenic *Escherichia coli* Recovered from Pig and Cattle Slaughterhouses

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Introduction: Pathogenic *Escherichia coli* (PEC) was considered important foodborne pathogens, and recognized as a significant public health problem. In most case, contaminated animal products may be responsible for PEC infections in humans. The animal carrying and shedding PEC and other pathogenic microorganisms in slaughterhouses is the main source of contamination.

Purpose: The aim of this study was to investigate the isolation rate of pathogenic *Escherichia coli* from pig and cattle slaughterhouses

Methods: The PEC was determined in pig carcasses (n = 245), cattle carcasses (n = 210), pig carcasses chilling room (n = 98), and cattle carcasses chilling room (n = 84), collected from 50 slaughterhouses in South Korea. In order to detect a virulence factor, we selected the target genes: *stx1* and *stx2* for STEC; *eaeA* for EPEC; *ipaH* for EIEC; *elt, estp*, and *esth* for ETEC; *aggR* for EAEC. The antibiotic susceptibility of PEC isolates was determined by the disk diffusion method according to NCCLS

Results: A total of 14 PEC isolates were isolated from 12 slaughterhouses; 2 of 245 of pig carcasses (0.82%), 8 of 210 of cattle carcasses (3.81%), 2 of 98 of pig carcasses chilling room samples (2.04%), and 2 of 84 of cattle carcasses chilling room samples (2.38%). Virulence genes of at least one PEC pathogroup was detected in 14 (2.20%) of the 637 samples, with 7 (1.10%) being positive for virulence genes of STEC, 6 (0.94%) of EPEC and 1 (0.16%) of ETEC. The antibiotic resistance observed was with tetracycline, streptomycin and chloramphenicol (14.29%) followed by ciprofloxacin (7.14%).

Significance: Pig and cattle carcasses and their storage condition should be monitored to prevent pathogenic *Escherichia coli*. The origin of infected slaughter animals should be identified and direct and cross-contamination of carcasses should be avoided by adhering to HACCP principles in association with good hygiene procedures (GHP).

P2-72 Species Diversity and Pheno- and Genotypic Antibiotic Resistance Patterns of Staphylococci Isolated from Ground Meats

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Introduction: Nowadays, infections caused by coagulase-positive staphylococci and coagulase-negative staphylococci (CoNS) are important public health problems in both developed and developing countries. Moreover, the transmission of antibiotic resistant strains of staphylococci between animal origin food products and humans has become a serious problem in the globalizing world.

Purpose: In this study we aimed to investigate the presence and prevalence of staphylococci in ground beef and lamb meat samples, to detect *16S rRNA*, *mec*A, *nuc*, *pvl* and *femA* genes and to determine pheno-/ genotypic antibiotic resistance profiles of obtained staphylococci isolates.

Methods: A total of 250 refrigerated ground meat samples were collected during a seven month period (September 2013 to March 2014) from randomly-selected retail stores in Diyarbakir, Turkey. Staphylococci were isolated using standard cultural methods and confirmed for species by the VITEK 2 compact identification system. Multiplex PCR and simplex PCR was performed to screen for some virulence genes and antibiotic resistance genes, and also phenotypic antibiotic resistance was tested for 24 antimicrobials.

Results: The prevalence of staphylococci was found to be higher in ground beef (86.4%) compared to ground lamb (62.4%). Eleven different staphylococci species were detected from 208 isolates. In 85 isolates, which were positive for *Staphylococcus aureus,* the presence of *femA*, *mecA* and *pvl* genes were 40%, 47% and 5.8%, respectively, while in 118 isolates positive for CoNS, rates were 0%, 10.1% and 0%, respectively. We determined that isolates had antibiotic resistances ranging from 0.9 - 85.5%.

Significance: Our study is the first study that reported detecting the *pvl* gene from MRSA isolates obtained from ground meat samples in Turkey. Also these study results show that ground meat samples are important vectors for staphylococci, which have pathogenic characteristics, in terms of food safety and subsequently public health, and also serve as an important reservoir of antibiotic resistant organisms.

P2-73 The Prevalence of *Salmonella* Isolated from Retail-level Chicken Parts with and without Skin

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Introduction: Even though in the United States the prevalence of *Salmonella* in chicken carcasses has decreased over the years from 16.3% in 2005 to 6.4% in 2011 (USDA/FSIS 2013), there is very limited information available in the published literature about the prevalence of *Salmonella* in skin-off versus skin-on chicken parts at the retail level.

Purpose: The aim of the present study was to determine the presence of *Salmonella* in chicken parts with and without skin collected from retail establishments in the metropolitan Atlanta (Georgia) area.

Methods: Retail packs of cut-up chicken parts (breast, thigh and drumstick) were purchased from supermarkets in the Atlanta metro area. Parts were collected from five counties in the metro area with number of samples proportional to the residence population in each county. At the supermarket, pairs of chicken breast packages and thighs (with skin-on and skin-off) were collected. Samples were paired production company and sell-by date. Skin-on drumstick samples were individually collected. All samples were tested for *Salmonella* presence using primary and delayed secondary selective enrichment standard methods.

Results: A total of 315 samples of each chicken part were collected and analyzed. *Salmonella* prevalence in skin-on and skin-off breast was 41.2% and 3.1%, respectively ($P < 0.05$); whereas, the prevalence in skin-on and skin-off thigh was 28.5%, 19.0%, respectively ($P > 0.05$). *Salmonella* prevalence on skin-on drumsticks was 25.3%.

Significance: Based on these preliminary findings, *Salmonella* prevalence in skin-on chicken meat parts is higher than skinless parts at the retail level, demonstrating an increased risk to consumer safety.

Poster

P2-74 *Clostridium difficile* in Retail Chicken Meat Parts and Liver in Turkey

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Introduction: Although there are studies conducted on the prevalence of *Clostridium difficile (Cl. difficile)* in meat products (especially ground beef and pork meat), there are few studies in the literature conducted on the presence of *Cl. difficile* in chicken meat parts and liver at the retail level.

Purpose: The objective of this study was to determine the presence and numbers of *Cl. difficile* in retail chicken meat parts and chicken liver samples using both qualitative and quantitative methods and to detect some genes such as *tpi*, *tcdA*, *tcdB*, *cdtA* and *cdtB* genes by PCR method.

Methods: A total of 310 chicken meat parts including leg quarter, breast, wing, drumstick and chicken liver samples were collected from supermarkets and butcher shops in two cities (Elazig and Diyarbakir) in Eastern Turkey. All samples were tested for the presence and numbers of *Cl. difficile* reported by Weese et al. (2009) with slight modifications. Multiplex PCR was performed to screen for *tpi, tcdA*, *tcdB*, *cdtA* and *cdtB* genes.

Results: *Cl. difficile* was isolated from 25 (8%) of 310 samples. The distribution of *Cl. difficile* in leg quarter, breast, wing, drumstick and liver samples were 12%, 8%, 6.6%, 5% and 7.5%, respectively. All of 25 *Cl. difficile* isolates were carried out *tpi* gene, of 32% *tcdA* and of 20% *tcdB* genes but none of isolates carried out *cdtA* or *cdtB* genes according to the PCR results.

Significance: This study is the first report of detection of *tcdA* and *tcdB* toxin genes in *Cl. difficile* isolated from chicken meats and liver in Turkey. Also, the present study showed that the number of *Cl. difficile* is low in retail chicken meat parts and liver, and it was isolated from the samples using the enrichment method except for one sample which had 20 spores/g.

P2-75 Prevalence, Multidrug Resistance and Biofilm Formation in *Escherichia coli* O157:H7 Isolates from Water Sources and Urine Samples of Bodija Abattoir Workers in Ibadan, Nigeria

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❖ ***Developing Scientist Competitor***

Introduction: *Escherichia coli* O157:H7 *is* a wide spread intestinal commensal found in human and animals. It is the most common cause of urinary tract infection. Biofilms formed by these bacteria allows for antimicrobial resistance and facilitate their spread and persistence.

Purpose: This study investigates the prevalence, antibiotics sensitivity profile and biofilm forming ability of *E.coli* O157:H7from water sources and urine samples of abattoir workers from the Bodija municipal abattoir, Ibadan, Nigeria for the dry and wet seasons.

Methods: Isolation of *E.coli* O157:H7 was made from eighty (80) water samples from wells and bore holes located within the abattoir and one hundred thirty-six urine samples from abattoir worker using conventional methods. Biofilms of *E.coli* O157:H7 were developed in Tryptose soy broth and Tryptose soy broth + 2% glucose and biofilm quantification was done using crystal violet binding assay. Disc diffusion method was used to determine the antibiotics sensitivity profile of the selected strains. Fifty questionnaires were administered to assess the knowledge and frequency of antibiotic usage among abattoir workers.

Results: The prevalence of *E.coli O157:H7* in water and urine are 1.25% and 5.88%, respectively. This prevalence was higher in the wet season compared to the dry season. Biofilm increased with incubation time until the 48 h. At 96 and 144 h, the biofilm mass reduced for all strains. This study revealed that 2% glucose had a significant effect on biofilm development at 48 h incubation. Most strains showed multidrug resistance. The isolates showed high sensitivity to Ofloxacin and Gentamicin but resistance to Cotrimoxazole, Augmentin and Amoxicillin. The questionnaire survey revealed that 94% of respondents abusively use antibiotics every day.

Significance: The production of biofilm and increasing resistance by these isolates to antibiotics presents a major risk and challenge in both human and animal medicine. Control of abusive usage by proper education of abattoir workers is strongly recommended.

P2-76 Tracking Cefotaxime Resistance in Beef Cattle from Birth to Slaughter Weight

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❖ ***Developing Scientist Competitor***

Introduction: The third-generation cephalosporins are used extensively in human medicine and to some extent in veterinary medicine therapeutically. The animal factors that affect the antibiotic resistance in general and cefotaxime resistance specifically are not understood.

Purpose: The purpose of this study was to evaluate animal and environmental factors that influence the prevalence of antibiotic resistant microorganisms in animals.

Methods: We tracked cefotaxime (a third-generation cephalosporin) resistance in beef calves right after their birth for one year. Samples were collected in a cohort of 300 beef calves from a multi-breed beef calf population derived from Brahman and Angus cattle. This study utilized a combination of culture-based and nucleic acid-based methods for the detection and enumeration cefotaxime resistant bacteria from the fecal samples. Data were analyzed by logistic regression methods using STATA software.

Results: Although the beef calves were never exposed to any of the cephalosporin antibiotics, the herd prevalence of cefotaxime resistance was high in the beef calves. The cefotaxime resistance was 61.07%, 50.7%, 68.57% and 6.25% in March, June, August and December, respectively. Cefotaxime resistance was not significantly associated with animal factors including breed, sex, castration or weight gain, but the influence of climate change is evidenced by the lowest prevalence of cefotaxime resistance in December samples.

Significance: Antibiotic use in animals is not the only factor that influences resistance. The present study found several factors influencing the prevalence and dynamics of cefotaxime resistance in beef cattle. Identification of all the factors will help to develop intervention strategies to control antibiotic resistance.

P2-77 Effect of Kosher Processing on Shiga Toxin-Producing *Escherichia coli* (STEC) and *Salmonella* on Surfaces of Fresh Beef and Its Quality

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Introduction: The main reason that people buy kosher products is for the impression of improved food quality and safety. However, both STEC and *Salmonella* may contaminate surfaces of fresh beef during slaughtering and many antimicrobial interventions cannot be applied due to kosher restrictions. Although the salting process to remove blood from the meat has antibacterial effects, the impact of the kosher beef salting process on foodborne pathogens has not been determined.

Purpose: To evaluate antimicrobial efficacy of kosher salt and quality of kosher beef during storage at refrigeration temperature.

Methods: Forty-two pieces of fresh beef were inoculated with cocktail mixtures of top seven STEC and *Salmonella* to approximately10^4 to 10^5 CFU/cm^2. Inoculated fresh beef was soaked with tap water for 45 min, salted (Kosher certified) for 45 min, and rinsed with water three times for 2 min each rinse. Enumeration for each kosher processing step was conducted to determine efficacy of salt in reducing target organisms after processing and after storage for 2 d at 2 to 4°C. The salt residue and lipid oxidation also were determined after processing and after storage for 7 d at 2 to 4°C.

Results: Water soaked alone or water soaked and chilled did not reduce the population of STEC and *Salmonella* on surfaces of fresh beef. Salt treatment alone reduced seven STEC strains and *Salmonella* ranging from 0.8 to 1.4 log cycle with additional 0.2 to 0.6 log cycle reduction after chilling for 2 d. The salt residue from kosher beef was 14 times higher than non-kosher beef. The amount of malondialdehyde from kosher beef increased three times compared to non-kosher beef during 7 d storage.

Significance: Kosher processing significantly reduced foodborne pathogens. However, the kosher process increased salt levels which could cause off-flavors from salt-accelerated lipid oxidation.

† USDA is an equal opportunity provider and employer.

P2-78 Prevalence of *Escherichia coli* in Small and Very Small Beef Slaughter Plants

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❖ ***Developing Scientist Competitor***

Introduction: The health and economic implications of foodborne illness continue to be key concerns of the 21st century. Restricting the transmission of pathogenic bacteria during the slaughter process is a critical step in preventing cross-contamination and avoiding foodborne illnesses related to beef and beef products.

Purpose: This study compares the transmission of coliforms and aerobic bacteria during beef slaughter in small and very small USDA-certified slaughter plants. This study is intended to provide small processors with a better understanding of intervention strategies, their effectiveness and the implications of inadequate interventions on foodborne outbreaks.

Methods: Unit operations (21 steps) in the slaughter process were identified for carcass, equipment and worker swabs. A total of 126 swabs were collected from each plant and samples were transported and analyzed on TSA and VRBA for enumeration within 24 h. All samples were incubated at 37°C for 18 - 24 h prior to enumerations.

Results: Data suggests that each unit operation from slaughter to fabrication of the beef carcasses reduced ($P < 0.05$) aerobic plate counts, total coliforms including *E. coli.* Comparison of a USDA-certified plant and a small processor indicate that there is a higher ($P < 0.05$) prevalence of coliforms and aerobic bacteria in the USDA-certified plant. Both slaughter plants demonstrated negative results during enumeration on VRBA in end product sampling, indicating the absence of coliforms. Microbial counts present after hide removal and prior to evisceration indicate transmission of bacteria via environmental factors, however, continued sampling is needed to clarify these correlations and the food safety gaps in beef slaughter.

Significance: The results of this study will be used to develop training modules for small processers and improve food safety in very small beef slaughter plants. Reducing foodborne illness will be achieved with continued open dialogue with small processers, strengthening extension and research efforts.

P2-79 Long-term Trends in the Incidence of Enterohemorrhagic *Escherichia coli* and *Salmonella* on Beef Trim and Ground Beef

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Introduction: The contamination of beef products with pathogenic bacteria is of public health concern. Beef processing plants have programs in place to reduce the occurrence of enterohemorrhagic *E. coli* (EHEC) and *Salmonella.* While there is seasonal variation in the incidence of these pathogens, we might hope that over a period of years, the frequency with which they are present would decrease due to continuous improvement in HACCP-based plant mitigation programs.

Purpose: To determine if there are significant changes in the occurrence of EHECs and *Salmonella* in beef trim and ground beef over a multi-year period.

Methods: During 10 calendar years (2005-2014), samples of beef trim from 31 processing plants were screened for the presence of EHECs and *Salmonella* using a PCR-based test. As the overall frequency of confirmed pathogen-positive samples is very low, we used data from the screening method. A total of 6,405,553 samples were tested.

Results: Of the 6,202,919 trim samples tested, 483,957 (7.8%) and 201,939 (3.3%) EHEC and *Salmonella* samples, respectively, were screen-positive. For 202,634 ground beef samples the corresponding positive samples and rates were 9426 (4.7%) and 32,949 (16.3%). During this period, EHEC in trim increased from 3.3% in 2005 to 4.9% in 2014 ($P < 0.05$) with a peak of 12.1% in 2012. The frequency of *Salmonella* in trim increased from 2.3% in 2005 to 4.5% ($P < 0.05$) in 2014. During the same period in RGB, EHEC increased from 0.3% to 8.5% in 2010 and decreased to 2.1% in 2014. *Salmonella* increased from 0.3% to 25.3% in 2011 but declined to 11.6% in 2014.

Significance: The frequency of screen-positive samples for EHECs and *Salmonella,* especially in ground beef, has significantly increased over this 7-year period suggesting that mitigation programs have not consistently improved.

P2-80 Application of Freeze-dried and Microencapsulated Extract of Co-products from Wine Processing on Oxidative Stability of Chicken Meat Pate

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Introduction: The production of wine and grape juice generates large amounts of co-products rich in phenolic compounds that can be used as natural antioxidants. The studies concerning natural antioxidants have multiplied with the increasing knowledge about the negative impact of synthetic antioxidants on human health.

Purpose: This study aimed to evaluate the effect of grape pomace extract derived from wine processing on oxidative stability of chicken meat pate.

Methods: Chicken meat (6.22 Kg) and chicken skin (1.53 Kg) were minced and homogenized with iced water (1.0 Kg), salt (0.05 Kg), carmine (0.003 Kg), curing salts (0.01 Kg) and sodium polyphosphate (0.02 Kg). This basic formulation was divided into 4 lots. The first was designated as the control and no additional ingredients were included (T1). The second lot was prepared by adding sodium eritorbate (3 $mg.g^{-1}$) (T2). The third lot received liophilized grape pomace extract (LGPE) (3 $mg.g^{-1}$) (T3). The fourth lot received grape pomace extract microencapsulated by atomizing spray-drying process (MGPE) (T4). Potential antioxidants in the test ingredients were determined as thiobarbituric acid reactive substances and expressed as mg of malonaldehyde/kg sample (MDA). Measurements were made on the day of their production and were analysed daily for a week of storage at 4°C.

Results: Lipid oxidation occurred in ascending order with time in all treatments. On the day of processing there was not significant difference in the malonaldehyde values between the pate containing liophilized extract and the pate containing microencapsulated extract ($p<0.05$). After 7 days' storage the pates treated with sodium eritorbate (2.244 mg $MDA.kg^{-1}$), liophilized grape pomace extract (1.677 mg $MDA.kg^{-1}$) and extract microencapsulated (2.029 mg $MDA.kg^{-1}$) had significantly lower values of malonaldehyde than the control pate (2.644 mg $MDA.kg^{-1}$).

Significance: Lipid oxidation was inhibited by the addition of both grape pomace extracts liophilized and microencapsulated.

P2-81 Prevalence of *Salmonella* and *Escherichia coli* O157 Found in Small-ruminants within the United States

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Introduction: *Escherichia coli* O157 and *Salmonella* are pathogens with food safety concerns and a known presence in ruminants. Information is known about the baseline of these organisms in cattle, but less research has been published about their prevalence in small-ruminants.

Purpose: The objective of this study was to determine the prevalence of *Salmonella* and *E. coli* O157 in small-ruminants across the United States.

Methods: Hide swabs (N = 167) and fecal samples (N = 169) were collected from sheep and goats at small abattoir facilities in New Mexico and Texas, from February through December 2014. Hide swabs were taken post exsanguination at four sampling locations (rump/inside leg round, midline/flank, foreshank, and neck/breast. Fecal samples were collected aseptically from the gastrointestinal tract post-evisceration. Hide swabs and fecal samples were analyzed for *Salmonella* using selective enrichment broths (Rappaport-Vassiliadis and Tetrathionate) and two different selective and differential media (Xylose Lysine Tergitol 4). *E. coli* O157 analysis was done by enrichment of hide swabs and fecal samples in Gram-negative broth with added antibiotics (8 µg/ml of vancomycin, 50 ng/ml of cefixime and 10 µg/ml of cefsulodin) to suppress background microflora. Samples were then subjected to standard immunomagnetic separation (IMS) procedures and plated onto CHROMagar O157 agar with 2.5 mg/l tellurite. A Chi-square (PROC FREQ) test was performed using SAS.

Results: *Salmonella* was detected in 21.56% (n = 36) of hide swabs and 9.47% (n = 16) of fecal samples. *E. coli* O157 was detected in 22.94% (n = 25) of fecal samples and 1.83% (n = 2) of hide swabs.

Significance: Pathogens in this study were readily isolated from these samples indicating a potential food safety problem if not properly controlled during slaughter. It will be important to investigate control measures for these pathogens during processing and validate their effectiveness in processing settings in order to ensure public health.

P2-82 Mitigating the Impact of Sanitizer Carry-over on Pathogen Monitoring

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Introduction: A number of chemicals in broiler processing aids are used to reduce or destroy pathogenic bacteria on processed carcasses. However, carry-over of active sanitizer to the analyzed rinse solution may cause false negative results and potentially confound regulatory oversight.

Purpose: This study was conducted to document antimicrobial carry over effect of cetylpyrinium chloride (CPC) in broiler carcass rinsate and test the effectiveness of a variety of neutralizing agents for counteracting such CPC activity.

Methods: Broiler carcasses were rinsed in Buffered Peptone Water (BPW) which was spiked with 10^7 CFU/ml of four serotypes of nalidixic acid-resistant *Salmonella*. CPC was added in amounts equal to what was previously found to be included in the rinse of a CPC treated and drained carcass. *Salmonella* cells were enumerated by plate count on bg-sulfa agar containing nalidixic acid. Four experimental neutralizers: lecithin, sodium polygalacturonate , poly(vinylsulfate) potassium salt and chondroitin sulfate sodium salt were tested to counteract carryover CPC in *Salmonella*-spiked carcass rinse. Three replicates for two separate trials, total n = 6, were performed for each neutralizer.

Results: Significantly ($P < 0.05$) fewer *Salmonella* were detected in un-neutralized CPC containing rinse (1.0 log) than in the water control (5.5 log). Lecithin, and poly(vinylsulfate) potassium salt were effective, resulting in numbers of *Salmonella* that were not different than in the CPC free control (5.5 log, $P > 0.05$). Chondroitin sulfate sodium salt was less effective (5.0 log) and sodium polygalacturonate was ineffective (0.3 log).

Significance: Findings indicate that the impact CPC used in poultry processing on detection of *Salmonella* from carcass rinses may be mitigated by incorporation of lecithin or poly(vinylsulfate) potassium salt in carcass rinse solutions.

P2-83 Characterizing Differences in Shiga Toxin-Producing *Escherichia coli* (STEC) Attachment to Pre-Rigor and Chilled Beef Carcass Surfaces

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Introduction: The United States Department of Agriculture declared seven Shiga Toxin-producing *E. coli* (STEC) serotypes to be adulterants in raw, non-intact beef products due to their severe health implications. STEC contamination of carcasses is most likely to occur during hide removal.

Purpose: This study evaluated efficiency of a mixed STEC inoculum to attach to raw beef carcass (lean or fat), and efficacy of 4.5% lactic acid (LA) or water (W) spray to reduce STEC populations, under four scenarios: (A) pre-rigor surface STEC inoculated (ca. 4 log CFU/cm^2), 30-min ambient temperature attachment, spray with LA or W; (B) pre-rigor inoculated, 24-h chilled attachment, spray; (C) tissue chilled 24 h, inoculated, 30-min attachment, spray; and (D) tissue chilled 24 h, rewarmed to 37°C, inoculated, 30-min attachment, spray.

Methods: Lean and fat tissues (230 cm^2) were collected from two fed cattle at harvest and assigned to the four scenarios, followed by post-inoculation ambient temperature W or LA spray. Tissue samples were collected pre- and post-treatment to enumerate STEC populations. The experiment was a completely randomized design with four replicates.

Results: STEC attachment levels to lean and fat tissues were similar across scenarios ($P > 0.05$). LA reduced STEC levels more effectively than water ($P \leq 0.05$). A significant treatment by scenario interaction was observed for STEC reductions, with LA being more effective in scenarios A and B (pre-rigor inoculation) than C and D (post-rigor inoculation).

Significance: LA spray is more effective for pre-rigor carcasses compared to chilled beef surfaces for reducing STEC. For laboratory studies, consideration must be given to when inoculum is applied to tissue surfaces to accurately determine effectiveness of antimicrobial treatments. Variability in pathogen inoculation protocols for carcasses and subprimals appears in the literature; complicating process control determinations and antimicrobial intervention comparisons. These findings provide guidance to processors and researchers regarding inoculation protocols and their potential impacts on microbiological results.

P2-84 Pre-Chill Antimicrobial Treatment to Enhance the Safety of Chicken Parts

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Introduction: There is an increase in microbial prevalence as a chicken carcass transitions from a broiler to cut-up parts. One hypothesis to explain this occurrence is that bacteria in water retained during the pre-chill processing step is released upon cut-up, leading to contamination of chicken parts.

Purpose: The objective of this study was to determine the effectiveness of applying an antimicrobial treatment in the pre-chill tank to reduce the prevalence of *E. coli, Campylobacter,* and *Salmonella* on chicken parts.

Methods: Commercially eviscerated broiler carcasses were collected and subjected to sequential pre-chill and cut-up procedures in a pilot plant. Selective enrichment and isolation of *Campylobacter* spp. and *Salmonella* spp., as well as enumeration of total aerobic bacteria and *E. coli*/coliforms, was performed using carcass rinses at the following points: before processing, after 15 minute pre-chill (22 - 25°C water), after 30 min chill (0 - 4°C water), and following cut up into parts. Six pre-chill, antimicrobial treatments were evaluated: 50 ppm chlorine, 50 ppm chlorine + 0.5% T-128, 20 ppm peracetic acid, 20 ppm peracetic acid + 0.5% T-128, 0.5% T-128, and water.

Results: Addition of 0.5% T-128 to water, 20 ppm peracetic acid, and 50 ppm chlorine during pre-chill resulted in a significant decrease by ~1.5 - 2.0 log ($P < 0.05$) in total aerobic bacteria and *E. coli*/coliforms following pre-chill. A significant increase by ~0.5 log ($P < 0.05$) following cut-up was only observed with *E. coli*/coliforms in the previously mentioned wash solutions.

Significance: Results of this study concluded that water retention in the pre-chill tank is likely not the cause of increased microbial prevalence on chicken parts and indicate the source may be attributed to other modes of contamination such as poor sanitation in processing facilities or cross-contamination by workers during cut-up. However, Smartwash T-128 may serve as an effective additive to wash water in the poultry industry.

P2-85 Radio-frequency Pasteurization of Inoculated Ground Beef Homogenate with *Escherichia coli*

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Introduction: Radio-frequency (RF) is an innovative emerging technology and highly effective in reducing microbial contamination. It has the potential for pasteurization and sterilization of foods for commercial applications. Decontamination of beef with RF heating and selected antimicrobials may have potential for preserving the efficacy of applied antimicrobial as well as the quality attributes of processed beef product.

Purpose: The objective of this study was to investigate the effects RF pasteurization on microbial inactivation of *E. coli* with selected antimicrobials in beef homogenate.

Methods: Ground beef homogenate was inoculated (cell concentration of 6 log CFU/ml) with five strains of non-pathogenic *Escherichia coli* (ATCC BAA-1427, BAA-1428, BAA-1429, BAA-1430, and BAA-1431, all cattle isolate). These strains were used as surrogates for the *E. coli* O157:H7. The inoculated beef homogenate was packaged in a retortable plastic bag and subjected to RF heating (50 and 55°C). The RF heating temperature was

monitored and recorded with a fiber optic probe. Microbial assays for coliform, mesophilic aerobic plate count (APC), and *E. coli* were performed to enumerate total coliform, *E. coli*. and APC counts were performed on 3M plates. The data was analyzed by two-way ANOVA (treatment × final temperature) using the JMP PRO 10 program.

Results: Results from this study revealed that RF pasteurization at 55°C of inoculated beef homogenates in the presence of 1.5% potassium bicarbonate with 0.5% citric acid resulted in a 6-log CFU/ml reduction of *E. coli* and mesophilic aerobic bacteria. Similar reduction in microbial population was observed with 2.5% lactic acid with 0.5% potassium bicarbonate combinations at 55°C temperature.

Significance: Decontamination of beef using antimicrobial intervention with RF heating may have potential for preserving the efficacy of applied antimicrobial as well as the quality attributes of beef products.

P2-86 Evaluation of *Escherichia coli* Biotype I Surrogates as Predictors of Non-O157:H7 Shiga Toxin-Producing *E. coli* (STEC) for Acid Resistance, Freezing, and Refrigerated Storage

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Introduction: The percentage of non-O157 serotype infections is increasing in many countries; therefore, acid interventions are often applied to beef as antimicrobial treatments. Six non-O157 STEC serotypes identified as O26, O111, O121, O145, O103, and O45 have been selected as target pathogens for comparison against the identified surrogate bacteria.

Purpose: To evaluate acid-resistance and storage conditions of potential surrogates for non-*E. coli* O157 STECs.

Methods: Non-O157 Shiga Toxin-producing *E. coli* (STECs) (ATCC # 2192, 2193, 2196, 2215, 2217 and 2219), *E. coli* biotype I surrogates (ATCC # BAA-1427, BAA-1428, BAA-1429, BAA-1430 and BAA-1431), and Rifampicin-resistant *E. coli* biotype I surrogates were cultured in tryptic soy broth (TSB) at 37°C for 18 h. Stationary and acid-adapted organisms were transferred into 10 ml of pre-warmed phosphate buffer saline (PBS) acidified with L-lactic acid at pH 2.5, 3.0 and 3.5. At pre-determined time intervals, enumeration was conducted to evaluate acid resistance. For freezing (-20 ± 0.5°C) and refrigerated (4 ± 0.5°C) storage, bacterial strains were enumerated on days 0, 7, 14, 21, 28, 60, and 90. Data were analyzed using GLM of SAS.

Results: For acid resistance, most *E. coli* biotype I microorganisms had similar ($P > 0.05$) reductions to the non-O157 STECs; however, there were some instances of greater ($P < 0.05$) reductions. Both refrigerated and frozen storage resulted in random differences between counts of *E. coli* biotype I microorganisms and non-O157 STECs; however, there were no notable trend or patterns observed. Findings for both acid resistance and storage conditions support previous research stating that surrogates may best be used as a cocktail rather than as single strains.

Significance: This research supports that *E. coli* biotype I microorganisms may serve as surrogates for acid resistance and storage conditions for these six non-O157 STECs.

P2-87 Validation of Thermal Cook Cycles for *Salmonella* Reduction on Whole Muscle Beef or Pork Slices under Simulated Industry Conditions

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Introduction: Validation of treat production processes for the elimination of *Salmonella* is needed due to the proposed regulations requiring risk-based preventative controls under the Food Safety Modernization Act.

Purpose: The objective of this study was to evaluate thermal cook cycle parameters in achieving a 7-log CFU/g reduction of *Salmonella* strains on whole muscle sliced beef or pork pieces.

Methods: Raw, whole muscle sliced beef or pork pieces were fully submersed into a *Salmonella* cocktail (*Salmonella* Heidelberg, *Salmonella* Typhimurium, *Salmonella* Enteritidis) for 60 sec, then placed onto racks for 30 min to allow attachment. Experimental cook cycle parameters were programmed into an Enviropak™ Minipak series commercial oven. One sample was pulled to determine concentration on pre-treated slices. Slices were put into the pre-heated oven. One sample was pulled for analysis at the end of each cook cycle step, then each hour past "Step 3" until the target water activity was met. The experiment was performed in duplicate. A 10 g sample was taken, serial dilutions were made, then plated on XLD with a thin TSA overlay. The plates were incubated for 37°C for 24 h and counted to determine surviving populations. Counts were converted to log CFU/g prior to statistical analysis. Samples with an inconclusive count were analyzed through culture confirmation and agglutination.

Results: The cook cycle showed a ($P < 0.05$) reduction of *Salmonella* by approximately 7.2 log CFU/g for beef slices and 7.3 log CFU/g for pork slices. However, viable *Salmonella* cells were detected after enrichment for most time points.

Significance: This study demonstrates a reduction of 7.0 log CFU/g *Salmonella* is achieved during the cook cycle, but that viable survivors remain. An increase in the relative humidity at the beginning of the cycle should be evaluated as a means to further reduce survivability.

P2-88 Validation of 5-log *Salmonella* Reduction on Moab Bones under Simulated Industry Conditions

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Introduction: The Food Safety Modernization Act includes proposed regulations for pet food producers that require risk-based preventative controls. Pet treat companies are under increased scrutiny due to recent recalls. Therefore, validation of treat production processes for the elimination of pathogens in these products is needed.

Purpose: To was to determine the reduction of *Salmonella* on inoculated Moab (knuckle) bones under simulated industry conditions.

Methods: Bones (n=10/rep) were full submersed into a *Salmonella* cocktail (*Salmonella* Heidelburg, *Salmonella* Typhimurium, and *Salmonella* Enteritidis) for 2 min, then set onto racks in the cooler for 30 min to allow for *Salmonella* attachment to occur. One sample was pulled to determine concentration on pre-treated bones. Bones were placed into a pre-heated Enviropak™ Minipak series commercial oven at 195°F for 240 h (10 d). One sample was pulled for analysis at the following time points: 24 h, 2, 4, 6, 8, and 10 d. The experiment was performed in duplicate. Serial dilutions were made and plated onto XLT4 agar with a thin TSA overlay. Plates were incubated at 37°C for 24 - 48 h then counted to determine surviving populations. Counts were converted to log CFU/bone prior to statistical analysis. Samples with no growth after 48 h of incubation were considered negative for *Salmonella*.

Results: The drying cycle reduced *Salmonella* populations by approximately 7.6 log CFU/bone after 24 h of drying, which is a significant ($P < 0.05$) reduction. All samples taken after 24 h of drying were negative for *Salmonella* ssp.

Significance: This validation of Moab bone processing demonstrates that a 7.0 log CFU/bone reduction can be achieved using a drying cycle of 195°F for 10 d. When this cycle is used within the plant, it would be effective in eliminating *Salmonella* ssp. The validation of these process steps are an important practice to fulfill the more rigorous requirements being developed for pet food regulations.

P2-89 Validation of the Efficacy of 3.41% Lactic Acid, 4.55% Lactic Acid, and 3.87% Purac Spray 80 under Simulated Industry Conditions

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Introduction: Lactic acid spray (LA) is frequently applied to beef carcasses as an antimicrobial intervention to reduce STECs and *Salmonella* that may be present.

Purpose: To determine the efficacy of 3.41% LA, 4.55% LA, and 3.87% Purac Spray 80 in reducing cocktail mixtures of 7 major serogroups of STEC considered adulterants by the USDA-FSIS and *Salmonella*.

Methods: Surface carcass samples were obtained from pre-rigor, freshly slaughtered cattle processed at a local slaughter facility. For each replication, n = 2 - 150 cm^2 pieces were used for: no treatment, 3.41% LA, 4.55% LA, and 3.87% Purac Spray 80, all heated to 125°F. The study was completed in duplicate. Pieces were submersed in either a cocktail of STEC or *Salmonella* for 1 min, then placed onto racks for 30 min to allow for attachment. Untreated pieces were swabbed (50 cm^2) to determine initial pathogen concentration. Immediately after attachment, the pieces were sprayed for 15 s in an industry equivalent CHAD spray cabinet. Treated samples were swabbed within 1 h of treatment to determine reductions on day 0. Pieces were stored at 0°C and separate areas were swabbed on days 1 and 2 to determine pathogen reductions. Swabs were stomached, serial dilutions were prepared and plated onto MacConkey or XLT4 agar, both with thin TSA overlay. Populations were converted to log CFU/cm^2prior to statistical analysis.

Results: Each treatment significantly ($P < 0.05$) reduced the concentration of *Salmonella* by approximately 2.0 log CFU/cm^2. STEC populations were significantly ($P < 0.05$) reduced by approximately 0.80 log CFU/cm^2 by 4.55% LA and Purac Spray 80 and by 0.60 log CFU/cm^2 by 3.41% LA. Significant reductions were noted for days 1 and 2 for both pathogens.

Significance: These data indicate that an intervention of lactic acid spray or Purac Spray does effectively reduce *Salmonella* and STECs on pre-rigor carcasses, thereby improving the safety of the product.

P2-90 Effect of pH and Water Activity on Inactivation of Non-O157 Shiga Toxin-Producing *Escherichia coli* (STECs) in Acidulated Beef Sausage by High Pressure Processing

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Introduction: High pressure processing has gained use in the meat industry to achieve pathogen reduction in products with minimal impact on product quality. The USDA-FSIS has declared six additional Shiga Toxin-producing *E. coli* (STEC) serogroups as adulterants in non-intact raw beef products in response to increasing human illness from these organisms.

Purpose: The objective of this study was to evaluate the effect of pH and water activity (a_w) on the efficacy of high pressure processing (HPP) for inactivation of non-O157 STEC in acidulated beef sausages.

Methods: A six-strain cocktail of non-O157 STECs (*E. coli* O26, O45, O103, O111, O121 and O145; ca. 7 log CFU/ml) was inoculated into irradiated, acidulated beef sausages of different pH (4.0 - 6.0) and a_w (0.950 - 0.980) values. Samples were pressure-treated at 586 MPa for 1 s, 1 min, 3 min and 5 min. STEC populations were enumerated on Petrifilm™ *E.coli*/Coliform Count Plates and Aerobic Count Plates.

Results: An STEC inoculation level of 6.96 ± 0.17 log CFU/g was achieved in sausages. Achieving the target pressure of 586 MPa (1 s) resulted in 0.90, 0.20, 0.39 and 0.44 log CFU/g reductions in STEC populations in beef (pH 6.0) with a_w values of 0.980, 0.970, 0.960 and 0.950, respectively. Increasing the pressure to 586 MPa (1 s) resulted in 0.90, 0.22, 0.46, 1.70 and 1.41 log CFU/g reductions in STEC populations in beef (a_w 0.980) with pH values of 6.0, 5.5, 5.0, 4.5 and 4.0, respectively. Greater STEC reductions were observed with increasing HPP processing time (586 MPa), decreasing pH and increasing a_w.

Significance: High pressure processing can be used as an effective method to achieve STEC reductions in beef sausages of various pH and a_w values.

P2-91 High Pressure Processing of Beef Summer Sausage to Reduce *Escherichia coli* Populations

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Introduction: The USDA-FSIS has declared *E. coli* O157:H7 and six additional serogroups of Shiga Toxin-producing *E. coli* (STEC-7) as adulterants in certain beef products. *E. coli* O157:H7 has been implicated in foodborne illness outbreaks related to fermented sausage products.

Purpose: The objective of this study was to utilize STEC surrogates to evaluate high pressure processing (HPP) as an alternative to thermal treatment to reduce STEC-7 in compliance with regulatory performance standards, with minimal effect on product quality.

Methods: A five-strain rifampicin-resistant, non-pathogenic *E. coli* cocktail (*E. coli* O157:H7 surrogates; BAA-1427, BAA-1428, BAA-1429, BAA-1430, BAA-1431), was used for inoculation. Sausages were fermented at ~108°F, and the product temperature was slowly increased to 130°F, cold showered, and chilled for 6 h in a cooler. Samples were collected post-fermentation (PF; pH 5.0), when product internal temperature reached 120°F (I-120°F), 130°F (I-130°F), and after chilling (PC). Sausages from each treatment were sliced, vacuum packaged and processed (HPP; 586 MPa) for 1 s, 1, 2, 3, 4 and 5 min. *E. coli* populations were enumerated on Petrifilm™ (Aerobic Plate Counts, *E. coli*/coliform).

Results: *E. coli* populations of ca. 7.4 log CFU/g were obtained in the batter subsequent to inoculation. *E. coli* reductions of 1.2, 1.3, 3.1 and 4.2 log CFU/g or cm^2 were achieved at PF, I-120°F, I-130°F and PC, respectively. HPP resulted in *E. coli* reductions of 2.6, 3.2 and > 3.4 (total reductions) log CFU/cm^2, at 1 s, 1 min and 2 min, respectively, in summer sausage (PC). HPP (1 s) of summer sausage that was heated to 130°F (no hold), resulted in > 6.7 log CFU/g or cm^2.

Significance: Currently, manufacturers rely on thermal processing to achieve the performance standard (5.0-log reduction) for *E. coli* O157:H7. HPP can be utilized as an alternative non-thermal lethality treatment to obtain the required reduction of *E. coli* O157:H7.

P2-92 Biofilm-forming Ability and Tolerance to Industrial Sanitizers of *Salmonella* spp. Isolated from Brazilian Poultry Processing Plants

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Poster

Introduction: Formation of biofilms by *Salmonella* spp. is a constant concern for the food industry due to their attachment to surfaces and risk of contamination of food products. Moreover, the biofilm-forming may reduce the biocidal effect of disinfectants.

Purpose: The aim this study was analyzed the correlation between biofilm formation in different materials and the presence of the genes *adrA* and *csgD.* Tolerance of the biofilm against common sanitizers used in the industry (chlorinated alkaline cleaners, peracetic acid, and the two combined) was also evaluated.

Methods: Biofilm-forming ability of 98 strains isolated from conveyor belts of poultry cutting rooms was evaluated in polystyrene plates, also was analyzed in 1-cm^2 polypropylene (PP) and polyurethane (PU) slides, and the slides were analyzed by scanning electron microscopy. Sanitizer efficiency was evaluated after 96-h incubation for biofilm formation, viable cells were removed from the surfaces soon after the treatment, and after reincubation for 96 h, by vortexing tubes with glass beads.

Results: Only one strain was a strong biofilm-producer in polystyrene, ability to produce microfilms was weak in 70% of strains, and moderate in 29% and both genes were found in all strains. Important differences in adhesion were observed between the materials, scanning electron microscopy showed that PU had a more irregular surface. No viable cells were recovered in PP slides treated with sanitizers, in PU the reduction in viable cell counts observed soon after sanitizer treatment was enough to consider that sanitizers were efficient, after reincubation, this difference was smaller for the treatment with peracetic acid.

Significance: The results of this study are a warning in food safety, due to the importance of the isolation of strains that are able to produce biofilms both on *in vitro* testing materials and in materials that are used as cutting surfaces in poultry processing plants.

P2-93 Surface Type and Inoculum Level Impacts the Integration of *Escherichia coli* Strain 0811 into Complex Biofilm Communities Formed under Simulated Meat Processing Conditions

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Poster

Introduction: Most biofilm studies involving bacterial foodborne pathogens have been conducted using monocultures. However, to better understand the persistence of these bacteria within food-processing environments, studies involving multi-species systems need to be implemented.

Purpose: To investigate the fate of *E. coli* strain 0811 (serotype O103) during the development of polymicrobic biofilms formed under simulating-meat-processing-conditions (SMPC).

Methods: Biofilms were grown on stainless steel (SS) and high density polyethylene (HDPE) coupons during SMPC for 12 days at 15°C in replicated trials. Homogenates derived from environmental swabs (floor drains from processing facility) were used to inoculate bioreactors. After 24 h *E. coli* was introduced at high or low levels (10^6 or 10^3 CFU/ml, respectively). Total planktonic bacteria and *E. coli* were monitored daily, whereas biofilms were analyzed during days 2, 5, 8, and 12. Community profiling was conducted by 16S rRNA gene sequencing.

Results: Biofilm cell counts for *E. coli* monocultures were greater ($P < 0.05$) on HDPE (1.5×10^7 CFU/cm^2) than SS (7.6×10^6 CFU/cm^2). However, when competing with environmental microflora, *E. coli* only survived (at low levels) if introduced at high levels. *E. coli* planktonic levels of 5.2×10^4 CFU/ml and 3.1×10^2 CFU/ml after 3 and 10 days, respectively, were maintained against a total bacterial population ranging up to 3×10^8 CFU/ml. On day 12 total counts for biofilm populations on HDPE (4.2×10^7 CFU/cm^2) exceeded those on SS (2.1×10^7 CFU/cm^2) ($P < 0.05$) while *E. coli* levels were 1.8×10^3 CFU/cm^2 on HDPE and not consistently detectable on SS. The HDPE community profile was dominated by *Lactococcus* spp. and *Yersinia* spp. (~70%) whereas on SS, *Yersinia* spp. (36%) and *Janthinobacterium* spp. (23%) were most prominent.

Significance: Although *E. coli* strain 0811 is a proficient biofilm-producer in monoculture it does not compete well within the complex biofilm community formed during our SMPC regimen.

P2-94 Isolation of *Lactobacillus* Strains from Siahmazgi Cheese to Study their Protective Behavior after Inoculation in Fermented-Sausage Model Medium

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Introduction: Lactic acid bacteria (LAB), economically an important group of bacteria for food production, has been used as starter culture in the production of fermented sausages. Isolation and screening of microorganisms from naturally occurring processes have been the most common means for obtaining beneficial cultures for scientific and commercial purposes. Fermented sausages have traditionally been produced by spontaneous ripening with LAB, since the indigenous LAB originating from fermented meats are well adapted to the conditions of meat fermentation.

Purpose: The aim of this study was to evaluate the LAB strains isolated from Siahmazgi cheese to determine their suitability for use as starter cultures in fermented-sausage production.

Methods: Seventy-one LAB were isolated from a sample of Siahmazgi, an Iranian traditional cheese. Lactobacilli were then screened for some technological properties such as rapid production of lactic acid, growth in different salt concentrations, gas production from carbohydrates, catalase activity and antimicrobial activity. Finally, the selected lactobacilli were tested for their growth profile in fluid model-medium modified according to the special conditions of fermented sausages.

Results: It was determined that these strains have particularly marked technological properties, being rapid acid producers and salt tolerant, and having antimicrobial activities against some Gram-positive and Gram-negative bacteria. Likewise they had capacity to grow, and to decrease pH values, under simulated conditions of fermented sausages. The lactobacilli were able to grow at a high salt concentration of 10%. They also showed antimicrobial activities against *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli.* Acceptable souring properties and good growth properties in simulated fermented sausage medium were found for these isolates with maximum number 7.99 log CFU/ml.

Significance: We concluded that the isolates exhibited the best technological properties to act as starter cultures for manufacturing fermented meat products. This research confirmed the protective effect of *Lactobacillus plantarum* in a model sausage environment.

P2-95 Fate of *Salmonella* Species, *Escherichia coli* O157:H7 and *Listeria monocytogenes* during the Manufacture of Dry Cured Westphalian Style Ham

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Introduction: Westphalian ham is a dry cured, Ready-to-Eat product that is manufactured without a lethal heat treatment. Hams are preserved by a process that involves curing with salts and spices, fermentation, smoking and drying. These processes have to be validated to successfully control the growth of pathogens.

Purpose: Examine the fate of *Salmonella* spp., *E. coli* O157:H7 and *L. monocytogenes* during the manufacture of dry cured Westphalian style ham.

Methods: Intact hams were surface inoculated with a three-pathogen cocktail resulting in a final population of at least 10^8 CFU/cm^2 of each pathogen and held at 4°C for 24 h prior to application of a dry rub containing salt, spices and fermentation starter culture of BactoFerm® C-P-77 or BactoFerm® C-P-77 and bio-protective anti-listeria culture SafePro® B-LC-48 combined and then subjected to a 70 day process of fermentation, smoking and drying. Enumeration of inoculated pathogens, pH, a_w and total viable counts on external surfaces were performed on both inoculated and uninoculated hams before any treatment and after inoculation, salting, smoking and drying.

Results: Both *Salmonella* spp. and *E. coli* O157:H7 decreased by at least 5 log CFU/cm^2 regardless of the bio-protective and/or fermentation culture used. *L. monocytogenes*, on the other hand, decreased by 4.41 and 5.64 log CFU/cm^2 on hams with fermentation culture or fermentation and bio-protective culture combined, respectively. The initial 28 day salting stage had the largest effect in reduction of the inoculated pathogens. Water activity of the product decreased from 0.98 (day 1) to 0.93 (fermentation culture) and 0.90 (combined starter culture) on day 70.

Significance: The Westphalian ham production process employed in this study is adequate for the reduction of *Salmonella* spp., *E. coli* O157:H7 and *L. monocytogenes* by at least 4.4 to 5.5 log CFU/cm^2. The salting step in the manufacture process is the most crucial step in pathogen reduction.

P2-96 Survival of Non-O157 STEC and *Escherichia coli* O157:H7 during the Manufacture of Dry Fermented Sausages

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Introduction: In response to *E. coli* O157:H7 outbreaks associated with DFS, regulatory agencies require processors to validate their production processes to demonstrate a 5-log reduction in *E. coli* O157:H7 numbers. More recently, non-O157 STEC have been responsible for outbreaks linked to fermented sausages.

Purpose: Compare the survival of the top six non-O157 STEC with *E. coli* O157:H7 during the manufacture of DFS.

Methods: Batches of uninoculated sausages and sausages containing individual strain of serotype O26, O103, O45, O111, O121, O14 and O157 at 10^7 CFU/g were produced. The sausages were subjected to fermentation (48 h) followed by ripening (72 h) and drying (34 days) to obtain dry salami. Microbiological (STEC and total plate counts), pH, a_w and MPr changes were monitored during the 39-day process. Changes in STEC numbers were analyzed by ANOVA for significance among serotypes and regression analysis to determine the rate of pathogen reduction.

Results: All serotypes showed a minimum of 5-log reduction in numbers by the end of the 39-day curing process. Significant pathogen reduction happened in the 48-h fermentation step ($P < 0.0001$), when the pH dropped from 5.6 to 4.7. Serotype O45 showed the highest reduction (1.95 log), while O26 had the least (0.97 log) reduction. The 72-h ripening step resulted in less than 1-log reduction. Regression analysis revealed that the most rapid pathogen reduction happened during the fermentation step followed by a slow but consistent reduction during the 34-day drying, with variation between serotypes, for example, O45 and O145 could not be enumerated past 27 days, while O157, O111 and O103 could be enumerated at 34 days even after the a_w had reached 0.80.

Significance: The results indicate that DFS manufacturing processes validated to demonstrate a 5-log reduction of *E. coli* O157:H7 could result in a similar reduction of the top six non-O157 STEC serotypes.

P2-97 Transfer of *Listeria monocytogenes* during Slicing of Cooked Ham

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Introduction: Slicing of processed Ready-to-Eat meat products at retail level can be a critical step for cross-contamination with pathogenic bacteria, especially *Listeria monocytogenes.* Predictive models can be helpful in establishing the best practices to minimize the transfer during this procedure.

Purpose: The study aimed at generating data on transfer of *Listeria monocytogenes* during slicing of cooked ham at retail, to be used in the development of a cross-contamination predictive model.

Methods: Experiments were carried out with pieces of cooked ham purchased in supermarkets and checked for absence of *Listeria monocytogenes* using ISO 11290-2:1998 method. Initially, a meat matrix was created in a manual meat slicer by slicing a *L. monocytogenes*-negative piece of ham. Another piece of ham was experimentally contaminated by immersion for 30 min in a suspension containing *L. monocytogenes* (8 log CFU/ml) and sliced, causing the experimental contamination of the slicer. Subsequently, new pieces of non-contaminated ham were sliced, until 40 (first trial) and 200 (second trial) slices were obtained. The extent of pathogen transfer (cross-contamination) was determined counting *L. monocytogenes* in all slices in the first trial and in the first ten sequential slices and then in every 5th/10th slice in the second trial.

Results: Two transfer scenarios were observed: counts of *Listeria monocytogenes* in the first slices were 5 log CFU/g and after the 25th slice the counts decreased gradually but formed a long tail. Even the last slice was *L. monocytogenes* positive.

Significance: These results confirm that slicing at retail level is an important source of cross-contamination of Ready-to-Eat meat products. These data, combined with other experiments with lower levels of experimental contamination, will be useful for the development of cross-contamination predictive models.

P2-98 Pathogen Reductions Associated with Traditional Processing of Landjäger: A Pilot Study

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❖ ***Developing Scientist Competitor***

Introduction: There is an increased need for regulatory compliance of non-heat treated, shelf-stable products. While a heat treatment is effective in reducing pathogens of interest, it may impart undesirable sensory characteristics in traditional, fermented, semi-dry sausages.

Purpose: The goal of this study was to determine if traditional processing (fermentation and drying; no heat step) would result in a 5 log CFU/g reduction of *Escherichia coli* O157:H7 (EC), *Listeria monocytogenes* (LM) and *Salmonella* Typhimurium (ST) in experimentally-inoculated Landjäger.

Methods: Pre-seasoned meat batter (4°C) containing pork, beef, and a commercial starter culture (*Pediococcus acidilactici, P. pentosaceus, Staphylococcus carnosus, S. xylosus,* and *Lactobacillus sakei),* was obtained from a sausage processor. The meat batter was divided proportionally and inoculated with individual pathogens or a pathogen cocktail of EC, ST, and LM. A negative control was made by inoculating sausage batter with 0.1% sterile buffer. Sausage links (~50 g each) were formed, pressed, fermented (24°C, for 72 h to pH ~4.8), cold smoked, and dried to a water activity of 0.88. Sausages were vacuum packaged in 3 mil plastic barrier film pouches and stored up to 20 days at ~23°C. Two sausages per treatment were randomly selected at each time point for microbial analysis, pH measurements, and water activity.

Results: Results suggest that fermentation and drying alone could reduce the pathogens > 3 log. LM was reduced 5.5 and 5.3 log CFU/g in individual- and cocktail-inoculated sausages, respectively. Similarly, ST was reduced 5.6 and 4 log CFU/g in individual- and cocktail-inoculated sausages, respectively. However, the process only achieved a ~4 and 3 log CFU/g reduction of EC in individual- or cocktail-inoculated sausages.

Significance: This study is the first to demonstrate that traditional processing of Landjäger, without a heat step, may result in a safe product. Future studies will address the impact of the traditional fermentation process on sausages made with all-pork formulations.

P2-99 Viability of *Listeria monocytogenes* on Boneless, Water-added Hams, Commercially Prepared with and without Food Grade Chemicals, during Extended Storage at 4°C and/or -2.2°C

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Introduction: The frequent handling and extended shelf life of deli-style hams justifies additional research to validate interventions to inhibit *Listeria monocytogenes* (*Lm)*.

Purpose: Determine viability of *Lm* on casing-cooked ham, formulated or surface treated with food grade antimicrobials, during refrigerated and/or frozen storage (i.e., sleeping at -2.2°C).

Methods: Hams were commercially prepared +/- potassium lactate and sodium diacetate (PM; 1.6%), buffered vinegar (BV; 2.2%), buffered vinegar and potassium lactate (VL; 1.7%), or potassium lactate, potassium acetate, and sodium diacetate (LAD; 1.7%), as well as +/- surface treated with lauric arginate ester (LAE; 44 ppm). In Phase I, hams (ca. 3.5 kg each) were sliced and inoculated (ca. 4.3 log *Lm*/slice), treated with LAE, and stored at either 4°C for 120 days or at -2.2°C for 90 days and then at 4°C for 120 days. In Phase II, inoculated/treated whole hams (ca. 1.0 kg each) were stored at -2.2°C for up to 6 months.

Results: In Phase I, without antimicrobials *Lm* increased by > 5.0 log CFU/slice within 90 days at 4°C; however, *Lm* increased slightly for hams formulated with PM and decreased by ca. 1.2 log CFU/slice when formulated with the other antimicrobials. For slices held/slept at -2.2°C and then stored at 4°C but not treated with LAE, *Lm* increased ca. 5.0 log CFU/slice for controls, whereas when formulated with antimicrobials, *Lm* decreased by ca. 1.5 log CFU/slice. For product treated with LAE, *Lm* increased ca. 4.5 log CFU/slice for controls, whereas when formulated with antimicrobials, *Lm* decreased by ca. 0.9 log CFU/slice for PM and by ca. 1.5 to 2.0 log CFU/slice for BV, LAD, and VL. In Phase II, *Lm* decreased by ca. 4.0 to 5.0 log CFU/ham when held/slept at -2.2°C.

Significance: Sleeping hams at -2.2°C was listericidal and inclusion of antimicrobials suppressed outgrowth of *Lm* during extended storage.

Poster

P2-100 Effect of Nitrite Concentration on *Clostridium perfringens* Growth during Extended Cooling of Cured Ham

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Introduction: The Food Safety and Inspection Service (FSIS) provides Appendix B as a guideline to define cooling parameters needed to limit *C. perfringens* growth to < 1-log increase in cured (100 ppm $NaNO_2$) meat and poultry products. Few published studies have compared the growth of *C. perfringens* in cured products formulated with nitrite concentrations greater than 100 ppm, along with extended cooling profiles which are relevant to products such as large diameter hams.

Purpose: To determine the inhibition of *Clostridium perfringens* growth in a 25-h biphasic cooling curve using various concentrations of nitrite.

Methods: Six treatments of ground ham were prepared with 0, 50, 75, 100, 150, or 200 ppm $NaNO_2$ and 547 ppm sodium erythorbate. Formulations were inoculated with *C. perfringens* (3-strain mixture) to yield 3 log CFU/g. Individual 50-g portions were vacuum-packed, cooked to 72°C, and cooled from 54.4°C to 26.7°C in 10 h, then from 26.7°C to 7.2°C in an additional 15 h. Triplicate samples were assayed for *C. perfringens* growth at 0, 5, 7.5, 10, and 25 h by plating onto tryptose-sulfite-cycloserine agar. The study was repeated three times.

Results: Populations of *C. perfringens* increased an average 6.4, 4.4, and 1.9 log at 7.5 h (~34°C) for the 0, 50, and 75 ppm nitrite treatments, respectively, but no additional growth was observed for the remaining 17.5 h cooling from 34 to 7.2°C. In contrast, samples with 100, 150, and 200 ppm nitrite plus 547 ppm erythorbate prevented < 1.0-log increase for the duration of the 25-h experiment.

Significance: The study showed that combinations of > 100 ppm $NaNO_2$ plus 547 ppm erythorbate are effective in inhibiting *C. perfringens* in ham through a 25-h cooling period. Therefore, it can be concluded cured meat and poultry products, such as ham, can be safely cooled following the longer cooling parameters identified in this study.

P2-101 Food Safety in Bacon by Cultures: Efficiency Proof by Challenge Testing and Metagenomics Analysis

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Introduction: Bio-protection is the practice of adding selected safe bacteria to foods in order to inhibit the growth of unwanted microorganisms. The process consists in substituting hurdles or adding additional ones that pathogens and spoilage bacteria cannot surmount while pursuing a clean labeling strategy. Through the inhibition of bacterial growth, microbial quality is improved but also color, texture, taste deviation could be avoided.

Purpose: This strategy was successfully used to improve the quality of cured bacon particularly to control *Listeria monocytogenes* and *Staphylococcus aureus* growth during its process and its shelf life.

Methods: To demonstrate the efficiency of SafePro® B-LC-77 (*Pediococcus acidilactici* and *Staphylococcus carnosus*) such a culture was applied (addition into the brine before its injection into pork bellies) during a bacon process. To check the right implantation of both strains from the SafePro®B-LC-77 metagenomic analyses were performed at the beginning and at the end of the bacon shelf life. In addition challenge tests were conducted to evaluate the growth potential of both pathogenic bacteria during the ripening (15 h at 95°F), the most sensitive step of the bacon process and the shelf life of the meat product.

Results: It was demonstrated that both strains from SafePro® B-LC-77 strongly dominate the endogenous flora from bacon during the whole shelf life. The addition of such an ingredient allowed a 2-log decrease of the *L. monocytogenes* concentration during the ripening step and another 2-log during the 28 days of storage at 68°F. In addition, the growth of *S. aureus* was also totally inhibited during the ripening and the bacon shelf life.

Significance: The use of culture can thus be considered as a natural anti-microbial agent to suppress and/or limit the growth of pathogenic bacteria in meat products.

P2-102 Assessment of Temperature at Recommended Methods of Determining Doneness in Egg Recipes

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Introduction: USDA and FDA recommend that egg dishes be cooked to an end-point temperature of 160°F. However, a nationwide survey found that only 5.3% of consumers used a food thermometer when preparing egg dishes. Reported methods for determining doneness by consumers included: cooking time, firmness of the product, if the product jiggled, color of the dish, or whether an inserted knife or toothpick came out clean.

Purpose: This study was designed to assess the potential safety of consumers reported methods for determining doneness of egg dishes.

Methods: After standardizing ingredients and measurements, chess pies, quiche, and breakfast casseroles were prepared and analyzed for doneness. Researchers recorded the time at which the dish reached 160°F, the consistency of the dishes at 160°F, temperature upon completion of the recommended cooking time, and time to "doneness." Various methods were used to determine if dishes were fully cooked.

Results: All replications of the chess pie and quiche reached the recommended temperature before the completion of the recommended cooking time but still had a liquid consistency at that temperature. Upon completion of the recommended cooking time the products were above 160°F, were set (did not "jiggle"), had browned, and both the knife and toothpick came out clean. The breakfast casserole failed to reach a safe temperature of 160°F within the recommended cooking time and was still liquid. The dish continued to cook until a safe temperature was reached, averaging another 12 minutes. At 160°F the casseroles were set, both the knife and toothpick came out clean and they were light brown in color.

Significance: Methods used by consumers to determine doneness of quiche and pies containing eggs appear to be safe. Breakfast casseroles needed to be cooked longer than the recommended time. Although alternate methods work, checking the internal temperature could ensure safe cooking.

P2-103 Overnight Incubation of Entire Broiler Carcass for Increased Detection of *Salmonella*

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Introduction: Broiler carcasses are generally sampled for presence of human pathogens such as *Salmonella* by whole carcass rinse and enrichment of a portion of the rinse. This may leave firmly attached cells out of the analysis potentially leading to false negative results.

Purpose: The objective of this study was to compare a traditional whole carcass rinse and culture of an aliquot to an overnight incubation of the entire carcass in the rinse liquid.

Methods: On each of 5 replicate sample days (5 flocks), 8 broiler carcasses were collected after chilling in a commercial slaughter plant. Each carcass was subjected to a whole carcass rinse in 400 ml buffered peptone water (BPW). A 30 ml aliquot was removed and placed in sterile cups. Cups and carcasses in broth were both incubated (35°C, 24 h) to pre-enrich for *Salmonella*. All incubated pre-enrichment broths were selectively enriched in RV and TT broth (42°C, 24 h) from which XLT4 and BGS plates were streaked and incubated (35°C, 24 h). Suspect colonies were confirmed as *Salmonella* by biochemical and serological methods.

Results: *Salmonella* was found on significantly ($P < 0.01$) more carcasses when sampled by carcass enrichment (29+/40) compared to aliquot enrichment (4+/40). Three of five flocks were found to have 1 or 2 positive carcasses by aliquot enrichment; in those flocks every carcass was positive when fully enriched. Even in flocks where all aliquot enrichments were negative, some carcasses (1 or 4) were found to be positive.

Significance: A broiler carcass sampling method that includes enriching the entire carcass allows detection of *Salmonella* that can be missed by enriching only an aliquot of the rinsate providing a more sensitive method.

P2-104 Thermal Resistance of Non-O157 Shiga Toxin-Producing *Escherichia coli* (STEC) in Irradiated, Raw Ground Beef

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Introduction: The USDA-FSIS declared six serogroups of Shiga Toxin-producing *Escherichia coli* (STECs) as adulterants in manufacturing trim and raw ground beef product components. Determining the thermal processing parameters (D- and *z*-values) will assist the beef industry in designing thermal processes to achieve regulatory performance standards.

Purpose: The objective of this study was to determine the thermal processing parameters for the STECs (cocktails of strains of the same serogroup), *E. coli* O157:H7 and two cocktails of STECs (containing one strain representing each serogroup) in irradiated, raw ground beef.

Methods: Cocktails of strains for each serogroup, cocktails of STECs (USDA-ARS and ATCC), and *E. coli* O157:H7 were grown individually in tryptic soy broth (TSB) and inoculated into raw ground beef (73% or 93% lean) to obtain ca. 7 log CFU/g. Inoculated ground beef (3 g) was vacuum-packaged in small vacuum bags and stored overnight at 4°C. The samples were submerged in a water bath at 54.4, 60.0, or 65.6°C, removed at prescribed time intervals, and surviving populations were enumerated using non-selective and selective media. Thermal resistance parameters were calculated.

Results: D-values for the six serogroups of STECs ranged from 28.32 to 45.02 min at 54.4°C, 1.96 to 2.94 min at 60.0°C, and 0.12 to 0.21 min at 65.6°C. These D-values of STECs were similar ($P > 0.05$) to *E. coli* O157:H7 values determined in this study at the same temperatures. D-values for O45 and O121 in 73% lean ground beef at 60.0°C were higher ($P \leq 0.05$) compared to *E. coli* O157:H7. Fat content of the ground beef (27 vs. 7%) did not affect ($P > 0.05$) D-values of the STECs.

Significance: Non-O157 STECs have similar heat resistance as *E. coli* O157:H7 in ground beef, regardless of fat level, and thus current validated thermal processes will be effective to mitigate the risk of STECs in beef.

P2-105 Destruction of Non-O157 Shiga Toxin-Producing *Escherichia coli* (STEC) in Salami by High Pressure Processing

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Introduction: Shiga Toxin-producing *E. coli* (STEC) have been implicated in outbreaks involving fermented beef products. Heating subsequent to fermentation is traditionally used to achieve the 5-log reduction. High pressure processing (HPP), if effective, could be used to achieve the desired lethality while minimizing thermally-associated product quality impacts.

Purpose: The objective of this study was to evaluate the efficacy of HPP for the destruction of *E. coli* in dry salami.

Methods: *E. coli* O121:H19 was grown in compatible solute-promoting media (Tryptic Soy Broth [TSB], and M9 medium containing glycine-betaine [M9-GB] or trehalose [M9-T]). Cells were harvested during stationary phase, inoculated onto sliced salami and vacuum packaged, stored for 9 d at 4°C, and then high pressure processed (600 MPa) after additional 0, 15, or 30 d refrigerated storage.

Results: *E. coli* O121 populations of 6.0, 5.24 and 4.81 log CFU/cm^2 were obtained subsequent to inoculation on salami slices for cells grown in TSB, M9-GB and M9-T, respectively. Refrigerated storage of inoculated salami for 15 and 30 days resulted in *E. coli* O121:H19 reductions of 1.47 and 1.89; 0.92 and 1.31; and 0.42 and 1.03 log CFU/cm^2, in salami inoculated with cultures grown in TSB, M9-GB and M9-T, respectively. *E. coli* reductions of 4.44, 3.15 and 3.18 log CFU/cm^2 were obtained by HPP (3 min) of vacuum-packaged salami slices inoculated with cells grown in TSB and stored for 0, 15 and 30 days, respectively. Lower reductions were observed on salami inoculated with *E. coli* O121:H19 prepared in M9 media (M9-GB and M9-T) and stored for 15 or 30 days.

Significance: Inoculum preparation methods and acclimatization of the cells is critical during conduct of validation studies for HPP. High pressure processing can be used to achieve STEC reductions in dry salami and other fermented sausages as an alternative to a traditional post-fermentation heating step.

P2-106 Mathematical Models to Describe Effect of Sodium Chloride on Inhibiting *Salmonella* and *Pseudomonas* Growth in Low-Sodium Nitrite Frankfurters

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❖ ***Developing Scientist Competitor***

Introduction: Productions of low-sodium nitrite meat products have been increased because sodium nitrite may form carcinogenic *N*-nitroso compounds. Sodium chloride concentrations in processed meats have also been decreased because of healthy diet. However, decreased sodium nitrite level in processed meats may allow bacterial growth.

Purpose: This study developed predictive models to describe the effect of sodium chloride on inhibiting *Pseudomonas* and *Salmonella* growth in low-sodium nitrite frankfurters.

Methods: A five-strain mixture of *Pseudomonas* and a five-strain mixture of *Salmonella* were inoculated on low-sodium nitrite (0 and 10 ppm) frankfurters formulated with sodium chloride (1.0, 1.25 and 1.5%) at 3 log CFU/g. *Pseudomonas* (cetrimide agar) and *Salmonella* cell counts (xylose lysine desoxycholate agar) were enumerated during storage at 4, 10, and 15°C for up to 60 days under aerobic and vacuum storage. The modified Gompertz model was fitted to the growth data to calculate maximum specific growth rate (μ_{max}; log CFU/g/h) and lag phase duration (*LPD*; h). The parameters were further analyzed with the square root model (μ_{max}) and a polynomial model (*LPD*) as a function of temperature and sodium chloride. The model performance was evaluated by root mean square error (*RMSE*).

Results: *Pseudomonas* growth was observed ($P < 0.05$) only under aerobic storage. In frankfurters at 0 and 10 ppm of sodium nitrite, μ_{max} of *Pseudomonas* decreased, but *LPD* increased as sodium chloride concentration increased. For both vacuum and aerobic storage, *Salmonella* growth was observed only at 10°C and 15°C, μ_{max} of *Salmonella* growth was higher ($P < 0.05$) at 0 ppm than 10 ppm of sodium nitrite, and the antimicrobial effect of sodium nitrite became more obvious as sodium chloride concentration increased. *RMSE* from validation was 0.521 - 1.019.

Significance: This result indicates that the developed models should be useful in describing the sodium chloride effects on inhibiting *Pseudomonas* and *Salmonella* growth in low-sodium nitrite frankfurters.

P2-107 Kinetic Model to Describe *L. monocytogenes* Growth in Bologna Sausage Formulated with Low $NaNO_2$ Concentration

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Introduction: Meat industry produces processed meat products formulated with low concentration of $NaNO_2$ because of health concern about $NaNO_2$, but low concentration of $NaNO_2$ in processed meat products may allow *Listeria monocytogenes* growth on bologna sausage.

Purpose: This study developed kinetic models to describe *L. monocytogenes* growth in bologna sausage formulated with low concentration of $NaNO_2$.

Methods: A mixture of *L. monocytogenes* strains NCCP10805, NCCP10808, NCCP10809, NCCP10810, and NCCP10943 was inoculated on bologna sausage formulated with $NaNO_2$ (0 and 10 ppm) and NaCl (1.0, 1.25 and 1.5%). The samples were then stored at 4°C (1440 h), 10°C (552 h) and 15°C (192 h) under aerobic and anaerobic conditions. *L. monocytogenes* cell counts were enumerated on PALCAM agar during storage. The modified Gompertz model was fitted to the *L. monocytogenes* growth data to calculate maximum specific growth rate (μ_{max}; log CFU/g/h) and lag phase duration (*LPD*; h). The temperature effect on μ_{max} and *LPD* were evaluated with the square root model and a polynomial model, respectively. Developed models were validated with observed data, and root mean square error (*RMSE*) was calculated.

Results: *L. monocytogenes* growth on bologna sausage was observed at 4 - 15°C at all concentrations of $NaNO_2$ and NaCl for both aerobic and anaerobic conditions ($P < 0.05$). Significant effect of $NaNO_2$ on μ_{max} and *LPD* values was not observed. However, the effect of $NaNO_2$ on μ_{max} and *LPD* increased when $NaNO_2$ was combined with NaCl. Validation result showed that *RMSE* was 0.330 - 1.768.

Significance: To lower $NaNO_2$ concentration in bologna sausage, NaCl should be combined with $NaNO_2$ to control *L. monocytogenes*, and the developed model should be useful in describing *L. monocytogenes* growth in low-$NaNO_2$ bologna sausage.

P2-108 Effects of Sodium Chloride, Sodium Phosphate, and Sodium Ascorbate on the Antibiotic Susceptibility of *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli*

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Introduction: Foodborne pathogens are exposed to sodium chloride, sodium phosphate, and sodium ascorbate. The food additives may be related to the antimicrobial susceptibility of foodborne pathogens.

Purpose: This study evaluated the effects of sodium chloride, sodium phosphate, and sodium ascorbate on antibiotic susceptibility of *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli.*

Methods: *L. monocytogenes* strains (NCCP10805, NCCP10806, NCCP10807, NCCP10808, NCCP10809, NCCP10810, NCCP10811, NCCP10920, and NCCP10943), *S. aureus* strains (ATCC13565, ATCC14458, ATCC23235, ATCC27664, and NCCP10826), and *E. coli* strains (NCCP10439, NCCP14037, NCCP14038, NCCP14039, and NCCP15661) were exposed to sodium chloride (0, 1.2, and 3.6%), sodium phosphate (0, 0.3, and 1.5%), or sodium ascorbate (0, 0.3, and 1.5%) supplemented in tryptic soy broth (TSB) at 35°C for 24 h, and 0.1 ml portions of the cultures were subcultured in correspondent TSB at 35°C for 24 h. This subculture was repeated ten times. Subsequently, bacterial cells were diluted to OD_{600} = 0.2, and the diluents were spread-plated on Mueller Hinton agar. Ten antibiotic disks (ampicillin, bacitracin, penicillin G, tigecycline, rifampicin, streptomycin, vancomycin, gentamicin, erythromycin, lincomycin) for *L. monocytogenes* and *S. aureus,* and ten disks (tigecycline, rifampicin, streptomycin, florfenicol, gentamicin, neomycin, tetracycline, amoxicillin, ceftiofur, chloramphenicol) for *E. coli* were placed on the plates, followed by incubation at 30°C (*L. monocytogenes*) and 35°C (*S. aureus* and *E. coli*) for 24 h. Clear zone (mm) was measured to evaluate antibiotic susceptibility.

Results: Clear zone diameters of *L. monocytogenes* NCCP10805, NCCP10806, NCCP 10809, NCCP10920, and NCCP10943 exposed to sodium phosphate were decreased ($P < 0.05$) to penicillin G, rifampicin, vancomycin, and erythromycin. However, the diameters of *S. aureus* and *E. coli* strains were not decreased.

Significance: These results indicate that sodium phosphate may decrease antibiotic susceptibility of *L. monocytogenes*.

P2-109 Probabilistic Model to Describe the Combination Effect of $NaNO_2$ and NaCl on *Salmonella* Growth Response

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❖ ***Developing Scientist Competitor***

Introduction: Because of health concern about sodium, low-$NaNO_2$ and low-NaCl meat products have been produced, but food safety for the products has not been evaluated.

Purpose: This study developed probabilistic models to describe *Salmonella* growth responses in low-$NaNO_2$ and low-NaCl meat products.

Methods: A five-strain mixture of *Salmonella* was inoculated in nutrient broth formulated with NaCl (0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, and 1.75%) and $NaNO_2$ (0, 15, 30, 45, 60, 75, 90, 105 and 120 ppm), and all samples were incubated at 4, 7, 10, 12, and 15°C under aerobic and anaerobic storage for up to 60 days. Growth (assigned the value of 1) or no growth (assigned the value of 0) for each combination was determined by turbidity. The growth response data were analyzed by a logistic regression to calculate the growth probability of *Salmonella* as a function of $NaNO_2$ and NaCl concentrations for each temperature. The predicted growth responses were compared with the growth response from frankfurters for validation.

Results: At 4 and 7°C, *Salmonella* growth was not observed at both aerobic and anaerobic conditions. Single application of low concentration of $NaNO_2$ did not inhibit *Salmonella* growth, but NaCl significantly ($P < 0.05$) inhibited *Salmonella* growth at 10, 12 and 15°C, regardless of presence of oxygen. When $NaNO_2$ was combined with NaCl, the probability of *Salmonella* growth was decreased. Validation result showed that concordance percentage between observed data and predicted data was 71%.

Significance: This study indicates that the developed probabilistic models should be useful in describing the combination effect of $NaNO_2$ and NaCl on inhibiting *Salmonella* growth in processed meat products.

P2-110 *Pseudomonas aeruginosa* DesB Plays a Role in *Staphylococcus aureus* Lysis through *pqs* Operon

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❖ ***Developing Scientist Competitor***

Introduction: *Pseudomonas aeruginosa,* is ubiquitous spoilage organism in foods, and also is the causative pathogen of serious infections which usually exists with other pathogens like *Staphylococcus aureus*. *P. aeruginosa* lyses *S. aureus* in multispecies environments. Various virulence factors produced by *P. aeruginosa* are closely associated with the growth hindrance and lysis of *S. aureus*. DesB, an aerobic desaturase, is previously reported as an important enzyme for *P. aeruginosa* virulence factor production. However, the role of DesB in *S. aureus* lysis by *P. aeruginosa* is not yet known.

Purpose: The objective of present study is to elucidate the role of DesB in the relationship between *P. aeruginosa* and *S. aureus*, especially in the lysis of *S. aureus* by *P. aeruginosa*.

Methods: To investigate the effect of DesB on *S. aureus* lysis, overnight cultures of wild type *P. aeruginosa* and its derived mutants were spotted on *S. aureus*-contained agar plates, and diameters of the clear zones were measured. Wild type *P. aeruginosa* (WT) or its *desB* mutant was co-cultured with *S. aureus*, and growth of each bacterium was monitored. Furthermore, in order to examine if *desB* regulates *pqsA* and its upstream regulator *mvfR* at transcriptional level, the expression levels of these genes in WT and *desB* mutant were compared by qRT-PCR.

Results: A zone of clearing, indicating a lysis of *S. aureus*, was relatively smaller in *desB* mutant than in WT. The growth of *S. aureus* cultured with WT was reduced more rapidly compared with the one with *desB* mutant. In qRT-PCR, *mvfR* was slightly less expressed in *desB* mutant, whereas *pqsA* expression was changed in a 50-fold decrease in *desB* mutant compared to WT. It suggests that PQS acts as a mediator of DesB-involving *S. aureus* lysis.

Significance: These results indicate that *P. aeruginosa* DesB contributes to PQS-mediated *S. aureus* lysis in multispecies environment.

P2-111 Growth of *E. coli* in Cooked Ground Beef as Affected by Temperature

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Introduction: *Escherichia coli (E. coli)* is a Gram negative bacteria that is commonly used as an indicator for fecal contamination. Several strains can be pathogenic and can cause serious foodborne illness. Since it is commonly found in ground beef, it is important to know its growth patterns at different temperatures in order to allow for safe food handling practices and avoid illness.

Purpose: To model the growth of *E. coli* in cooked ground beef at different temperatures over time.

Methods: Cooked ground beef was inoculated with *E. coli* ATCC 11229 and stored at four different temperatures (25, 35, 50, and 65°C) for 9 h. The beef was sampled every hour and plated on 3M™ Petrifilm™ *E. coli*/Coliform Count Plates, which were later enumerated for *E. coli* colonies.

Results: Two-log increases were seen at 25°C after 9 h, 35°C after 4.75 h, and 50°C after 3.5 h, but 65°C contained no detectable CFUs after 1 h. The most rapid growth was seen at 50°C, however other studies reported *E. coli* growth above 45°C to be irreproducible.

Significance: These results provide information on the growth of *E. coli* in cooked ground beef and can provide a basis for manufacturers in determining the microbial load of the product and safe holding time procedures. These findings also provide validation for USDA HACCP principles and guidelines.

P2-112 Effect of Thermal Adaptation on Thermal Inactivation Rates of *Salmonella* in Roast Beef at Low Cook Temperatures

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Introduction: USDA, FSIS Appendix A is widely used as validation support for thermal processes for meats. However, recent validation studies suggest that long come-up times observed in low-temperature cook processes may allow *Salmonella* to become heat-adapted and more thermal resistant.

Purpose: To compare the isothermal inactivation rate of *Salmonella* in a model roast beef system conditioned under three different temperature profiles immediately prior to cooking at 54.4°C.

Methods: Ground roast beef batter was inoculated with 8 log CFU/g *Salmonella* (5-strain mixture). One-g samples of inoculated meat were flattened into a thin film (0.5 - 1.0 mm thickness) in moisture-impermeable pouches and vacuum-packaged. Inoculated samples were then held under one of three conditions (not adapted; 4°C for 3 hours; ramp-up from 4°C to 54.4°C over 3 hours) prior to cooking at 54.4°C in a water bath. Triplicate samples were removed at 0, 10, 20, 30, 40, 60, and 90 min, immediately chilled to ≤ 4°C, and then enumerated for *Salmonella* survival using XLD with thin layer overlay of nonselective media to enhance recovery of injured cells. The study was replicated three times.

Results: Populations of *Salmonella* decreased 1.2 - 2.8 log at the end of the 3-hour ramp-up period from 4°C to 54.4°C compared to inoculation levels, whereas counts were unchanged in the other two treatments. D-values (calculated from linear regression on log reduction from the beginning of the 54.4°C cook process) were 23.4, 15.2 and 13.0 minutes for the thermal-adapted, cold-adapted, and non-adapted cells. However, when considering the initial inoculum in the roast beef batter, the final surviving populations were not significantly different among the three treatments.

Significance: Sub-lethal heat exposure associated with low-temperature cook cycles can enhance the thermal resistance of *Salmonella*; however, additional studies are needed to determine the practical implication for industry. Additionally, caution should be exercised when applying results from a model system to industrial processes.

P2-113 Survival of *Penicillium* Species Conidia during Thermal Processing (Frying and Baking) of Frozen Breaded Chicken

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Introduction: Fungal spoilage is a cause of approximately 1 - 1.5 % losses in the frozen chicken nuggets segment. The growth of fungi may also result in mycotoxin production by some species. Psychrophilic species of *Penicillium* are the main spoilers of frozen chicken nuggets.

Purpose: The objective of this study was to evaluate the effects of frying and baking of frozen chicken nuggets on Penicillia.

Methods: *P. commune* (NGT 16/12), *P. polonicum* (NGT NGT 23/12 and 33/12), *P. glabrum* (NGT NGT 29/12 and 35/12), *P. solitum* (NGT 30/12) and *P. crustosum* (NGT 51/12) previously isolated from spoiled frozen chicken nuggets were used in this study. Conidia solutions of each species were prepared after growth in appropriate conditions and washing and centrifugation procedures. Then, the ability of conidia from these fungi to survive to thermal shock (80°C/30 min) in phosphate buffer (PB) at pH 7.2 in thermal death tubes (TDTs) was assessed. After, each fungus was inoculated separately in frozen chicken nuggets and further submitted to: frying, baking and the combined treatment (frying and baking). The number of decimal reductions caused by these treatments was determined.

Results: None of the strains was able to survive to heat shock in heat resistant, and all of them were inactivated with heat shock in PB. On the other hand, the conidia of *P. commune*, *P. polonicum* NGT 23/12, *P. solitum* and *P. crustosum* inoculated chicken nuggets and subjected to heat treatments were able to survive to the combined treatment. The number of decimal reductions in Penicilia counts varied from 1.8 to 5.2 log CFU/g.

Significance: The results found highlight the need for the use of raw materials of high mycological quality, because Penicilia may be able to survive heat treatment employed during nugget processing and further spoil the frozen chicken nuggets during shelf life.

P2-114 Resistance of Different Probiotic *Bacillus* during Creamy Curd Cheese Manufacturing Process

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Introduction: The creamy curd cheese (CC) is a typical Brazilian cheese consumed often, which makes it an attractive matrix for delivering probiotics. Despite this, during CC processing, because of high temperature used, probiotic lactic acid bacteria will not survive. In this context, the greater resistance of spores to external stresses is a fundamental property for innovative probiotic formulations in the functional food market.

Purpose: To evaluate the resistance of *Bacillus* probiotics in the different stages of the technological process of cream curd cheese manufacturing.

Methods: Probiotic *Bacillus* strains (*Bacillus coagulans* Lactospore; *Bacillus coagulans* GBI-30, 6086; *Bacillus subtilis* PB6; *Bacillus subtilis* PXN1; *Bacillus flexus* HK1) spores were added at different stages of cream curd cheese production process: prior to pasteurization, prior to coagulation, and prior to curd fusion. During all stages, samples were collected and spores were further enumerated using appropriate culture media and thermal shock, as indicated by suppliers of the strains. The number of survivors for each step studied was calculated based on initial inoculum level and spores recovered after each processing step.

Results: In the steps of pasteurization, coagulation and curd fusion, *Bacillus coagulans* GBI-30, 6086 showed better resistance in comparison to other strains tested ($P < 0.05$). However, at the step of curd coagulation, *Bacillus coagulans* Lactospore, *Bacillus subtilis* PB6 and, *Bacillus flexus* HK1, presented similar resistance to *Bacillus coagulans* GBI-30, 6086 ($P > 0.05$). At this step, *Bacillus subtilis* PXN1 strain was the least resistant. Thus, among the five strains analyzed, *Bacillus coagulans* GBI-30 consistently showed good resistance at all stages of the process.

Significance: In this study it has been found that probiotic *Bacillus* can be a very important option for delivering probiotics through foods processed under harsh conditions. Data indicates that the GRAS strain *Bacillus coagulans* GBI-30, 6086 seems to be the best option for delivering probiotics through CC.

P2-115 Microbiological Evaluation of Poultry Product Packages from Grocery Stores in Nashville, Tennessee

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Introduction: Our previous study indicated that there is a high chance of contamination and transfer of meat juice from packages to hands and food contact surfaces during shopping and handling of raw poultry products. Further evaluations into the microbiological contamination on the raw poultry packages are necessary for assessments of consumer exposure.

Purpose: This study investigated the extent of microbiological contaminations on the surfaces of raw poultry packages purchased from local grocery stores.

Methods: Packages of raw poultry products were purchased from thirty-five grocery stores in metropolitan area of Nashville, Tennessee. Three packages were purchased from each store including whole chicken, chicken breast, and ground turkey. Information on the brands and packaging types was recorded and package conditions were inspected to note any leaking of meat juices. Microbiological contaminations on the packages were evaluated.

Results: Of the 105 packages, 24 had aerobic plate count (APC) higher than 10^6 CFU on the packages and 18 had coliform count (CF) and 8 had *E. coli* count (EC) higher than 10^3 CFU on the packages. *Campylobacter* was found on 7 and pathogenic *E. coli* was found on 6 of the packages while no *Salmonella* was found. There was no significant difference on the bacteria counts among the product and package types. However, leaking packages had higher bacteria counts; the average APC, CF, and EC were 3.9×10^6, 1.3×10^3, and 4.2×10^2 CFU, respectively. Of the 8 leaking packages, 5 had either *Campylobacter* or pathogenic *E. coli* and 2 had both found on the surfaces.

Significance: *Campylobacter* and pathogenic *E. coli* are more often found on poultry packages than *Salmonella*. Leaking packages clearly increase the risk of cross-contamination.

P2-116 Survival of *Salmonella* on the Kitchen and Food Package Surfaces Contaminated with Raw Meat Juice

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Introduction: Meat juice from raw poultry products can carry foodborne pathogens which have been known to cause illness through contaminations of kitchen surfaces and food items during handling or storage. Survival rates of *Salmonella* in such conditions are indispensable for assessing risk of consumer exposure.

Purpose: This study compared the survival rates of *Salmonella* in raw chicken juice on kitchen surfaces and food packages at room temperature and refrigerated conditions.

Methods: Cultures of *Salmonella* Typhimurium were inoculated in raw meat juice and spread on the surfaces of kitchen countertops, refrigerator shelves, cereal box, potato chip bag, bread bag, and milk carton that were kept at room or refrigerated temperature. The numbers of viable *Salmonella* were continuously monitored by enumeration on XLT-4 selective agar. The experiments were repeated and all samples were replicated.

Results: The results showed a steady decline in viable rates of *Salmonella* at both conditions, although slower in refrigerator. Regression analyses were used to predict the decline rate in each of the conditions. On the surfaces of countertop, chip bag and cereal box at room temperature, numbers of *Salmonella* reduced to one hundredth of the initial levels in 16.6, 2.8 and 2.0 hours, respectively. On the surface of hands, it only took 10.1 minutes to decease to that level. In contrast, the rates of decline were much slower on the surfaces of refrigerator shelves, bread bag and milk carton in the refrigerator where it took 222.7, 43.5 and 23.7 hours, respectively, to achieve the same level of reduction.

Significance: Because *Salmonella* can survive days in refrigerated conditions, risk of cross-contamination to other foods is higher in the refrigerator than at the room temperature. Precautions are needed to prevent cross contamination when handling and storing raw poultry products in the refrigerator.

P2-117 Prevalence of *Salmonella* and *Listeria* in Meat and Poultry Influenced by Various Market Settings in Ho Chi Minh City of Vietnam

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❖ ***Developing Scientist Competitor***

Introduction: *Salmonella* and *Listeria* are the leading cause of foodborne illness. Various market settings in the developing countries do not use refrigeration and expose fresh meat and poultry to pathogenic contamination, posing serious challenges to food security.

Purpose: To determine *Salmonella* and *Listeria* prevalence of fresh meat and poultry in various market settings in Ho Chi Minh City (HCMC), Vietnam.

Methods: Five whole muscle beef, pork, and chicken (whole bird) samples were aseptically purchased in two supermarkets (SM), indoor markets (IM), and open markets (OM) at opening and closing (N = 180). Samples were rinsed in 90 ml of buffered peptone water, which was used for microbiological testing. *Salmonella* was detected as red colonies with yellow halo, using *Salmonella* Enrichment Broth™ and Petrifilm™ *Salmonella* Express Systems™ (3M USA, St. Paul, MN). *Listeria* was detected as blue/green colonies, using Demi-Fraser Broth™ (3M USA, St. Paul, MN) and ALOA agar (bioMérieux, St. Louis, MO). Statistical significance was determined at $P \leq 0.05$.

Results: *Salmonella* prevalence in beef was similar among market types at opening (50.00 to 62.50%; $P \geq 0.54$), however, was greater in SM and IM at closing (90.00 and 100.00%, respectively, $P \leq 0.01$). *Salmonella* prevalence in pork differed between IM and OM at opening and closing ($P = 0.04$), however, was similar in chicken among market types or sampling times ($P > 0.05$). *Listeria* prevalence in beef was similar among market types at opening (87.50 to 100.00%, respectively; $P \geq 0.25$), however, was lower in SM at closing (70%, $P < 0.01$). *Listeria* prevalence in pork and chicken did not differ among market types or sampling times ($P > 0.11$).

Significance: The data indicate that consumers in HCMC, Vietnam are exposed to great risks of *Salmonella* and *Listeria*, which depend on market types and time of purchase.

Poster

P2-118 Process Analysis of Poultry Slaughtering in Three Abattoirs: Influence of Process Stages on the Microbiological Contamination of Carcasses

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Introduction: For assessment of the slaughter process performance, operations increasing or decreasing microbial loads on carcasses must be identified. With regard to slaughtered broilers, such examinations must also include *Campylobacter*. Reductions of *Campylobacter* counts on carcasses might cause a decrease in associated human cases.

Purpose: The aim of this study was to investigate the effects of selected slaughter process stages on the microbiological contamination of broiler carcasses in three large-scale Swiss abattoirs.

Methods: Broiler carcasses from three abattoirs were examined at five process stages (scalding, plucking, evisceration, washing, chilling) for total viable counts (TVC), Enterobacteriaceae, E*scherichia coli* and *Campylobacter*. At each stage and abattoir, 90 carcasses (pooled neck and breast skin) from 30 flocks were sampled. In addition, 48 carcasses sampled before scalding and 156 scalding water samples were examined for TVC and *Campylobacter*.

Results: With regard to TVC, Enterobacteriaceae and *E. coli*, distinct reductions were evident from delivered broilers to chilled carcasses and trends of respective results were comparable in the abattoirs (mean TVC at abattoirs: 7.6 – 7.8 log CFU/g before scalding, 6.0 – 6.5 log CFU/g after scalding, 4.6 – 4.9 log CFU/g after plucking, 4.2 – 4.4 log CFU/g in the chiller). With regard to *Campylobacter* counts on broiler carcasses, abattoir-specific differences after scalding were striking (probably associated with varying temperature/time expositions), plucking slightly increased the counts, whereas results remained mainly constant at the following stages (mean *Campylobacter* counts at abattoirs: 2.3 – 3.3 log CFU/g after scalding, 2.7 – 3.6 log CFU/g after plucking, 2.5 – 3.4 log CFU/g in the chiller).

Significance: Such abattoir-specific data form the basis for implementing targeted and sustainable measures at selected slaughter process stages (cost-benefit analysis) in order to reduce carcass contaminations and the introduction of pathogenic bacteria into the food chain.

Poster

P2-119 Changes in Food Safety Practices at Egg Products Plants from 2004–2014

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Introduction: From 2003-2012, the number of eggs broken for further processing in the U.S. increased by 16%. Egg products are increasingly being incorporated into other processed food products. As consumption of egg products has risen, their safety and wholesomeness have become more important for public health and to regulators.

Purpose: The purpose of this study was to assess technological and food safety practices of the egg products industry, and how these practices have changed over the past ten years.

Methods: We conducted a national survey of egg products plants (n = 57, response rate of 72%) using a multimodal approach, allowing respondents to choose between a paper or web survey, and contacting the plants using telephone, mail, and e-mail. The questionnaire asked about operational and sanitation practices, microbiological testing practices, food safety training for employees, and other food safety and marketing practices.

Results: The percentage of egg products plants using advanced pasteurization technologies and an integrated, computerized processing system increased by almost 30 percentage points since 2004. Although a written HACCP plan is not currently required, 93% of plants have one for at least one production step. Plants have an average of 6 employees with formal HACCP training, and 90% of plants have management employees trained in HACCP. Most plants (93%) conduct voluntary microbiological testing. Plants that conduct microbiological testing before pasteurization increased by almost 30 percentage points since 2004. The largest increase in testing was for *Listeria* species, with a 21 percentage point increase from 2004 to 2014.

Significance: The results of the nationwide survey, and their comparison to a previous survey conducted in 2004, indicate advances in food safety technologies and practices employed in egg products plants. The study findings can identify areas in which improvements are needed, as well as guide regulatory policy making and inform regulatory impact analysis.

P2-120 Consumers' Cooking Practices for Eggs and Poultry Place Them at Risk for Foodborne Illness

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❖ ***Developing Scientist Competitor***

Introduction: Previous research has shown that many consumers do not follow recommended food safety practices for cooking poultry and eggs, which can lead to exposure to *Salmonella* and *Campylobacter*. Prior research has been done through surveys and interviews, rather than observation.

Purpose: The objective of this project was to determine if consumers follow food safety guidelines when cooking poultry and eggs.

Methods: Consumers (n = 101) divided between three locations (Manhattan, KS; Kansas City area; Nashville, TN) were observed as they prepared a baked chicken breast, a pan fried ground turkey patty, a fried egg, and scrambled eggs. Out of view of the consumers, observers measured the endpoint temperature for the cooked products within 30 seconds after the consumers indicated they were finished cooking.

Results: Thermometer use while cooking was low in all of the products; only 37% for the chicken breasts, and 22% for the turkey patties, with no thermometer usage for fried or scrambled eggs. Only 77% of the chicken and 69% of the turkey was cooked to a safe temperature (165°F), while 77% of scrambled and 49% of fried eggs reached a safe temperature (160°F). Unfortunately, thermometer use did not insure cooking to the recommended temperature. Safe hand-washing was noted in only 40% of respondents after handling the chicken breast and 44% after handling the ground turkey patty. This decreased to 15% after handling raw eggs for fried eggs, and 17% for scrambled eggs.

Significance: These results show that there is a high prevalence of unsafe behaviors (undercooking and poor hand-washing technique) when cooking poultry and eggs, and a great need for improvement in consumer behavior with poultry and eggs. Consumers also need to be educated on proper thermometer usage in addition to simply using thermometers.

P2-121 *Salmonella enterica* Serovar Enteritidis-specific Genes That Contribute to Survival in Egg Albumen

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❖ ***Developing Scientist Competitor***

Introduction: An increasing number of outbreaks of gastroenteritis caused by *Salmonella enterica* serovar Enteritidis have been linked to the consumption of eggs in recent years. The molecular mechanisms by which this pathogen associates and survives in the egg albumen are generally unknown.

Purpose: To identify *S. enterica* Enteritidis-specific genes, which are absent in the genomes of serovar *Salmonella* Typhimurium and Typhi, relevant to its survival in the egg albumen.

Methods: Using comparative genomic analysis, we found 94 genes in *S. enterica* Enteritidis which are absent in the genomes of Typhimurium and Typhi. Among these genes, 25 in-frame gene deletion mutants were constructed in Enteritidis PT4. Overnight cultures of wild-type PT4 and each mutant were adjusted to a final concentration of 10^5 CFU/ml and thoroughly mixed with 5 ml of organic, antibiotic-free chicken egg albumen. Samples were incubated at 37°C for up to 72 h. Samples were taken at 0, 24, 48, and 72 h and serial dilutions were plated onto LB and XLD agars. Data were analyzed using Prism GraphPad and R statistical software. A *P*-value of less than 0.05 was considered significant.

Results: The *Salmonella* Enteritidis gene deletion mutants which had significantly lower survival in egg-albumen than the wild-type strain (greater than 3 log CFU) were *sen0998* and *sen0276* (less than 2 log CFU) at 48 h incubation, indicating that these genes may contribute to *S. enterica* survival in egg albumin. At 72 h, the wild-type and all gene deletion mutants declined from 5 log CFU to less than 1 log CFU at 37°C incubation.

Significance: Constructed gene deletion mutants will help determine the relative functions of different genes in *Salmonella* Enteritidis. The data of this study will aid in understanding the pathogenicity of *Salmonella* Enteritidis and will help in the prevention of outbreaks associated with egg albumen.

P2-122 Impact of Alternative Antimicrobial Commercial Egg Washes on Reducing *Salmonella* Contamination

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❖ ***Developing Scientist Competitor***

Introduction: Table eggs are washed with an alkaline detergent (approx. pH 11) followed by a chlorine rinse. Minimum wash water temperature is 32°C, but frequently as high as 49°C. Wash temperature and an antimicrobial rinse are required by regulation, but wash pH is not specified. At pH 11, little, if any, free chlorine is available in the final rinse. Using a chlorine stabilizer (e.g., T-128) in the wash may help maintain chlorine effectiveness. The heated wash warms eggs and slows cooling, which can encourage microbial growth.

Purpose: The objective was to determine the effectiveness of four egg wash treatments to decrease *Salmonella* contamination: chlorine+T-128 at pH 6.0 at both ambient temperature (approx. 20°C) or 49°C and alkaline wash at pH 11 at both ambient temperature or 49°C.

Methods: Eggs were drop inoculated with *Salmonella* Typhimurium and *Salmonella* Enteritidis and were washed with each of the treatments. All wash treatments were followed by a chlorine rinse. Two control treatments were also evaluated: inoculated, not washed and inoculated, washed with 20°C water. Post-wash *Salmonella* counts were compared to determine intervention effectiveness.

Results: *Salmonella* counts were reduced by similar levels when washed with chlorine+T-128 at both temperatures and with the alkaline wash at the high temperature (~5 log CFU/ml egg shell emulsion reduction); these treatments were not significantly ($P > 0.05$) different from each other. The counts were significantly ($P < 0.05$) lower than the 20°C alkaline treatment and 20°C water treatment counts (~3.2-log CFU/ml reduction).

Significance: Results show that acidic washes may provide the same results as the traditional egg wash and that, for the acidic washes, ambient temperatures were just as effective as heated washes. The traditional basic egg wash does not show the same antimicrobial effectiveness when applied at ambient temperatures.

P2-123 Artisanal Italian Salami and Soppresse: Identification of Control Strategies to Manage Microbiological Risks

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Introduction: Foodborne outbreaks have been associated to the consumption of traditional raw pork products such as salami and soppresse, manufactured without starter cultures and ripened in not a standardized environment.

Purpose: In order to avoid the marketing of potentially at-risk salami/soppresse, a study has been performed with the aims to investigate the production process, collect information on microbiological contamination of salami/soppresse along the production process and identify control measures easily applicable by the producers (FBO) with the supervision and control of the Competent Authority (CA).

Methods: Since 2009, samples have been collected along the farm to fork chain in order to estimate the prevalence of several foodborne pathogens (*Salmonella* spp., *Campylobacter* spp., *E. coli* O157, *Listeria monocytogenes*) and to record parameters such as pH, a_w and weight decrease which define the goodness of the ripening process.

Results: Microbiological analyses showed that the main risk was represented by *L. monocytogenes* which was detected in 10% (30/288) of salami/soppresse during season 2009-2010. During the sampling period 2010-2013, *L. monocytogenes* was detected in 5% (34/654) of batches (minced meat samples). However, the pathogen was detected at level above 10 CFU/g in less than 1% (2/48) of salami/soppresse at the end of the ripening period and thus before consumption. Moreover, it was possible to establish a weight loss of at least 25% in order to have an $a_w \leq 0.92$ for salami and soppresse, which according to European Regulation CE 2073/2005 defines products considered unable to support the growth of *L. monocytogenes*.

Significance: A control strategy was defined based on the identification of positive/negative batches by the CA and the monitoring of the weight decrease in salami/soppresse by FBO; products were allowed to be marketed if coming from negative batches and with a weight decrease of 25%.

P2-124 Domestic Consumer-style Storage and Thawing Practices: Effect on *Salmonella* in Poultry-based Meat Preparations

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Introduction: Time-temperature abuse has been reported as one of the most common contributory factors in salmonellosis strong-evidence outbreaks. Studies on the temperature of domestic refrigerators showed that from 55 to 80% of refrigerators operate at a temperature > 5°C; moreover 46 to 60% of consumers preferred to thaw food on the kitchen countertop instead of the refrigerator.

Purpose: The purpose of the study was to record the effect of different domestic refrigeration temperatures and thawing methods on the presence and numbers of *Salmonella* spp. in poultry-based meat preparations.

Methods: Burgers, sausages and ready-to-cook kebabs were submitted to: i) storage at three refrigerator temperatures (4°C, 8 or 12°C), with or without prior temperature abuse (25°C for 2 hours simulating shop to home transport) and ii) thawing according to two methods (overnight in refrigerator at 8°C versus on the kitchen countertop at 23°C). Storage tests were carried out on naturally or artificially (*Salmonella* Typhimurium at ca. 10 CFU/g) contaminated products while freezing/thawing tests were conducted only on artificially contaminated products (*Salmonella* Typhimurium at ca. 10, 100 and 1000 CFU/g).

Results: Artificially contaminated products showed substantial growth of *Salmonella* Typhimurium at 12°C (from ca. 8 MPN/g to > 710 MPN/g) in kebabs after 7 and 10 days but more moderate growth in sausages (from ca. 14 MPN/g to a maximum of 96 MPN/g after 9 days). Storage of naturally contaminated burgers or sausages (contamination ≤ 1 MPN/g) and short temperature abuse (2 h at 25°C) did not facilitate an increase in *Salmonella* presence and numbers. Thawing overnight in the refrigerator led to either a moderate reduction or no change *Salmonella* Typhimurium numbers in meats.

Significance: Time-temperature abuse can cause a substantial increase of *Salmonella* numbers in some types of meats, thus efforts for dissemination of consumer guidelines on correct storage and handling of food need to be continued.

Poster

P2-125 Determination of Genetic Diversity of the *Escherichia coli* Population on Beef Trimmings from a Large Beef Packaging Plant

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Introduction: Presence of *Escherichia coli* in beef is indicative of possible contaminations of enteric pathogens of fecal origin such as verotoxigenic *E. coli* of the serotype O157:H7. Thus testing beef trimmings for *E. coli* can be useful in examining the effectiveness of routine slaughter/dressing operations in beef packaging plants.

Purpose: To determine genetic diversity and phylogenetic grouping of *E. coli* strains isolated from beef trimmings.

Methods: Forty beef trim samples were collected from a beef packing plant during each of three separate visits and enriched for *E. coli* in tryptone soy broth supplemented with 20 ppm novobiocin. Selected *E. coli* isolates were purified by streaking on selective agar and confirmed using real-time PCR before being genotyped using multiple-locus variable-number tandem-repeat analysis (MLVA). Phylogenetic group of each *E. coli* genotype was determined.

Results: The three visits each yielded 30, 118 and 17 *E. coli* isolates, and 4, 6 and 4 genotypes, respectively. Overall, nine distinct genotypes were identified, of which genotypes 263 and 89 were predominant, contributing to 50.3 and 37.5 percent of total isolates, respectively. Genotype 263 was recovered from samples obtained from all three visits, while 89 was recovered during the second and third visits. Genotypes 115 and 270 were recovered during the first as well as second visit and other genotypes were isolated during one visit only. Genotypes 89, 115, 177, and 178 were also previously isolated from hide-on and/or skinned carcasses. All genotypes belonged to phylogenetic group A_0 which is commonly found in cattle.

Significance: Despite that phylogenetic grouping showed all genotypes originated from cattle, *E. coli* isolates from beef trimmings lacked genetic diversity that was observed on hide-on carcasses. Coincidently, identification of same genotypes over multiple sampling dates suggests that certain genotypes may become persistent strains in a plant and contribute to post-kill floor contamination.

P2-126 Analysis of ALLRTE and RTE001 Sampling Results for *Listeria monocytogenes*, Calendar Years 2005 through 2012

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Introduction: The Food Safety and Inspection Service (FSIS) used sample data from the ALLRTE and RTE001 sampling projects to examine *Listeria monocytogenes* (*Lm*) contamination of Ready-to-Eat (RTE) meat and poultry products from federally-regulated establishments. ALLRTE consists of random samples for all types of RTE products, while RTE001 represents a risk-based sampling project for post-lethality exposed RTE products.

Purpose: To analyze ALLRTE and RTE001 *Lm* sampling data collected over an 8-year period.

Methods: FSIS evaluated results of Lm testing of meat and poultry product samples collected under the ALLRTE and RTE001 sampling projects for 2005-2012. Samples were tested in accordance with FSIS's Microbiology Laboratory Guidebook.

Results: FSIS analyzed 24,388 ALLRTE samples collected from 3,007 establishments and 66,674 RTE001 samples collected from 2,776 establishments. ALLRTE samples were 0.38% *Lm*-positive (range, 0.18-0.64%), and RTE001 samples were 0.42% *Lm*-positive (range, 0.28%-0.71%). Percent positive rates decreased markedly from 2005–2008 and have remained relatively stable in subsequent years. In 2012, ALLRTE and RTE001 percentage positive rates were 0.18% and 0.34%, respectively. Of 3,007 ALLRTE establishments, 99 produced at least one *Lm*-positive sample (3.3%), while of 2,776 RTE001 establishments, 249 had at least one *Lm*-positive sample (9.0%). The 372 positive samples identified (92 ALLRTE, 280 RTE001) encompassed diverse RTE products, including deli meats, hot dogs, sausages, salads/spreads/pates, sliced/diced/shredded products and meat plus non-meat multicomponent products. Most of the *Lm*-positive samples were obtained from small (57%) or very small (40%) establishments based on HACCP size categories. In addition, 71% of the positive samples were obtained from establishments using only sanitation methods without additional control measures.

Significance: This detailed data analysis helps guide changes in policies, regulations, inspection procedures and enforcement actions relevant to the prevention of *Lm* contamination in RTE products in producing establishments. In January 2013, ALLRTE and RTE001 were replaced by two new product sampling projects.

P2-127 Analysis of Laboratory Test Results from the Automated Import Information System (AIIS), July 2007 to May 2012

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Introduction: AIIS was a computerized tracking system used by the Food Safety and Inspection Service (FSIS) between 2002 and 2012 to monitor meat and poultry imports. Along with imported meat/poultry products being inspected in the country of origin and reinspected visually before being released in the United States, AIIS selected certain shipments (lots) of these imports for additional reinspection tasks, such as testing for various microbiological pathogens or residues. Lot selection was statistically based on the annual volume of shipments from the exporting country. Effective May 2012, AIIS functions were replaced by the Imports module of the Public Health Information System (PHIS).

Purpose: To summarize microbiological and residue testing results on imports samples selected by AIIS, July 2007 - May 2012.

Methods: Imports samples collected under AIIS were tested for *Escherichia coli* O157:H7 (*Ec*), *Listeria monocytogenes* (*Lm*) and *Salmonella* spp., plus 14 different chemical residues in accordance with FSIS standard laboratory procedures.

Results: Testing was performed on 37,009 samples from 22,739 unique lots, representing over 394 million pounds of imports from 29 countries. About 93% of the tests were scheduled inspections, with 3% unscheduled and 4% under intensified status. A total of 22,553 samples were tested for microbial pathogens, with 48 samples positive (0.21% of samples tested). Twenty of these were raw beef samples positive for *Ec*, while another 26 were *Lm*-positive Ready-to-Eat (RTE) meat (beef and pork) and poultry products. There were 16,454 samples from the 29 countries tested for chemical residues, with 44 samples from 5 countries classified as violative positives (0.27%). Almost all these samples were positive for Avermectins, detected in both raw and RTE beef samples.

Significance: AIIS data have guided improvements in policies, regulations, inspection procedures and enforcement actions relevant to testing of imported meat and poultry samples for microbiological or residue contamination under PHIS.

P2-128 Survival and Metabolic Activity of Lux-marked *Escherichia coli* O157:H7 in Different Types of Milk

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Introduction: *Escherichia coli* O157:H7 is a potentially lethal pathogen which has been responsible for several outbreaks of milk-borne illness in recent years.

Purpose: The aim of this study was to improve our understanding of the pathogen's behavior in different types of milk.

Methods: This study was to evaluate the survival and metabolic activity (indexed by bioluminescence) of a chromosomally lux-marked strain of *E. coli* O157:H7 in raw, pasteurized and microfiltered pasteurized milk at 4 and 20°C for up to 14 d.

Results: Results showed that the population of *E. coli* O157:H7 and its metabolic activity decreased in all samples during storage at 4°C, with no significant differences in numbers observed between the different milk types; but metabolic activity was significantly higher ($P < 0.05$) in the microfiltered pasteurized milk than that in raw milk. At 20°C, survival and metabolic activity were significantly lower in raw milk compared with pasteurized milk.

Significance: Examining the role of incubation temperature has practical significance in understanding how *E. coli* O157:H7 and other aerobic cells behave in the food chain, from retailer fridge storage to the consumer home where the greatest risk of human infection occurs.

P2-129 Qualitative and Quantitative Production of Biogenic Amines by Bacteriocinogenic Lactic Acid Bacteria from Raw Goat Milk

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Introduction: Biogenic Amines (BA) are basic compounds produced mainly by lactic acid bacteria (LAB) that could interfere with the quality and safety of fermented foods. Once ingested at high levels, BA can cause toxicological problems in the consumers. The use of some LAB as starters or biopreservatives in foods demands caution because some of them can be potential BA producers.

Purpose: To search the presence and expression of BA genes in bacteriocinogenic LAB, and to quantify this produced substances.

Methods: Eight *Enterococcus* and six *Lactococcus* previously isolated from raw goat milk and capable to produce bacteriocins were selected and submitted to PCR to identify genes for tyrosine (*tdc*), histidine (*hdc*) and ornithine (*odc*) decarboxylase. Their expression was identified using modified MRS (mMRS) agar containing the precursor amino-acids and a pH indicator. The production quantification was accessed by HPLC.

Results: None of the isolates carried the *odc* gene or produced putrescine. Seven *Enterococcus* and three *Lactococcus* were positive for *tdc*, and six *Enterococcus* and two *Lactococcus* were identified as tyramine producers using mMRS. Based on HPLC results, three *Enterococcus* produced high amounts of tyramine (maximum of 4.3 mg/kg) and only one *Lactococcus* produced 2.44 mg/kg of tyramine. However, one *Enterococcus* and one *Lactococcus* produced tyramine using mMRS, and both isolates did not present positive results for *tdc*. Only one *Enterococcus* carried the *hdc* gene, but did not produce histamine by mMRS or by HPLC.

Significance: The results demonstrated that bacteriocinogenic LAB strains are capable to produce virulence factors, such as BA. Also, *Lactococcus* isolates presented BA genes and were able to produced BA, even being usually considered as safe to be applied in foods. Finally, BA production should be quantified because only at high amounts can be a risk to human health.

Acknowledgments: CNPq, CAPES, FAPEMIG

P2-130 Cytotoxicity of Bacteriocins Produced by Lactic Acid Bacteria Isolated from Goat Milk

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Introduction: Bacteriocins produced by LAB are peptides that present antimicrobial activity. These peptides have biotechnological potential in food safety as biopreservatives in control of spoilage and pathogenic microorganisms. Application of bacteriocins in food demands a careful evaluation of their toxic properties, ensuring the safety for their use as food additives in industrial scale.

Purpose: The aim of this study was to analyze the cytotoxicity of partially purified bacteriocins produced by six bacterial strains, obtained from goat milk.

Methods: Bacteriocins produced by *Lactococcus lactis* subsp. *lactis* (GLc03 and GLc05) and *Enterococcus durans* (GEn09, GEn12, GEn14 and GEn17) were partially purified from 12-h cultures grown in MRS broth at 37°C by precipitation with ammonium sulfate and hydrophobic chromatography on SepPakC18. Vero cells derived from monkey kidney were seeded in 96-well plate and subsequently treated with increasing concentrations of studied bacteriocins. Two days after initial treatment, MTT was added to cells and colorimetric intensity was obtained at wavelength of 490 nm. The obtained values were converted into percentage of viability, and compared to negative control.

Results: The tested bacteriocin concentrations, ranged from 6 µg/ml to 480 µg/ml, showed different levels of cytotoxicity. The lowest cytotoxicity values were 172 µg/ml and 295 µg/ml, corresponding to the bacteriocins produced by *E. durans* GEn09 and *L. lactis* subsp. *lactis* GLc03, respectively. The cell line viability remained higher than 80% when treated with maximum concentrations of the bacteriocins produced by other tested strains (GLc05, GEn12, GEn14 and GEn17) and therefore it was not possible to estimate the value CC_{50}. All strains showed cell viability above 80% when tested at concentration of 6 µg/ml.

Significance: Obtained results indicated low cytotoxicity of the tested bacteriocins, indicating that these peptides can be considered as safe and highlighting their potential use as biopreservatives in food. Acknowledgments: CAPES, CNPq and FAPEMIG.

P2-131 Antibiotic Resistance of *Listeria monocytogenes* Isolated from Meat Processing Environments, Foods, and Clinical Cases in Brazil

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Introduction: In the last years, studies showed large increase in the description of antibiotic resistant strains. The emergence of antimicrobial resistance has serious consequences for public health, which makes monitoring, an important measure to be taken.

Purpose: Assess the antimicrobial resistance of *Listeria monocytogenes* obtained in different sources and Brazilian regions.

Methods: A total of 137 *L. monocytogenes* isolates, from 11 different States of Brazil were analyzed in this study. They were identified by biochemical tests and molecular serogrouping. The presence of virulence markers genes (*inlA, inlC, inlJ*) was also checked. And all strains were subjected to phenotypical analysis to characterize their resistance against 12 antimicrobials.

Results: The isolates were classified into serogroups 4b, 4a, 4c, or 4d (46%), 1/2c or 3c (27%), 1/2a or 3a (13.9%), and 1/2b or 3b (13.1%). Virulence markers were detected in all isolates, and most of tested isolates presented sensitivity to the majority of antimicrobials, but presented resistance or intermediate resistance to clindamycin (88.3%) and oxacillin (73.7%).

Significance: This study showed the prevalence of pathogenic serogroups among isolates. Despite having susceptibility to most of the antibiotics used to treat Listeriosis, the presence of high antimicrobial resistance to oxacillin and clindamycin is a serious concern for public health, and more in depth research is needed to better understand the mechanisms of antimicrobial resistance.

Acknowledgments: CNPq, CAPES, and FAPEMIG.

P2-132 UV Tolerance of Spoilage Microorganisms and Acid-shocked and Acid-adapted *E. coli* in Apple Juice Treated with a Commercial UV Juice-processing Unit

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Introduction: The enhanced thermal tolerance and survival responses of *Escherichia coli* O157:H7 in acid and acidified food products is a major safety concern in the production of low-pH products, including beverages. However, little is known about this phenomenon when using ultraviolet (UV) light treatments. Previous studies have shown that spoilage microorganisms present higher UV tolerance compared to pathogenic bacteria, but limited information has been published regarding the UV doses required to control these organisms when using commercial UV juice-processing units.

Purpose: We studied the effects of strains, acid shock and acid adaptation on the UV tolerance of *E. coli* O157:H7, as well as the UV tolerance of selected spoilage microorganisms using a commercial UV juice-processing unit for the treatment of apple juice.

Methods: The log reduction of five strains (*E. coli* O157:H7 C7927, ATCC® 35150™, ATCC® 43895™, ATCC® 43889™ and *E. coli* ATCC® 25922™) at three physiological states (unadapted-control, acid-adapted, and acid-shocked cells) was determined in apple juice treated with a *CiderSure* 3500 UV reactor and under the conditions stated in current FDA regulations. The UV tolerance of three spoilage microorganisms (*Aspergillus niger, Penicillium commune,* and *Alicyclobacillus acidoterrestris*) was also determined at UV doses between 0 and 70 $mJ \cdot cm^{-2}$. Trials were performed in triplicate.

Results: A higher than 5-log reduction with *E. coli* was achieved under all tested conditions. A significant effect of strain ($P = 0.01$) was observed, but the physiological state did not affect UV inactivation ($P = 0.96$). Total reductions of 3.3, 3.8 and 5.0 logs with *Aspergillus*, *Penicillium* and *Alicyclobacillus*, respectively, at 70 $mJ \cdot cm^{-2}$were observed.

Significance: These findings suggest that the use of unadapted *E. coli* is adequate to conduct challenge studies using UV light technologies, and support the relevance of a hurdle approach to extend the shelf life of UV treated beverages.

P2-133 Associations between Farm Management Practices and Spore Counts Provide Novel Information about Spore Populations in Raw Milk

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Introduction: Bacterial spores are important contaminants of dairy products, given their unique capacity to survive unfavorable environmental conditions. Therefore reducing levels of spores in raw ingredients, namely raw milk, remains key to dairy product quality and safety.

Purpose: The goal of this study was to enumerate and identify bacterial spores in bulk tank raw milk.

Methods: Bulk tank raw milk samples and corresponding surveys examining farm management practices (n = 198) were collected from 33 farms, sampled every other month for 1 year. Raw milk samples were spore pasteurized (80°C for 12 min) to enumerate psychrotolerant, mesophilic, and thermophilic spore-forming bacteria, and PCR amplification of the *rpoB* gene was used for isolate identification. *Bacillus cereus* Group isolates were screened for i) the presence of toxin encoding genes, and ii) for hemolysis on sheep's blood agar.

Results: Overall, psychrotolerant, mesophilic, and thermophilic spores were detected (> 10 spores/ml) in 1% (2/198), 74% (147/198) and 58% (115/198) of samples, respectively. Different management practices were associated with mesophilic and thermophilic spore levels, with the exception of herd size. *Bacillus licheniformis* was the most frequently isolated organism, representing 48% (313/654) of all isolates. Genes encoding the non-hemolytic enterotoxin and enteric FM toxins were present in all *B. cereus* Group allelic types (n = 10), while genes encoding the hemolysin BL toxin were absent for 5 out of 10 of the *B. cereus* Group allelic types, as determined by PCR screening. All representative *B.* cereus Group strains, except one, were hemolytic on sheep's blood agar.

Significance: The results presented here provide relevant baseline spore counts in raw milk, and associations between management practices and spore counts that could be used to reduce spore contamination of raw milk for enhanced quality and safety.

P2-134 Comparative Proteomics Analysis by iTRAQ-2DLC-MS/MS Provides Insight into the Key Proteins Involved in *Cronobacter* Species Biofilm Formation

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Introduction: *Cronobacter* spp., an opportunistic foodborne pathogen, can cause high mortality in neonatal and premature infants because this pathogen is associated with powdered infant formula (PIF) contamination. Biofilm formation is recognized as the most effective means to help *Cronobacter* survive throughout the long shelf life of PIF, thus promoting resistance to cleaning agents and disinfectants.

Purpose: To find the key proteins involved in *Cronobacter* spp. biofilm formation and invasion.

Methods: Crystal violet staining and fluorescence microscopy analyses were used to compare the biofilm formation ability among different *Cronobacter* strains and to discover the key proteins that are involved in *Cronobacter* biofilm formation. Two-dimensional liquid chromatography–tandem mass spectrometry, which was coupled with isobaric tags for relative and absolute quantification (iTRAQ) labeling, was employed to quantitatively identify the proteins that were differentially expressed in the weak biofilm former *C. sakazakii* ATCC29544 compared to the strong biofilm former *C. dubliniensis* DSM 18707.

Results: In total, 1190 differentially expressed proteins were detected. Gene ontology analysis indicated that these differentially expressed proteins are related to biological binding, cell structure, signal transduction, cell adhesion, and cellular interaction. Among these differential proteins, the expression levels of 448 non-redundant proteins were altered significantly, and 177 of these proteins were differentially expressed by more than 5-fold, with 81 up-regulated proteins and 96 down-regulated proteins.

Significance: In this study, the proteins that are attributed to the differential biofilm formation of *Cronobacter* strains were screened using the iTRAQ approach of quantitative proteomic analysis. Several of these proteins were selected to elucidate the mechanism of biofilm formation in *Cronobacter* spp.

P2-135 Food Safety of Farmstead Cheese Processors in Pennsylvania: An Initial Needs Assessment

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Introduction: Every year, roughly 48 million people get sick, 128,000 are hospitalized, and 3,000 die because of foodborne illness. In the U. S., from 1998 to 2011, 90 outbreaks were attributed to cheese, causing 1,083 illnesses, 229 hospitalizations, and 6 deaths. Raw milk cheeses, characteristically made by farmstead dairy processors, have been involved in several of these outbreaks; but little is known about this audience's food safety practices.

Purpose: The main goal of this study was to conduct a needs assessment addressing food safety issues at farmstead cheese establishments in Pennsylvania, using information obtained from dairy inspectors, cheesemakers, on-site observations, and environmental sampling.

Methods: State dairy inspectors (n = 12) were surveyed to identify the most common "out of compliance" issues during inspections conducted over the last 4 years. Another survey was developed and disseminated to farmstead cheesemakers (n = 10) to assess their food safety knowledge, behavior, and attitudes. Five (5) farmstead dairy establishments also were visited and observed during cheesemaking sessions to evaluate food safety and sanitation issues. Finally, environmental samples from a processing facility were taken on two different days and analyzed using ATP bioluminescence and microbiological methods.

Results: The inspector surveys (n = 6), the observations during cheesemaking (n = 5), and environmental sampling (n = 23) results suggest that basic sanitation is a concern and there is room for improvement. Additionally, the farmstead dairy processors indicated that their knowledge, attitude, and behavior were good or very good in the areas of sanitation (70%) and food safety (65%).

Significance: These findings indicate that farmstead cheese processors may lack basic food safety practices and demonstrate gaps in their food safety knowledge, attitudes, and behaviors. As such, there appears to be a need for food safety training, with an emphasis on sanitation, for this underserved audience.

P2-136 Genotypic and Phenotypic Characterization of Enterotoxigenic Methicillin-resistant *Staphylococcus aureus* Isolates from Bovine Raw Milk in Korea

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❖ ***Developing Scientist Competitor***

Introduction: *Staphylococcus aureus* is one of the major etiological pathogen of bovine mastitis. Also, methicillin-resistant *S. aureus* (MRSA) has been constantly isolated from bovine milk and is known as the causative agents of various clinical infectious diseases. The presence of MRSA in milk can be regarded as a potential hazard for public health.

Purpose: We investigated the antimicrobial resistance, virulence and genotype of MRSA isolated from bovine raw milk in Gyeonggi province of Korea.

Methods: A total of 1,222 raw milk samples were collected from 47 dairy farms from Gyeonggi province of Korea, during 2011 to 2012. Of these samples, 643 milk samples that had more than 500,000 SCC/ml were further examined for the isolation of mastitis-causing Staphylococci. The *spa* typing, SCC*mec* typing and PFGE were also analyzed. Antimicrobial resistance was determined using a disk-diffusion test and MIC determination by broth dilution test were also performed according to the CLSI guidelines.

Results: All MRSA isolates showed four or more drug resistant and four PFGE types (A-D). The most frequently detected staphylococcal enterotoxin (SE) gene profile was *seg*, *sel*, *sek*, *sem*, *sen* and *seo* genes (20 isolates, 87%). None of the MRSA carried the *vanA*, *vanB*, *eta*, *etb*, *tsst,* and *PVL* genes. Nine of the 23 MRSA origination from three farms belonged to SCC*mec* type IVa-t324. Just three isolates from the same dairy farm of the SCC*mec* type II-t002.

Significance: Our results show that there are significant similarities in genotypic characteristics among MRSA isolates from different farms in Korea. And there is a possibility of clonal transmission of enterotoxigenic MRSA from one dairy farm to another dairy farm. Investigating distributions of MRSA in dairy herds is important for establishing infection control strategies by providing basic information.

P2-137 Culture Independent Analysis of Contaminated Yogurt via Next Generation Sequencing

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Introduction: In September 2013, select lots of Greek yogurt in US marketplaces were suspected of being contaminated with an unknown biological agent. Consumer complaints ranged from odd taste and smell from their yogurts to a few reported that they became ill after consuming this product. The suspected lots were recalled by the manufacturer and further investigation led to the identity of the contamination a mold, *Mucor circinelloides*. The company stated that this mold is commonly found in plants and it not harmful for human consumption.

Purpose: We have developed a culture-free analytical tool in order to detect unknown contaminants in foods containing live microbials. In addition to identifying unknown biologics, we can explore the known contents of these products and ensure proper safety and labeling in rapid time.

Methods: Utilizing whole genome sequencing, we have developed a pipeline that includes DNA isolation, data production, and in-house data analyses for identification of microbes in dietary supplements and functional foods. Analyses performed on this food commodity included strain identification from the yogurt samples sent from FDA's Office of Regulatory Affairs Pacific Regional Lab (PRL).

Results: From the analytical work, two types of mold, *Mucor circinelloides* and *Rhizopus*, were identified. This result is different from what was provided by the company in their reported analysis. In addition, the presence of the mold in these samples caused a shift in their bacterial population.

Significance: This project determined the identity of mold in a contaminated food product using whole genome sequencing and also determined a shift in the microbes present in the yogurt when contaminated. This method will be very useful for identification of contaminants in foods containing live microbials to ensure proper labeling and safety of our food supply.

P2-138 Characterization of *B. cereus* in Extended Shelf-life (ESL) Milk

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❖ ***Developing Scientist Competitor***

Poster

Introduction: Despite the existence of Food Safety Management Systems, *B. cereus* spore contamination continues to be a global concern more so in mild heat treated products including dairy products. Unlike most ESL milk products that are based on ultra-pasteurization (94 - 100°C) a recently introduced milk product on the South African market is produced by pasteurization (72 - 73°C), bactofugation and aseptic cold filling.

Purpose: The objective of this research was to isolate *B. cereus* from the entire ESL milk processing chain, processing equipment (filler nozzles) and the final chill stored ESL milk product to determine the characteristics and similarities of *B. cereus* strains isolated.

Methods: Spore formers were isolated at selected sampling locations using standard plating methods. Isolates were identified using biochemical, morphological tests and MALDI-TOF-MS. $(GTG)_5$-Rep-PCR fingerprinting was performed to determine similarities between the *B. cereus* isolates. A discriminatory PCR was carried out to identify psychrotrophic strains of *B. cereus.*

Results: MALDI TOF MS dendrogram showed that there were six groups of *B. cereus*. All *B. cereus* showed ability to digest blood agar and were positive on proteolytic activity. $(GTG)_5$-Rep-PCR revealed the presence of two major clusters with subgroups. Results of the discriminatory PCR showed the presence of csp*A* gene in all isolates therefore indicating that the isolates were psychrotrophs. Isolates were sequenced using the 16S RNA gene to confirm the identity of strains.

Significance: The close relationship between isolates from the ESL milk product and those from filler nozzles shows processing equipment is a source of product contamination with psychrotrophic *B. cereus* which is of both safety and spoilage concern. This research can be utilized for validation of Food Safety Management Systems and risk assessment of *B. cereus* in ESL milk.

P2-139 Efficacy of High Pressure Pasteurization as a Kill Step for *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in Low and High Acid Juices and Almond Milk

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Introduction: High pressure pasteurization (HPP) is a cold pasteurization process used extensively in the juice industry as a kill step to achieve the 5-log reduction of pertinent pathogens mandated by Juice HACCP (21CFR120). The efficacy of a standard HPP treatment to achieve a 5-log reduction of vegetative pathogenic microorganisms in a variety of juice and beverage products was evaluated.

Purpose: Evaluate effectiveness of HPP to achieve a 5-log reduction of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in Carrot Mix Juice (pH = 4.12), Green Juice (pH = 3.86), Apple Juice (pH = 3.64), Carrot Juice (pH = 6.41), Coconut Water (pH = 5.37), and Almond Milk (pH = 8.43).

Methods: Products were separately inoculated with *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes*. Inoculated products were HPP-treated at 85,000 psi for 180 seconds. Log reductions were determined immediately after HPP and after 24 h of storage at 4°C post-HPP using MPN methodology.

Results: For high acid juices (pH < 4.6), log reductions > 5 logs were achieved for all organisms in all products after 24 h of storage post-HPP. However, immediately after HPP, log reduction of *E. coli* O157:H7 in Apple Juice was 4.82 ± 0.48 logs (n = 3). For low acid products (pH > 4.6), log reductions > 5 logs were achieved for *Salmonella* and *L. monocytogenes* in Coconut Water and Almond Milk. However, a 5-log reduction of *E. coli* O157:H7 was not achieved in either of these products. HPP was the least effective for Carrot Juice, in which log reductions of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* were 2.31 ± 0.16, 1.79 ± 0.00, and 3.91 ± 0.30 (n = 3), respectively.

Significance: The results of this study raise questions regarding the ability of HPP (as tested) to achieve the required 5-log reduction of *E. coli* O157:H7 in low acid juices or Almond Milk. For high acid juices, products may require an additional 24 h hold post-HPP to achieve a 5-log reduction of *E. coli* O157:H7.

P2-140 Evaluation of Analyst Proficiency and Laboratory Performance in Testing Pathogens in Infant Formula Based on a Proficiency Study

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Introduction: Understanding analyst/laboratory performance in the detection of pathogens in infant formula can affect enforcement of regulations. Sources of error in detection can be traced to analytical techniques, analysts, and random effects. Quantifying these sources can be used to improve quality assurance and safety.

Purpose: Evaluate proficiency test (PT) data to assess analytical performance in detecting pathogens.

Methods: Commercial liquid and powdered infant formula samples (LIF, PIF) were inoculated at two levels in duplicate with cocktails of Gram positive and negative organisms and with one or more of the following pathogens: *Staphylococcus aureus, Bacillus cereus*, *Salmonella* Typhimurium*, Listeria monocytogenes*, *Shigella flexneri*, *Cronobacter sakazakii*, and *Escherichia coli* O157:H7. The inoculation procedures, mixing methods, and homogeneity/stability were tested according to ISO 17043 and ISO 13528. A total of 135 analysts from 59 laboratories (LIF) and 29 analysts from 19 laboratories (PIF) tested for one or more pathogens based on preferred methods.

Results: Interlaboratory comparisons from proficiency testing data were performed based on ISO 22117 guidelines. Qualitative results are summarized as percentages of correct results with total observations in parentheses for LIF/PIF: *Staphylococcus* 100%/88% (292/60), *Bacillus* 88%/88% (200/40), *Salmonella* 98%/94% (380/72), *Listeria* 100%/95% (380/64), *Shigella* 90%/83% (220/60), *Cronobacter* 99%/96% (104/24), and O157 96%/73% (368/48). Specificity (r_{SP}) and sensitivity rates (r_{SE}) were calculated according to ISO 22117. For low inoculation levels, r_{SP} ranged from 96.3 - 100% and r_{SE} ranged from 75.6 - 100%. For high inoculum levels r_{SE} was 92 - 100%. The lowest r_{SE} values were found in low inoculum samples of *Bacillus*, 84%, and *Shigella*, 75.6%; indicating these samples were the most challenging.

Significance: This study shows how PT data can be used to ensure laboratories have the capability to detect key food pathogens. It also demonstrates the value of expansive, matrix-relevant PTs with realistic microbiological composition to generate method performance data in rapid and economical ways.

P2-141 Aerobic Count and Coliform/*E. coli* Method Comparison in Pasteurized Milk Containers

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Introduction: The Pasteurized Milk Ordinance (PMO) specifies food safety procedures and is managed by the National Conference on Interstate Milk Shipments (NCIMS). PMO Section 7, Item 11p requires Single Service Containers and Closures to be manufactured in a facility in compliance with Appendix J of the PMO. Appendix J contains bacteriological testing requirements and standards for Containers and Closures using approved test methods.

Purpose: The goal of this study is to compare the newly developed simplified methods, Charm Peel Plate EC HV (total coliform) and Peel Plate AC (aerobic count), to the NCIMS/FDA reference methods according to NCIMS laboratory committee procedures.

Methods: Gallon milk containers were tested in accordance with NCIMS/ FDA form 2400i Pasteurized Milk Containers using the 100 ml nutrient broth rinse method. The containers were divided into six (6) groups of five (5) containers each. Five (5) groups were inoculated with *P. aeruginosa* and *K. pneumonia* and/or *E. coli* at various levels. After broth addition and agitation, each container was plated using methods for coliform (Peel Plate EC HV, AOAC 996.02 and Violet Red Bile Agar confirmed with BGLB broth) and methods for aerobic plate (Peel Plate AC, AOAC 986.33 and Standard Plate Count Agar). Coliform tests were incubated at 32 ± 2°C for 24 ± 2 h and aerobic bacteria 32 ± 2°C tests 48 ± 3 h.

Results: In the method comparisons, results were evaluated for repeatability (S_r) and by paired-*t*-test for statistical difference using > 0.5 log. The Peel Plate EC HV and Peel Plate AC results were not significantly different, less than 0.2 log difference at each concentration, from the NCIMS/FDA reference pour plate and film methods for both coliform count and aerobic plate count.

Significance: The comparative analyses with Peel Plate AC and Peel Plate EC HVS show that these methods are at least equivalent to NCIMS/FDA approved reference methods and meets NCIMS acceptance criteria for single service container and closure testing according to Appendix J of the PMO.

P2-142 The Use of Kefir Supernatant to Control *Cronobacter sakazakii* in Experimentally Contaminated Powdered Infant Formula

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Introduction: Kefir is a type of fermented milk containing lactic acid bacteria, acetic acid bacteria, and yeast.

Purpose: In this study, we evaluated the antimicrobial activity of kefir supernatant against *Cronobacter sakazakii* in powdered infant formula (PIF).

Methods: Antibacterial activity was assessed by the spot-on-lawn method, agar well diffusion assay, measurement of optical density of culture broths. The antimicrobial activity of kefir supernatant to experimentally contaminated PIF was also investigated by plate count method.

Results: In a spot-on-lawn test, 20 *C. sakazakii* strains, including 10 clinical isolates and 10 food isolates, were completely inhibited by kefir supernatant. In an agar well diffusion test, significant differences were observed between the diameters of inhibition zones obtained by kefir supernatant and culture supernatants obtained from *Lactobacillus kefiri* and *Candida kefyr*, as well as solutions of lactic acid, acetic acid, and ethyl alcohol ($P < 0.05$). In addition, the growth of *C. sakazakii* in nutrient broth containing different volumes of kefir supernatant was evaluated by spectrophotometry. The addition of 100 µl of kefir supernatant into 1 ml of nutrient broth completely inhibited the growth of and killed *C. sakazakii*. Finally, we applied the antimicrobial activity of kefir supernatant to experimentally contaminated PIF. Remarkably, no viable *C. sakazakii* remained in the PIF rehydrated with 30% kefir supernatant solution.

Significance: In conclusion, kefir could be a great option to control *C. sakazakii* in PIF owing to its advantages of reduction in antibiotic use and its well-established safety for oral consumption.

P2-143 Evaluation of Microbiological Contamination Levels of Small- and Medium-sized Dairy Farms in Korea

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Introduction: Dairy farming is a class of agricultural enterprise for long-term production of milk, which is processed for eventual sale of a dairy product. In Korea, small- and mid-sized dairy farms have been increased gradually to reach 30-50% of total dairy farms.

Purpose: In the present study, we investigated the microbiological contamination levels of small- and mid-sized dairy farms in Korea for suggesting managing and safety plans for the small- and mid-sized dairy farms. Also we verified effectiveness of teat washing and milk pasteurization as production process for control of microbiological hazard.

Methods: A total of 55 swab samples and 55 dairy products samples were collected between August and October 2014 from dairy farms located in Gyeong-gi and Jeon-la province. Mesophilic aerobic bacteria, coliforms and *Escherichia coli* were enumerated using 3M Petrifilm™. *Salmonella*, *Staphylococcus aureus, Listeria monocytogenes, Enterococcus faecalis* and *Enterococcus faecium* were isolated according to the methods provided by

Korean Food Code. In addition, the antibiotic susceptibility of isolated bacteria was determined by the disk diffusion method according to CLSI guideline.

Results: Mesophilic aerobic bacteria, coliforms and *Escherichia coli* were significantly reduced after teat washing and milk pasteurization. A total of 1 *Salmonella*, 12 *S. aureus*, 18 *E. faecalis*, and 18 *E. faecium* were isolated. Of these, 85.7% were from the samples collected samples obtained before teat washing and milk pasteurization. The difference in the antibiotic resistance patterns of milk isolates and cured cheese isolates was observed suggesting that cured cheeses contamination would occur after pasteurization production process.

Significance: Based on the results, we suggest that milk pasteurization and teat washing arethe most important processes for reducing the microbiological hazard in final dairy product. Furthermore, it is needed to prevent post-pasteurization contamination to ensure the safety of final dairy products.

P2-144 Characterization and Source Tracking of *E. coli* in Bulk Milk

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Introduction: Pathogenic strains of *E. coli* are among the common causes of foodborne disease outbreak in many parts of the world. Understanding the characteristics of *E. coli* isolates and its pathogens in food is important for risk assessment and is also a critical step for diagnostic and epidemiological purposes.

Purpose: The purpose of this study was to characterize *E. coli* in bulk milk for safety and microbial source tracking.

Methods: A total of 258 bulk milk samples were collected from purchase points in 8 different geographical regions around South Africa. 3M *E. coli* petrifilm plates were used for detection, isolation and enumeration of *E. coli*. Matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was used for rapid identification of the *E. coli* isolates. Virulence factors determination, antimicrobial agent susceptibility tests, detection of ESBL producing capacity and serotyping of the *E. coli* isolates was performed.

Results: There was a significant difference ($P < 0.05$) in *E. coli* counts among the regions. *E. coli* (121) isolates were detected and isolated from 36.4% of the milk samples. More than 15% of the *E. coli* isolates were positive for virulence genes *stx1*/*stx2* and 25.6% (n = 121) gave positive results for the O157:H7 serotype. Antimicrobial resistant *E. coli* isolates were detected in 70% of the milk samples, of which 36.8% of *stx1*/*stx2* positive *E. coli* showed multi-drug resistance. Presence of ESBL capacity was observed in 20.6% of the isolates. Cluster analysis using GTG_5 finger printing revealed clustering of the isolates irrespective of origin.

Significance: The information from the current research can be useful in modelling the risk from pathogenic *E. coli* in bulk milk sold in the informal market in South Africa.

P2-145 Pressure Change Technology – An Innovative Method for the Inactivation of Spoilage Microorganisms in Wine and Fruit Juices

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Introduction: The pressure change technology is a novel technology for inactivating microorganisms in temperature sensitive beverages like fruit juices and wine without adding chemicals like sulfur dioxide. The beverage is pressurized and mixed with a compressed inert gas. The gas diffuses into microbial cells and after instantaneous pressure release the gas expands which leads to cell rupture.

Purpose: The main objective was to determine the effect of incubation time of yeast, the main spoilage microorganisms in fruit juices and wine, on inactivation in order to define the most promising process step in wine manufacturing for the application of the technology.

Methods: *Saccharomyces cerevisiae* was incubated at 23°C in red grape juice for 0, 1, 2, 6 or 9 days. The fermented grape juice was treated in a pressure chamber at 500 bar with nitrogen at different temperatures (10, 25, 40°C) in triplicate, respectively. The viable count before and after decompression was determined by plating on wort agar and incubation at 30°C for 3 - 5 days.

Results: The resistance of yeast decreased for all process temperatures until an incubation time of two days (end of exponential growing phase). Afterwards in the stationary phase the resistance increased significantly. For an incubation time of 2 days the highest viable count reduction was found at 25°C (-1.0 ± 0.1 log) while at 10°C and 40°C the reduction was slightly lower (-0.8 ± 0.2 log and -0.7 ± 0.1 log, respectively) indicating that the process temperature had a minor effect.

Significance: The pressure change technology is a valuable tool for reducing spoilage microorganisms in wine and fruit juice while retaining the aroma and flavor. Most promising is the application for the inactivation of yeast in the transition from exponential to stationary growing phase to stop the fermentation in the wine making process without adding sulfur dioxide.

P2-146 Thermal Inactivation of *Listeria monocytogenes* and *Salmonella* Species in Flavored Cream Cheese Formulations Cooked at 65°C

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Introduction: Unpasteurized or contaminated ingredients may introduce pathogens into cream cheese formulations. Therefore, additional cooking and hot-fill may be useful to ensure safety.

Purpose: To determine the thermal inactivation rates of *Listeria monocytogenes* and *Salmonella* in various cream cheese spreads heated at 65°C for 10 min.

Methods: Three varieties of cream cheese spread were formulated to represent a wide range of products including 47 - 60% moisture, 0.7 - 1.6% salt, a_w 0.94 - 0.97, pH 4.9 - 5.3, and fat 19 - 23%. For each formulation, ingredients were heated with agitation to 65°C, and then inoculated with a 5-strain mix of either *L. monocytogenes* or *Salmonella* to yield 8 log CFU/g. Triplicate samples were assayed at 0, 2, 4, 6, 8 and 10 min for *L. monocytogenes* or *Salmonella* populations by enumerating on modified Oxford agar or xylose-lysine-deoxycholate agar, respectively, with thin layer overlay of nonselective media to enhance recovery of injured cells. The survivor curves were modeled using GInaFiT software.

Results: Data confirmed that *Salmonella* was more heat sensitive than *L. monocytogenes* in the cream cheese formulations tested. Cooking formulations at 65°C yielded > 6-log kill of *L. monocytogenes* and *Salmonella* within 6 and 2 minutes, respectively, for all three formulations. Data analysis using log-linear regression provided higher R^2 values compared to using the Weibull model; D-values ranged from 22 to 57 s for *L. monocytogenes* and 18 s for *Salmonella* for all treatments. Inactivation of *L. monocytogenes* was most rapid in the mushroom formulation (60%

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moisture, a_w 0.97, pH 4.9; D-value 22 s) where > 5.0-log CFU/g reduction was achieved in 2 min, whereas similar reduction was observed at 4 min for chocolate (48% moisture, a_w 0.96, pH 5.3; D-value 57 s) and cranberry-orange (47% moisture, a_w 0.94, pH 4.9; D-value 53 s) formulations.

Significance: The D-values generated by this study provide manufacturers guidelines for designing thermal processes for hot-filled cream cheese.

P2-147 Risk Assessment of Raw Caprine and Bovine Milk Produced and Consumed in the Willamette Valley of Oregon

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Introduction: Asymptomatic cows and goats sporadically shed zoonotic microbial pathogens into milk leading to numerous outbreaks of foodborne illness linked to raw milk consumption. Despite these known risks, the demand for raw dairy products remains high and state laws regarding retail sale are becoming increasingly lenient. Effective testing strategies to detect contaminated milk coupled education efforts targeting raw milk producers are necessary to reduce risks associated with raw milk.

Purpose: Understand attitudes, knowledge, and production practices of raw dairy producers to identify opportunities for extension education and determine daily changes in microbiological quality and safety of raw milk from bovine and caprine dairies.

Methods: Surveys were conducted to assess producer knowledge, attitude, and production practices using commercially available software (Qualtrics, Provo UT). Two caprine and two bovine dairies in the Willamette Valley of Oregon provided sequential daily raw bulk tank milk samples for 30 days. Milk samples were analyzed for aerobic and coliform counts and for the presence of *L. monocytogenes, Salmonella* spp., and Shiga Toxin-producing *E. coli* (STEC) using a multiplex real-time PCR assay targeting *inv*A, *stx*1*, stx*2*,* and *iap* genes coupled with cultural methods.

Results: Thirty-four Oregon dairy producers responded to the survey, 16 organic and 18 conventional farms with diverse responses for record keeping, sanitation, and motivation to produce raw milk. From the 30-day sampling period, two samples from a single bovine dairy were positive for STEC and one sample from a caprine dairy was positive for *L. monocytogenes*.

Significance: Production practices, attitudes, and knowledge among raw milk producers in Oregon are variable. Clinically healthy animals sporadically shed zoonotic pathogens confirming a risk to raw bovine and caprine milk consumers. Standard testing frequencies are insufficient to detect sporadic shedding events.

P2-148 Detection of *Salmonella* Species in Mexican-style Cheese with PCR-based Technology

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Introduction: Mexican-style cheese is a group of perishable products that can be a vehicle of human pathogens such as *Salmonella* spp. Detection time of pathogens in this food type is crucial because of its short shelf life. Some commercial PCR-based technology provides a protocol for qualitative detection *of Salmonella* in approximately 26 hours as an alternative to the traditional method.

Purpose: The purpose of the study was to evaluate the detection limits for *Salmonella* spp. using a PCR-based technology in Mexican-style cheeses.

Methods: *Salmonella* Montevideo (ATCC 8387) resistant to rifampicin was inoculated in two types of Mexican-style cheese (panela and oaxaca cheese; 25 g portions) with 4, 28 and 68 cells. Pathogen detection was performed according to the ANSR protocol and traditional methodology. At the end of enrichment, *Salmonella* was quantified on tryptic soy agar supplemented with rifampicin. Assays were performed in triplicate.

Results: *Salmonella* was detected in the inoculated cheese samples in all cases. The concentration of *Salmonella* after enrichment was 8 log CFU/ml in oaxaca cheese and 6 log CFU/ml in panela cheese. Total plate count of oaxaca and panela cheese were 6.3 and 8.9 log CFU/g, respectively. Associated microbiota in panela cheese could be hindering *Salmonella* growth; however, ANSR technology was able to detect it.

Significance: PCR-based technology was able to detect *Salmonella* in panela and oaxaca cheese, offering a reliable alternative to traditional methodology.

P2-149 A Unique Rapid Detection and Quantification Assay for Total Count of Yeasts and Molds in Dairy Products Based on Multiplex Real-Time PCR

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Introduction: Ubiquitous in the environment, foods can easily become contaminated with yeasts and molds becoming predominant spoilers, particularly in dairy products. Thus, their presence and amount are regularly monitored. Conventional methods can take ≤ 14 days. A rapid test would economically benefit the dairy industry with faster product release.

Purpose: Validation of the sensitivity, robustness and specificity of a lyophilized real-time PCR assay (the **food**proof®Yeast & Mold Quantification LyoKit) for detection and quantification of yeasts and molds in dairy products.

Methods: Ten different dairy sample types were diluted 1:10 and spiked with yeasts and molds at specific concentrations of ≤ 6 x 10^3 CFU/g. Eight hundred µl of homogenized sample was treated with Reagent D for live/dead cell differentiation. Live cell DNA was extracted and real-time PCR performed. Live/dead differentiation efficiency, sensitivity (DNA and cell spiked samples), specificity (inclusivity and exclusivity), and robustness were determined for the new **food**proof® Yeast and Mold Quantification LyoKit, and compared to the classical ISO-method (ISO 6611).

Results: Genomic DNA from 15 yeast and mold species was tested with 11 replicates. One hundred percent of replicates were positive, even at 0.39 GE in all dairy samples. All sample types spiked with 10^2 and 10^3 CFU/g *Yarrowia lipolytica, Aspergillus niger, Candida kefyr,* and *Hypopichia burtonii* showed appropriate positive signal. All samples spiked with 6000, 600, 60 and 6 CFU/g showed positive results with a deviation of < 1 ct. Thus, quantification was successful (LOD of < 10 CFU/g). Comparison to ISO 6611 showed good correlation independent of matrix type. Specificity results showed 100% success for inclusivity (290 strains from 260 species) and exclusivity testing (> 60 bacteria, plants and mammal cells).

Significance: Validation of the new commercial kit showed results in > 4 hours equivalent to or better than ISO 6611 for the detection and quantification of yeasts and molds in dairy products.

P2-150 Comparison of Different Enumeration Protocols for Indicator Microorganisms in Water and Sediment

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Introduction: Water quality, such as irrigation water quality, becomes more and more important for food safety. *Escherichia coli* and Enterobacteriaceae have been used as indicator organisms to monitor the potential fecal contamination of water. Previous studies have shown that both the *E. coli* and the Enterobacteriaceae counts might be significantly different when water is sampled at different times of the day. It has also been proposed that sediments might contain higher levels of indicator microorganisms than the surface water, and may be one of the reasons causing fluctuations reported in bacterial enumeration.

Purpose: To better understand the fluctuations seen from bacterial enumeration and the role sediments may play in water quality monitoring, this study chose two lakes in the state of Alabama as models and examined their water quality.

Methods: At each lake, two sampling spots were chosen. Fifty ml of surface water and 25 g of sediment from each sample site were collected and plated using 3M™ Coliform/*E. coli* Petrifilm and 3M™ Enterobacteriaceae Petrifilm. The concentrations of the indicator microorganisms were enumerated following the manufacture's manual. In addition, one ml of surface water was collected using a disposable Transfer Pipet and plated using *E. coli* Coliscan Easygel® for parallel comparison with the 3M method.

Results: Results showed that both the sampling time (morning vs. afternoon) and sample types (sediment vs. surface water) impacted the enumeration results ($P < 0.05$). Because the Enterobacteriaceae group covers bacteria such as *Salmonella* and *Shigella*, the numbers of Enterobacteriaceae in water and sediment were always higher than the total *E. coli*/Coliform ($P< 0.05$).

Significance: The results of this study indicate that a more accurate sampling method and sampling schedule are still needed in order to better monitor water quality, especially the water used for irrigation purposes and direct body contact.

P2-151 Natural Occurrence of Foodborne Pathogens in Native Pecan Production and the Influence of Cattle Grazing

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Introduction: Foodborne illness outbreaks associated with tree nut consumption highlight the need for a basic understanding on the natural occurrence of foodborne pathogens in tree nut production and the potential contributing factors, including cattle grazing, which may lead to such contamination.

Purpose: The objectives of this study were to assess the natural occurrence of two major foodborne pathogens, Shiga Toxin-producing *Escherichia coli* (STEC) and *Salmonella,* in native pecan production and the influence of cattle grazing in such incidence.

Methods: Samples of soil, cattle feces, pecans (from the ground and harvested), and wild animal fecal swabs were collected from eight pecan production orchards (four cattle grazed and four without) at pre-harvest (two months before harvesting) and during harvest. Swabs from harvesting equipment surfaces were also collected during harvest. Isolation and detection of STEC and *Salmonella* were performed by enrichment, selective enrichment/plating, and multiplex PCR. Molecular typing of isolates was conducted by pulsed-field gel electrophoresis (PFGE).

Results: While both pathogens were detected in pecan production orchards, significantly ($P < 0.05$) higher numbers of STEC-positive samples were obtained. *Salmonella* was detected in pre-harvest (17%) and harvest (8%) soil samples from a non-grazed orchard as well as cattle feces (17%) in grazed orchards at pre-harvest; however, no *Salmonella* was detected in harvested pecans, regardless whether the orchards were grazed by cattle or not. Surprisingly, the percentages of STEC-positive pecans (from the ground or harvested) were not significantly different between grazed and nongrazed orchards, with 33% ground pecans and 5% harvested pecans from nongrazed orchards and 8.3% ground and 17.5% harvested pecans from grazed orchards tested positive for STEC.

Significance: The results of our research show the presence of human pathogens in native pecan production and the complicated contributing sources to such contamination, including sources other than cattle grazing.

P2-152 Developing Effective Consumer Protection Measures for Trade in Seafood: A Study of the Situation in Oman

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Introduction: Trade in seafood brings positive benefits but presents challenges. Food protection measures recommended by international organizations are frequently embraced by major importing countries but they also impose additional safety requirements which lead to rejections as exporters fail to meet the standards. Adopting the highest standards for a national seafood control system can be an excessive burden so frequently countries adopt a dual system with separate controls for the international markets and those for the majority of the products for the more local markets. With regional liberalization also occurring, deciding on appropriate protection measures is complex.

Purpose: The study examines the safety and quality issues facing the seafood industry in the Sultanate of Oman, a member of the regional Gulf Cooperation Council (GCC), as it builds its export markets. We aim to identify the marketing channels and the structural barriers and provide recommendations for enhancing consumer protection in the country and its international markets.

Methods: Data and information were collected from literature reviews, reports and related studies. A semi-structure questionnaire was used to collect data from different officials in the Omani seafood control system.

Results: The study shows that in Oman seafood is often exposed to a lengthy distribution chain and, given its perishability, its quality and safety declines. Controls in Oman are shared across various government agencies with attempts made to increase export approved premises and improve those serving the domestic market. Along with other GCC countries, there should be moves to a fully risk-based control system.

Significance: The issues faced by Oman are typical of those faced by many developing countries attempting to build an export trade. The results can be used by many similar countries seeking to compete successfully in the global market whilst enhancing their domestic food protection.

P2-153 Survey of Hygiene Practices in the Ready-to-Eat Meats Slicing Sector in Supermarkets in São Paulo, Brazil

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Introduction: Meat slicers are among the most difficult items to clean. Poor hygiene practices in the Ready-to-Eat meat products slicing sectors in retail foodservice establishments may cause cross-contamination with undesired microorganisms, affecting their safety and shelf life. Proper risk assessments related to this type of cross-contamination depend on information of these practices.

Purpose: To conduct a survey of the hygiene and handling practices adopted in establishments where Ready-to-Eat meat products are sliced.

Methods: A questionnaire was developed based on Brazilian legislation related to SSOP and GMP, validated and applied in 10 randomly selected supermarkets in the city of São Paulo, Brazil. Questions addressed mainly hygiene practices during food handling and slicing, personal and environmental hygiene, and documentation.

Results: Eighty percent of the evaluated supermarkets followed the SSOP recommended by the Brazilian legislation, and had a trained employee responsible for the adoption of GMP in the slicing sector. However, the GMP manual was not available for consultation in 30% of the establishments. Also, in 30% of the establishments, slicers were not cleaned with water and detergent. In 40% of establishments, the cleaning was performed only once per day, at the end of the day, while in 60% the machines were cleaned at every shift change, usually twice a day. Only 40% documented the frequency of cleaning and used sanitizers for disinfection of slicers. Seventy percent alcohol was the most common sanitizer. Only 40% of the employees sanitized their hands before entering the slicing sector. In 20%, the temperature of refrigerators did not follow the regulations (-4°C to 4°C).

Significance: This survey contributed with bits of information on hygiene practices adopted in the Ready-to-Eat meat products slicing sectors in some Brazilian supermarkets, contributing with data for proper assessment of risks associated to these products.

P2-154 FDA's Regulatory Approach to Preserving the Safety and Effectiveness of Medically Important Antimicrobials Used in Food-producing Animals

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Introduction: Medically important antimicrobials (MAs) are used in veterinary medicine to treat, control, and prevent diseases in food-producing animals (FAs). In addition, some MAs are currently approved for production purposes (*e.g.*, increased rate of weight gain or improved feed efficiency). All antimicrobial uses carry a risk of antimicrobial resistance development; however, production uses may particularly be a contributing factor in the rise of antimicrobial resistance due to their frequent administration to entire FA herds or flocks at low doses and for prolonged durations. FDA thinks that such production uses of MAs are not judicious.

Purpose: This presentation provides an up-to-date understanding of FDA's regulatory activities surrounding MAs in FAs.

Methods: FDA's current risk-based regulatory approach, as detailed in Guidance for Industry (GFI) #152, is to evaluate potential human health risks associated with proposed uses of antimicrobial drugs in FAs. All production uses of MAs were approved prior to the publication of GFI #152; therefore, these products were not evaluated using the current approach with respect to antimicrobial resistance. To begin a reconciliation of these concerns, FDA published GFI #209 and GFI #213. GFI #209 provides the following framework to ensure judicious use of antimicrobials in FAs: 1) limit use of MAs in FAs to those that are considered necessary for assuring animal health, and 2) limit such uses in FAs through veterinarian oversight. GFI #213 provides recommendations on how to implement the strategies outlined in GFI #209.

Results: To date, all sponsors of some 293 affected applications have confirmed their intent to comply with the recommendations outlined in GFI #213. Additionally, 36 actions have been taken by sponsors on these affected applications.

Significance: In summary, FDA thinks using MAs judiciously is critical to minimize the selection and dissemination of antimicrobial resistance from FAs and to help preserve their effectiveness.

P2-155 "Free Riders" and Weak Economic Incentives to Control Foodborne Pathogens

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Introduction: Why haven't scientific innovations to test and control pathogens from farm to fork been uniformly adopted by companies? How can stronger economic incentives improve foodborne pathogen control?

Purpose: Private market incentives for food safety are weak, because the causative pathogen and the food company are rarely linked to the 47.8 million U.S. acute foodborne illnesses each year. Similarly, regulatory programs at FDA and USDA link only an estimated 0.5% of acute foodborne illnesses to the food and its company. What actions by Federal regulators could strengthen the linkage and thereby strengthen the economic incentives for foodborne pathogen control?

Methods: Economic policy analysis evaluates the costs and public health benefits of options to limit the burden of disease due to foodborne pathogens in the U.S. marketplace, estimated at $78 billion annually by Robert Scharff. Advances in testing methods have created new faster, cheaper ways to identify specific pathogen serotypes and thus created new options for pathogen identification, linkage to companies, and control options.

Results: The building blocks of a Federal system to increase economic incentives and accountability for food companies to control foodborne pathogens are: 1) requiring pathogen tests from farm to fork, 2) requiring government access to all private pathogen test results (as a condition for selling food in the U.S. marketplace, 3) setting strict pathogen performance standards that require yearly improvement, 4) recording all legal liability cases and out-of-court settlements involving foodborne pathogens in a public database, 5) expanding epidemiologic research to identify and quantify acute human illnesses for CDC's "unknown" foodborne pathogens (80% of illnesses) and the Long Term Health Outcomes of acute foodborne illnesses (acknowledged by CDC but not enumerated), and 6) creating an integrated nationwide database to link pathogens to specific food products and to the companies that supply these contaminated foods.

Significance: Creation and enforcement of this six step Federal program will give food companies stronger economic incentives to control pathogens and will level the playing field. **Currently, bad-actor companies who cause foodborne illness are given a "free ride" as these companies are not held accountable for damage they inflict on U.S. consumers. This "free ride" hurts good-actor companies that do invest in superior pathogen control.** Finally, the costs of implementing these six steps are much less than the estimated $78 billion annual U.S. public health burden due to foodborne pathogens causing both acute and Long Term Health Outcomes.

P2-156 Design of a Semi-quantitative Self-assessment Tool for Evaluation of Readiness Level With the Proposed Produce Standards of the Food Safety Modernization Act (FSMA)

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Introduction: As one of the components of the Food Safety Modernization Act (FSMA) of 2011, FDA published the proposed Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption in 2013, a preventive approach to improve food safety in produce.

Purpose: To design a semi-quantitative self-assessment tool to evaluate the preparedness level of produce producers to satisfy the proposed Produce Standards (PS) of FDA.

Methods: Requirements proposed in the PS were organized into subparts, and separate questionnaires were created on the basis of each specific requirement. To assess the readiness level, a color-coded scale from 0 to 5 was designed. The user assigns a particular qualification, and

the instrument automatically computes the score and percentage of relative compliance for each individual section as well as the overall set of rules. Using a series of hyperlinks, the final scores are displayed as a graph that represents a global evaluation of the activities and practices in the operation, contrasted to the specific requirements of the proposed rule. To facilitate the application by different countries, the tool was created in English and Spanish. The ease and effectiveness of the instrument was evaluated in six different produce companies in Central America.

Results: Producers were able to understand the tool, and identify aspects that require more attention. The instrument provided scores that represent the implementation level of the proposed rule. Produce companies were able to create a plan of action and use the numerical scales as indicators to follow the progress of the improvements.

Significance: This tool provides valuable information to producers, auditors, technical support specialist, and government to better implement the PS. Producers need to assess the preparedness level of their operations to potentially comply with the rule once implemented, so that their access to the US market is not compromised.

P2-157 Antimicrobial Resistance Profiles and Biofilm Formation of *Escherichia coli* Strains Isolated from Produce-Farm Environments in Northeast Mexico

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Introduction: Antibiotic treatment for diarrheal infections caused by *Escherichia coli* has changed in recent years. This is due in part to bacterial resistance to antimicrobials usually applied in treatments for human diseases and in animal production. On the other hand, biofilm formation is considered an important virulence factor, which could enhance survival and pathogenesis.

Purpose: To determine the antibiotic resistance profiles and biofilm formation of *E. coli* isolates prevailing in farm environments in Northeast Mexico.

Methods: One hundred and seventy-two isolates of *E. coli* were obtained from tomato and jalapeño farm environments (produce, farmworkers' hands, soil and water). The Kirby Bauer diffusion disc method was used to determine antibiotic resistance against nalidixic acid (30 µg), tetracycline (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ampicillin (10 µg), trimethoprim/sulfamethoxazole (25 µg), cefotaxime (30 µg), ceftazidime (30 µg) and chloramphenicol (30 µg). Biofilm formation was quantified in microplates with broth after staining with crystal violet and measured spectrophotometrically. The biofilm formed was classified as strong, moderate or weak.

Results: A higher percentage of antibiotic resistance of isolates was observed against ampicillin (25% of isolates), and tetracycline (24% of isolates), followed by ceftazidime (21%), chloramphenicol (13%), cefotaxime (12%), nalidixic acid (10%), gentamicin (9%) and trimethoprim/sulfamethoxazole (7%). All strains were sensitive to ciprofloxacin. Higher resistance was observed in strains isolated from tomato, 38% of these isolates were resistant to ampicillin, whereas the isolates from jalapeño pepper were more resistant to tetracycline (23%). Biofilm production was observed by most strains (approximately 70%), 40% of the isolates produced a strong biofilm.

Significance: Information about antibiotic resistance or sensitivity and biofilm formation of wild *E. coli* strains is important for the establishment of control measures and for administration of appropriate antibiotics by health institutions.

P2-158 The Detection of Carbapenemase-producing *Escherichia coli* and Antibiotic Resistance

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Introduction: The emergence of Carbapenem-resistant *Enterobacteriaceae (CRE)* is a rising public concern. It is often associated with multidrug resistances which leave few therapeutic options. Most CRE infections are associated with people in hospitals and health care facility.

Purpose: The purpose of this study was to detect the presence of carbapenemase-producing *Escherichia coli* from fresh vegetables and characterize their antibiotic resistance.

Methods: Seventy-six confirmed *E. coli* samples were randomly chosen and tested for the presence of New-Delhi *Metallo-β-lactamase* (NDM) using polymerase chain reaction (PCR). Strains that showed the presence of the NDM were further tested for antibiotic resistant against seven antibiotics (Penicillin, Kanamycin, Vancomycin, Methicillin, Ceftazidime, Imipenem, and Biapenem) using the spiral gradient test (SGE).

Results: Thirty-two percent of the samples produced NDM. The MIC range was 0.4 - 16 (µg/ml) and the interpretation of the MIC value was determined according to the Clinical and Laboratory Standard Institute M100-S16 document. Overall, all tested samples where susceptible to Kanamycin. Ninety-six were resistant to Imipenem. Methicillin, Penicillin, and Vancomycin, had 82 - 88% resistant, ≤ 30% susceptibility, and no intermediate. Ceftazidine was 32% resistant and 32% susceptible to the tested samples. However, the tested samples showed 25% intermediate resistance to Ceftazidime. Biapenem had an effect on the tested samples but there were no criteria to interpret the results.

Significance: The results suggest that carbapenemase-producing *E. coli* is present in vegetables with potential to be resistant to commonly used antibiotics. Therefore additional safety protocols should be implemented to track vegetables and ensure their safety.

P2-159 Prevalence, Multidrug Resistance and Biofilm Forming Ability of *E. coli* O157:H7 Isolates of Fecal, Milk and Water Samples at a Major Abattoir, Ibadan, Nigeria

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Introduction: Multi-drug resistant Enterohemorrhagic *Escherichia coli* (EHEC) of O157:H7 serotype is a worldwide zoonotic pathogen responsible for causing severe human diseases. This pathogen has shown biofilm forming properties, reducing the microbial safety of foods.

Purpose: This study determined the prevalence, antibiotic susceptibility patterns and biofilm forming abilities of *E. coli* O157:H7 from feces and milk of slaughtered cattle and abattoir water samples during two climatic seasons at the Bodija municipal abattoir, Ibadan, Nigeria.

Methods: A total number of 196 fecal, 34 milk and 61 water samples were assessed and isolation was made using standard cultural and serological methods. Susceptibility to eight antimicrobials was tested using disc diffusion method. Biofilm was assayed on microtiter plates in two media (Tryptose soy broth and Tryptone soy broth + 2% glucose) at 37°C for 48, 96 and 144 h incubation and quantified using the crystal violet binding assay.

Results: A total prevalence rate of 20.6%, 6.6% and 4.9% were recorded for milk, fecal and water samples, respectively. Prevalence of *E. coli* O157:H7 was significantly higher during wet than in the dry season ($P < 0.05$). All isolates were resistant to one or multiple antibiotics, but highest resistance (95.7%) was to Augmentin and Amoxicillin (87%) while Ofloxacin (87%) and Gentamycin (78.3%) were the most sensitive. The strains produced higher biofilm masses after 48 h incubation especially with glucose (0.45 ± 0.02 to 0.46 ± 0.07).

Significance: These results indicate that feces and milk of slaughtered cattle and water used for processing in the abattoir may be important sources of *E. coli* O157:H7 contamination of beef, especially during the rainy season if strict decontamination strategies are not taken. Presence of glucose sources would increase their biofilm forming abilities.

P2-160 Comparative Genomics of *Escherichia coli* Isolated from Soil Fertilized with Litter of Chicken Fed with or without Tetracycline

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Introduction: The persistence of antibiotic resistant bacteria in agricultural soils is a critical food safety issue.

Purpose: We analyzed and compared the genomes of *Escherichia coli* from soils fertilized with litter of chickens fed with or without tetracycline-supplemented diet to determine their antimicrobial resistance, virulence and persistence.

Methods: The genomes of 13 *E. coli* strains collected over several months (8 from soil fertilized with litter of tetracycline-fed chickens and 5 from that of control diet) were sequenced by Illumina MiSeq. Sequence processing and assembly were performed using Spades, before annotating them using RAST. Known virulence and antibiotic resistance genes were detected by BLASTN. A whole-genome phylogenetic tree was built to define the relationships among strains.

Results: The genome size ranged from 5.0 to 5.3 Mbp, with a G+C content of 50.4 to 51.2%. The number of protein-coding genes and mobile genetic elements varied from 4905 to 5357, and 40 to 136, respectively. All 13 *E. coli* strains harbored *tetD*, bla_{CMY-2} and *ampC* genes while *tetA* or *tetB* and bla_{TEM} were the most frequently found genes in strains recovered from the tetracycline treatment regardless of the collection time (August - March). Strains isolated at different months appeared to contain similar resistance genes, suggesting their maintenance over time. Virulence genes such as *malX*, *ompT*, *traT* and *iss* were found in all 13 strains. Furthermore, our data point toward possible horizontal genes transfer. Phylogenetic analysis showed genetic relationships between strains, indicating their persistence in soils over time.

Significance: Our results reveal that *E. coli* strains from chicken litter harboring multiple antibiotic resistance and virulence genes persist in agricultural soils regardless of tetracycline feed-supplementation. Such *E. coli* could constitute a reservoir of antibiotic resistance/virulence genes and present a food safety risk.

P2-161 Development of the Fly 'Crop Vessel Assay' to Elevate Growth of *Escherichia coli* O157:H7 in the House Fly, *Musca domestica*

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Introduction: The house fly, *Musca domestica* can transmit human pathogens including *Escherichia coli* O157:H7 through regurgitation of ingested bacteria from the crop. The crop is a foregut organ of house fly which stores the excess ingested nutrients. Interactions between the ingested bacteria and the crop have a direct influence on bacteria persistence, survival and ultimately fly vector competence.

Purpose: The development of an *in situ* crop vessel assay to investigate bacterial growth within fly crops up to 48 h post-ingestion.

Methods: Flies were fasted for 12 h prior to feeding *E. coli* ATCC 43895 with pEGFP. Flies were fed bacteria suspended in sterile M9 media with 1% glucose, ampicillin and IPTG. Red food coloring was added to confirm that flies had consumed the bacteria. After feeding flies *E. coli* O157:H7, the flies with red abdomens were chilled at 0°C**;** 70% ethanol was used to externally disinfect the fly. Crops were removed and maintained in sterile phosphate buffered saline in microtiter plates held at 32°C. For each time point, five crops were homogenized individually using a tissue grinder and bacterial levels (CFU/crop) were monitored using plate counts. Confocal microscopy of intact crops was used to monitor biofilm development.

Results: There was no statistical increase in cell numbers (CFU/crop) over the 48 h incubation period. Microscopy shows that upon prolonged incubation, GFP-expressing *E. coli* within the crop produced biofilms. This method showed greater reproducibility in studying bacterial interactions with the crop, than using a live fly feeding study.

Significance: This assay is a reproducible *in situ* assay that can be used to study the biofilm formation of *E. coli* O157:H7 within the crop of *M. domestica*. This system will allow further studies to better study the relationship and dissemination of *E. coli* O157:H7 from the environment to food by house fly.

P2-162 Characterization of Equine-associated *Escherichia coli* Isolates in South Korea

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Introduction: *Escherichia coli* (*E. coli*) is a part of the normal microflora of the gastrointestinal tract of animals and humans. According to the increased percentage of GDP, many people want to go out to enjoy their leisure time such as horseback-riding and horse meat. Imogen Johns *et al* reported that the isolation rate of ESBLs-producing *E. coli* from horse samples was over 60 percent.

Purpose: In this study, we examined the cross-transmission of antibiotic resistant (AR) *E. coli* between horses and their environments.

Methods: Isolated putative colonies from 3,078 swab samples were confirmed as *E. coli* by PCR method and antibiotic sensitivity of *E. coli* isolates was determined by a disk diffusion test with the 16 antibiotic disks according to the CLSI. In addition, PCR was performed to all isolates resistant to ampicillin, tetracycline, streptomycin and trimethoprim/sulfamethoxazole for screening the presence of the individual resistance genes. The genetic relatedness among the AR *E. coli* isolates was determined by PFGE.

Results: A total of 143 *E. coli* isolates (4.6%) were obtained from the swab samples and 30 isolates (21.0%) were resistant to at least one antibiotic. Most of AR *E. coli* isolates carried the corresponding resistance genes with minor exceptions. PFGE analysis with the 29 AR *E. coli* isolates showed that 2 clone sets identical in their molecular patterns were isolated from the 2 different sampling places.

Significance: The PFGE data indicated the possibility for cross-transmission of AR *E. coli* clones between horses and their environments. Therefore, we should examine the prevalence and antibiogram of equine-associated *E. coli* constantly.

P2-163 Shiga Toxin Production by Different *Escherichia coli* Serotypes Grown under High Osmolarity Conditions

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Introduction: Shiga Toxin-producing *E. coli* (STEC) include serotypes with different levels of pathogenicity to humans. The toxins produced by STEC (Shiga Toxin 1 and 2), and their subtypes, are one of their major pathogenic attributes. Ultraviolet light, certain antibiotics, and low iron levels have been shown to affect toxin production, but little is known about how other environmental factors affect growth and toxin production among STEC.

Purpose: The purpose of this study is to assess various phenotypic conditions defined through the Biolog platform.

Methods: The Phenotypic Microarray (PM) was used in this study. We focused on the PM-9 plate to determine the effect of different osmolyte/ionic conditions on the growth and toxin production among STEC. PM-9 plates were inoculated following manufactures instructions, and data was analyzed using Biolog software to determine growth kinetics. The presence of Shiga Toxin from PM-9 filtrates was determined by ELISA.

Results: Our preliminary results show a significant variability on the survival of different STEC to salt (NaCl). The 2011 German outbreak strain *E. coli* O104:H4, is highly osmotolerant and survives well up to 6% NaCl (W/V). However, *E. coli* O157:H7 strain EDL933 cannot grow above 3%, and EC4045, from the spinach outbreak, grows well up to 4%. We also observed differences on the Shiga Toxin produced under these stress conditions among the different serotypes. Shiga Toxin was detected from *E. coli* O104:H4 cultures grown on media supplemented with 6% NaCl.

Significance: Salt is widely used as food preservative in meat processing. Although the effect of salt in the survival and toxin production of *E. coli* O157:H7 is well known, little work has been done on other STEC serotypes that can also present a threat to public health.

P2-164 T4 Bacteriophage-resistant *Escherichia* Variants Display Altered Phenotypes and Growth in a Model Food System

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Introduction: Bacteriophages are readily found in foods, where they constantly interact with bacteria. These interactions may lead to consequences regarding the fitness of bacterial pathogens as they grow in foods.

Purpose: The objective of this study was to determine how bacteriophage resistance in *Escherichia coli* affects growth and survival in foods, using a model system.

Methods: Bacteriophage T4 *E. coli* mutants were produced by growing *E. coli* K-12, in broth culture, in the presence of high concentrations of bacteriophage T4. Bacteriophage resistant mutants were confirmed by spotting the broth cultures on TSA plates, followed by addition of T4 bacteriophage. Colonies that grew in the presence of T4 were isolated and tested for altered biochemical properties using API 20E test strips, and altered susceptibility to the antibiotic kanamycin. In addition, several bacteriophage resistant mutants were evaluated for their ability to grow in beef slurry following an 8-hour incubation at 25°C.

Results: Eight T4 bacteriophage resistant *E. coli* K-12 mutants (A to H) were isolated. These variants did not display any biochemical changes by API20E or differences in kanamycin susceptibility compared to wild-type K-12. Interestingly, of three mutants tested, one (G) showed no growth in beef slurry after 8 hours.

Significance: These results demonstrate that bacteriophage interactions may alter the fitness of *E. coli* in foods. These results are important in light of the increasing use of bacteriophages as antimicrobials in foods.

P2-165 Effects of Temperature and Benzoic Acid on 5-log Reduction Times for Pathogenic *Escherichia coli* Strains in Acidified Pickled Vegetables

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Introduction: The survival of enteropathogenic *Escherichia coli* strains in acid and acidified foods depends on a variety of environmental factors. Previous studies have focused on 'worst case scenario' (10° C) conditions. Data on the relative effects of environmental factors on cell survival (i.e., temperature and benzoic acid) are lacking.

Purpose: The purpose of the this study was to determine the relative effects of temperature, acetic acid and benzoic acid concentration on 5-log reduction times for EHEC under conditions typical of acidified vegetables.

Methods: A cocktail of 5 *E. coli* O157:H7 strains was subjected to 1% and 2% acetic acid solutions, with and without 0.1% benzoic acid at 10, 20, and 30°C under anaerobic conditions at pH 3.5 using brined cucumbers as a representative pickled vegetable. Surviving cells were recovered on non-selective tryptic soy agar at 37°C for 48 h. Survivor curves from 3 or more independent replications were analyzed with SAS (PROC MIXED) and Matlab software to determine and compare 5-log reduction times.

Results: For 2% acetic acid data, increasing temperature (10°, 20°, and 30°C) resulted in a 10-fold decrease in 5-log reduction times with and without benzoate. The 5-log reduction time with 0.1% benzoate at 10°C was 73 ± 5 hours *vs* 8 ± 0.22 h at 30°C, and similarly, 328 ± 12.5 hours *vs* 28.6 ± 0.9 hours without benzoate. For 20 and 30°C data, the interactions of the effect of benzoate temperature or acetic acid on the 5-log reduction times were found to be significant ($P < 0.05$).

Significance: The data show the importance of benzoate and increasing temperature for decreasing 5-log reduction times for pathogenic *E. coli* strains in acidified vegetable products. Results may be useful for estimating 5-log reduction times based on product formulation and storage conditions.

P2-166 Comparative Analysis of Generic and Pathogenic *E. coli* following a Synthetic Gastric Juice Challenge

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❖ ***Developing Scientist Competitor***

Introduction: Enteric bacteria are often exposed to environmental stress and acidic conditions prior to causing infection. Conditions that allow an organism to survive on a leaf surface and then cause disease are not fully understood.

Purpose: In this study *E. coli* isolates that previously exhibited environmental fitness were subjected to gastric juice and compared to laboratory-control strains.

Methods: *E. coli* isolates (2) were recovered from manure and leafy greens at 10 days post inoculation from previously conducted field trials or from survival in wildlife feces over 12 months (O104:H4). Five *E. coli* isolates, including 2 laboratory-control strains, were individually compared for survival on spinach leaves (0 or 1 h) first and then in synthetic gastric juice pH 2.0 (0.5 or 2 h) or in 0.1% peptone water. Spinach plants (n = 6 of 3 pooled plants) were inoculated with 7 log CFU per strain and recovered bacteria enumerated on TBX agar.

Results: Bacteria were recovered from spinach plants and the field isolates survived exposure to gastric juice significantly better than control strains despite presence of *rpoS* in the controls ($P < 0.003$). All isolates decreased from initial concentrations by an average of 1.7 - 2.6 ± 0.3 log CFU after plant recovery. Laboratory-control strains of *rpoS* positive and deficient *E. coli*, decreased by an average of 3.91 and 3.99 log CFU after gastric juice exposure ($P = 0.008$); while field isolates and O104:H4 decreased by an average of 0.79,1.20, and 0.82 log CFU after 2 h of gastric acid exposure. No differences were observed between gastric juice exposure times. No significant decrease was observed in any strain following leaf recovery and 2 h in peptone water. Research extending time points will be conducted.

Significance: Field isolates survived on leaves and in synthetic gastric juice better than laboratory-strains, suggesting these isolates have enhanced persistence which merits further attention.

P2-167 Characterization of Bacteriophage Integrases in *Escherichia coli* Reveals the Presence of Prophages Originating from the Foodborne Pathogens *Salmonella enterica* and *Shigella flexneri*

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Introduction: Temperate phages constitute a main source of genetic diversity associated with the virulence of many foodborne bacterial pathogens, and these phages are known to encode toxins, effector proteins, and adhesion factors, and to favor the dissemination of antibiotic resistance genes and other mobile genetic elements such as pathogenicity islands. Within the Enterobacteriaceae, the integration of prophages into their bacterial hosts is mediated by site-specific tyrosine integrases.

Purpose: The objective of this study was to characterize the types of prophage integrases contained within the fecal indicator bacteria *Escherichia coli*, as a measure of the presence and diversity of prophages that may disseminate virulence factors.

Methods: One thousand bacterial isolates were obtained from feces of wildlife collected from bovine feedlots in Colorado, USA. Isolates were confirmed as *E. coli* through cultural methods and biochemical tests. PCR and gel electrophoresis was used to assess phage integrase gene diversity in *E. coli*, by using degenerate primer sets designed against all known enteric phage tyrosine integrase sequences located in GenBank.

Results: Of 27 *E. coli* isolates selected for further study, fourteen (51%) were observed to contain at least one tyrosine integrase, three (11%) isolates contained two tyrosine integrases, and three isolates (11%) had three integrases. Further analysis showed that 11 (41%) of the *E. coli* isolates contained phages originating from *Salmonella enterica* and *Shigella flexneri,* with two isolates potentially containing multiple phages originating from *Salmonella enterica*. Several phages were induced by the antibiotics mitomycin C and streptonigrin.

Significance: This study highlights the diversity of temperate phages in *E. coli*, and demonstrates that many of these phages originate from enteric foodborne pathogens in which temperate phages are known to transduce toxin and antibiotic resistance genes. The use of tyrosine integrases as a marker for phage diversity in bacterial species of foodborne importance is also significant.

P2-168 Characterization and Infection Receptor Analysis of Bacteriophages for *Escherichia coli* O157:H7 and non-O157 Shiga Toxin-Producing *E. coli*

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Introduction: Shiga Toxin-producing *Escherichia coli* (STEC) are one of the pathogenic *E. coli* and produce Shiga Toxins. Although *E. coli* O157:H7 has been involved in most of outbreaks in the world, non-O157 STEC serotypes are also associated with outbreaks and severe human illness.

Purpose: The objective of this study was to isolate bacteriophages for *E. coli* O157:H7 and non-O157 STEC and compare physiological characteristics. Also, infection receptor analysis of bacteriophages for *E. coli* O157:H7 and non-O157 STEC, which might be associated with phage-host recognition, was conducted.

Methods: Seven bacteriophages for *E. coli* O157:H7 and non-O157 STEC were isolated from various environmental samples, respectively, and characterized using plaque assay. Also, random mutant library was constructed by transposon mutagenesis, and phage resistant mutants were screened for bacteriophage receptor identification.

Results: As the result, *E. coli* O157:H7 bacteriophages, which belonged to *Siphoviridae* family, were specific only to 24 *E. coli* O157:H7 strains with 95% infection rate, while non-O157 STEC bacteriophages, which belonged to *Myoviridae* family, showed broad host range in 10 non-O157 STEC strains, 24 *E. coli* O157:H7 strains and 51 non-pathogenic *E. coli* strains. In stability test, only 22% and 11% of *E. coli* O157:H7 bacteriophages averagely decreased under pH 3 and 70% ethanol for an hour, respectively, while 48% and 56% of non-O157 STEC bacteriophages averagely decreased under the same conditions, respectively. In infection receptor analysis, *E. coli* O157:H7 bacteriophages recognized O-antigen as a receptor when they infected *E. coli* O157:H7 ATCC 43890, and non-O157 STEC bacteriophages recognized core as a receptor when they infected *E. coli* O103 NCCP 13937.

Significance: In conclusion, *E. coli* O157:H7 and non-O157 STEC bacteriophages might have different morphological characteristics and host spectrum, and *E. coli* O157:H7 bacteriophages might be more stable than non-O157 STEC bacteriophages under harsh conditions of acidic pH and organic solvent. Also, each receptor of two bacteriophage groups could result from different part of lipopolysaccharide.

P2-169 Inflammatory Immune Response of Rat Orally Fed with *E. coli* Bacteriophages

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Introduction: Bacteriophage is an emerging agent as an effective alternative to conventional biocontrol for the food safety. However, its safety when applied to foods and humans still remains a concern.

Purpose: In the present study, inflammatory responses against five *E. coli* bacteriophages in rats was investigated.

Methods: Ten rats were orally fed with 8 log PFU/ml of the bacteriophage cocktail per day for periods of up to 4 weeks and sacrificed.

Results: Bacteriophage killing specific bacteria has received interest in applying sanitizers or phage therapy, however, its safety has been in doubt. This study investigated the safety of bacteriophage by determining inflammatory immune response of rats treated with bacteriophage. The rats were administrated with the bacteriophage cocktail of log 8 PFU for *E. coli* a day for four weeks. Feed consumption and efficiency and body weight were measured and there were no significant differences compared to the control group, and the phage was not detected in the serum collected before autopsy. For the histopathological examination, the notable changes were not observed in tissue of liver, kidney, and spleen. However, measure of organ weight showed some differences from the liver of males and the ovary (left) of females. The remarkable changes were not observed at 12 different cytokines in the serums of the rats. Among mRNA expression of proinflammatory cytokines of COX-2, IL-6, and TNF-α in the liver, kidney, and

spleen, COX-2 mRNA expression was increased 2.4-fold, which was a significant difference only in spleen. Therefore, this study showed that there were slight changes in the proinflammatory response of rats fed with the bacteriophage.

Significance: Therefore, the *E. coli* bacteriophages may potentially induce inflammatory responses in rats.

P2-170 Quantification and Comparison of *Salmonella* Typhimurium and Heidelberg Biofilm Formation on Plastic Coupons

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❖ ***Developing Scientist Competitor***

Introduction: Plastic containers that are used to transport and store food products are subject to biofilm formation once exposed to microorganisms. Biofilms can survive harsh environmental conditions, and once established, these bacterial communities can result in widespread contamination, which will ultimately lead to foodborne illness.

Purpose: The purpose of this study was to quantify and compare *Salmonella* Typhimurium and Heidelberg biofilm formation on plastic coupons by conventional microbiological methods.

Methods: *Salmonella* Typhimurium and Heidelberg were inoculated into separate 40 ml sterile specimen cups containing enrichment broth and plastic coupons, and the cups were incubated for 24 h at 37°C, 110 rpm. Subsamples (obtained at 0, 4, 8, 12, and 24 h) were diluted and plated in duplicate to determine the CFU/ml of unattached cells, and following 24 h of growth, coupons were rinsed with deionized water and vigorously vortexed for 1 min with 3 g of glass beads and 20 ml of phosphate buffer saline (pH 7.4) to remove attached cells.

Results: The percent CFU/ml recovered (biofilm concentration/starting concentration) from the plastic coupons for *Salmonella* Typhimurium and Heidelberg was (0.09 ± 0.01%) and (0.06 ± 0.15%), respectively.

Significance: This research provides useful information regarding biofilm formation on plastic, and will ultimately educate food producers that consider reusing plastic containers for the transportation and storage of food products, especially fresh produce.

P2-171 Prevalence and Diversity of *Salmonella* Phages Isolated from Animal Farms and Wastewater of a Seafood Processing Plant

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❖ ***Developing Scientist Competitor***

Introduction: *Salmonella* spp. are pathogenic bacteria that can cause a serious public health concern worldwide. Previous studies have indicated the association between *Salmonella* hosts and *Salmonella* phages mostly in the farm environments. Characterization of *Salmonella* phages from diverse sources are thus needed to better understand the prevalence, diversity, and the phage-host dynamics among these sources.

Purpose: The aim of this study was to determine the prevalence and the host range of *Salmonella* phages from different food-associated environments, i.e., animal farms and a seafood processing plant.

Methods: Five feces samples were collected from a chicken farm, a swine farm, and a goat farm and five samples were obtained from wastewater treatment ponds of a seafood processing plant. Samples were enriched with a cocktail containing 15 serovars of *Salmonella enterica* for phage isolation. Purified and tittered phages were used to perform the host range on 25 *Salmonella* isolates (23 serovars) and one *E. coli*. Clustering analysis was performed using the lysis patterns from three replicates.

Results: All samples from animal farms and a seafood processing plant were positive for phages, yielding eight and 42 *Salmonella* phages, respectively. Host range characterization of those phages showed that phages could be clustered into three groups: (i) 18 phages (36%) representing narrow host range (lysing 1 to 5 host strains), (ii) 20 phages (40%) represent intermediate host range (lysing 2 to 14 host strains) and (iii) 12 phages (24%) representing broad host range (lysing 10 to 24 host strains). The majority of phages from farms showed broad host range as compared to phages from a food processing plant.

Significance: This study indicates that *Salmonella* phages isolated from animals feces have broader host range than those from the food processing plant environment, suggesting the diversity of the *Salmonella* hosts predominantly present in each source.

P2-172 Prevalence, Serovars and Antimicrobial Resistance of *Salmonella* from Australian Cattle Populations at Slaughter

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Introduction: Antimicrobial agents are used in cattle production systems for the prevention and control of bacterial associated diseases. Australia is the world's third largest exporter of beef; however, it does not have an ongoing surveillance system for AMR *Salmonella* in cattle or foods derived from these animals.

Purpose: To determine the prevalence, serovars and phenotypic AMR status of *Salmonella* from a range of cattle classes.

Methods: A total of 1500 fecal samples from adult beef (628), young beef (286), adult dairy (128), young dairy (143) and veal calves (315) were tested for *Salmonella* using a Dynabead IMS protocol. The resulting *Salmonella* isolates were serotyped using molecular and conventional serotyping methods and were subsequently phenotypically assessed for resistance to 17 antimicrobials using microbroth dilution and the Sensititre apparatus.

Results: *Salmonella* were isolated from 65 (10.4%) adult beef, 39 (13.6%) young beef, 23 (18.0%) adult dairy, 44 (30.8%) young dairy and 45 (14.3%) veal calves. Thirty-seven *Salmonella* serovars were identified with Typhimurium comprising between 20 and 50% of isolates across each of the cattle classes. Anatum, Bovismorbificans and Saintpaul were the only other serovars present in > 10% of isolates in any cattle class. Greater than 96% of all isolates were susceptible to all antimicrobials tested. AMR was only observed in isolates from beef cattle and were to antimicrobials of low importance to human medicine.

Significance: Although some differences in prevalence and AMR between cattle classes were observed, there is minimal evidence that specific production practices are responsible for disproportionate contributions to AMR development and in general resistance to antimicrobials of critical and high importance in human medicine was low regardless of the isolate source.

P2-173 Internalization of *Salmonella* in *Arachis hypogaea*, Peanut Plants and Seed Pods

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❖ ***Developing Scientist Competitor***

Introduction: Recent outbreaks involving *Salmonella* in peanut products has prompted scientists to investigate the routes of contamination of *Salmonella* into peanuts. One potential route that has yet to be studied in detail is the internalization of *Salmonella* cells into peanut plants and seedpods from soil.

Purpose: The objective of this research was to assess and characterize the possible routes of *Salmonella* internalization into peanut seedpods and plant tissues.

Methods: The internalization of *Salmonella* Typhimurium was studied by exposing peanut seedpods to cell suspensions and inoculated soil containing 7.0 log CFU/g at different temperatures and variable times (n = 3 - 6). For sterile soil experiments, moisture level and soil type were studied. Internalized cells were determined by swabbing and rinsing the interior surfaces of the seedpod. External and internalized cells were quantified using differential tryptic soy agar. Peanut plants were also grown in inoculated soil and the presence of *Salmonella* in the roots, stems, and leaves was determined.

Results: Using cell suspensions, the average count of internalized cells after 22-h exposure ranged from 3.4 log CFU/pod at 16°C to 6.4 log CFU/pod at 37°C which resulted in rates of internalization of 0.07 and 13.7%, respectively. This trend was consistent with 4-h exposure results. Pods exposed to *Salmonella*-inoculated soils at < 10% moisture content had no detectable (< 10 CFU/pod) internalized cells, but when water content was > 15% the mean internal count was 4.2 log CFU/pod. Plants that were seeded in inoculated soils had detectable levels of *Salmonella* inside of roots and stems.

Significance: The direct uptake of *Salmonella* by peanut seedpods indicated a potential route for product contamination is influenced by moisture and temperature. This work suggests that peanut seedpods and whole plants are susceptible to *Salmonella* infiltration, which indicates an entry point into a peanut processing facility from the growing field.

P2-174 Comparison of Attachment of *Salmonella* Enteritidis and Typhimurium on Black Pepper, Almond and Hazelnut

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❖ ***Developing Scientist Competitor***

Introduction: *Salmonella enterica* Enteritidis and Typhimurium are the two most common serovars that cause foodborne illness in the U. S. Outbreaks of these serovars have been attributed to low moisture foods such as peanuts, infant formula, and chocolate. However, little is known about the mechanisms of attachment on low moisture food surface.

Purpose: To evaluate the influence of black pepper, almond, and hazelnut surface characteristics on the attachment of *Salmonella* Enteritidis and Typhimurium.

Methods: *Salmonella* Enteritidis PT4 and Typhimurium LT2 were inoculated onto the surfaces of black pepper, almonds and hazelnuts at 10^9 CFU/g. After air drying for 2 h, samples were incubated at 25°C and 60% humidity. At 0, 1, 3, 7 and 14 d, samples were recovered with agitation at 200 rpm to remove loose or non-attached bacteria. To remove more firmly attached bacteria, the samples were then vortexed at 2000 rpm with sterile glass beads. Samples were serially diluted onto BHI and XLD agars. Statistical analyses were conducted using Excel and Prism GraphPad; a *P*-value less than 0.05 was considered significant.

Results: Significant differences were found in the attachment of Enteritidis and Typhimurium on the surface of black pepper, almonds and hazelnuts. For black pepper, the *D*-values (in days) were 24.62 ± 4.51 vs. 6.44 ± 0.42 on BHI and 23.34 ± 3.25 vs. 6.22 ± 0.55 on XLD for LT2 vs. PT4, respectively. For attachment on almonds, D-values were 18.12 ± 7.94 vs. 3.53 ± 0.39 on BHI and 15.35 ± 4.69 vs. 3.26 ± 0.28 on XLD for LT2 vs. PT4, respectively. For attachment on hazelnuts, D-values were 7.49 ± 1.37 vs. 4.37 ± 1.15 on BHI and 7.12 ± 1.38 vs. 4.16 ± 0.97 on XLD for LT2 vs. PT4, respectively.

Significance: The data obtained can be used to better understand the physiology of *S. enterica* on low moisture foods and aid in developing effective control measures to reduce pathogen contamination.

P2-175 Validation of Baking to Control *Salmonella* Serovars in Hamburger Bun Manufacturing and Evaluation of *Saccharomyces cerevisiae* and *Enterococcus faecium* ATCC 8459 as Nonpathogenic Surrogate Indicators for Process Verification

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Introduction: The Food Safety Modernization Act (FSMA) mandates a shift in the food industry's focus to establishing preventative controls and proactively reducing relevant foodborne hazards. Title 1 (Sec. 103) calls for facilities to establish documented hazard analyses and risk-based preventative controls as verification of product safety. To facilitate this process, relevant hazards and appropriate critical limits must be specified for each food product or category.

Purpose: This study was conducted to validate a commercial baking process for hamburger buns to control *Salmonella* spp. contamination and to determine the appropriateness of using non-pathogenic surrogates (*Enterococcus faecium* or *Saccharomyces cerevisiae*) for in-plant baking validation studies.

Methods: Flour was inoculated separately with three stationary phase *Salmonella* serotypes (Typhimurium, Newport or Senftenberg), *Enterococcus faecium* ATCC 8459, or *Saccharomyces cerevisiae* (baker's yeast). Dough was formed, proofed and baked to mimic commercial bun manufacturing conditions. Target microbial populations were enumerated using selective and injury-recovery media in re-dried inoculated flour, pre- and post-proof dough, after defined baking times (9, 11, and 13 min) at a ~218°C oven temperature, and after a 30 min ambient-air chill period following 13 min of baking.

Results: A ≥ 6-log CFU/g reduction for all baking times for all target bacteria was observed. *E. faecium* demonstrated greater thermal resistance compared to *Salmonella* spp., making it a suitable surrogate for commercial baking process validation studies, while *S. cerevisiae* demonstrated lower heat resistance than *Salmonella* spp. and *E. faecium* during initial baking trials and was deemed an inappropriate surrogate.

Significance: The experimental protocol developed and the *Salmonella* spp. reductions reported will assist the baking industry in conducting future process validations with additional product types and will assist bakers in complying with FSMA regulations.

P2-176 Bacteriocin Formation by Dominant Aerobic Sporeformers Isolated from Traditional *Maari*

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Introduction: *Maari* is an alkaline fermented condiment made from baobab seeds. However, the fermentation is spontaneous, uncontrolled resulting in products inconsistent in quality attributes.

Purpose: The purpose is to examine the dominant aerobic sporeformers during traditional *maari* productions, in order to select starter cultures.

Methods: The dominant microorganisms isolated from *maari* were screened for antimicrobial activity by agar well diffusion method. Then, SDS-PAGE and PCR were used to characterize the antimicrobial substances. Finally, the kinetics of bacteriocin production and the effect of aeration on bacteriocin production were studied.

Results: Three *Bacillus subtilis* strains (B3, B122 and B222) in particular had antimicrobial activity against some Gram positive organisms and were selected for further studies. It was found that the antimicrobial substances produced were heat-stable, insensitive to catalase, sensitive to protease and trypsin but resistant to the proteolytic action of papain and proteinase K and equally active at pH values ranging from 3 to 11. Bacteriocin secretion started in late exponential growth phase and maximum activity was detected during the stationary growth phase. Maximum production of bacteriocin was observed under reduced aeration. Specific primers were used to screen isolates B3, B122 and B222 for genes involved in the synthesis of the bacteriocins subtilosin A, subtilin, sublancin and ericin. Amplicons of the expected sizes were detected for *iywB, sboA, sboX, albA* and *spaS* involved in the biosynthesis of subtilosin and subtilin, respectively.

Significance: The present study contributes to the selection of *Bacillus* strains to be used as starter cultures for controlled production of *maari.*

P2-177 Ultraviolet Radiation Resistance and Injury of Long-term-survival Phase Cells of *Salmonella* Typhimurium ATCC 14028 in 0.85% Saline and Apple Juice

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Introduction: Under adverse conditions in nature or food processing environments bacteria may enter a long-term-survival (LTS) phase where numbers of viable cells remain stable for months or years. In this LTS phase, pathogens may develop resistance to antimicrobial interventions.

Purpose: This study investigated the resistance of LTS phase *Salmonella enterica* serovar Typhimurium ATCC 14028 to ultraviolet (UV) radiation in 0.85% (wt/vol) saline and in pasteurized apple juice. The extent of sub-lethal injury in LTS phase survivors was also investigated.

Methods: LTS phase *Salmonella* Typhimurium cells were cultured in tryptic soy broth with 0.6% yeast extract (TSBYE) at 35°C for 14 days. Exponential- and stationary-phase cells grown in TSBYE (35°C) for 4 h and 24 h, respectively, served as control. Cells (10^7 CFU/ml) from each physiological state were exposed to UV light in saline (80 μW/cm^2) and apple juice (820 μW/cm^2). The *Salmonella* Typhimurium survivors were enumerated by plating diluted (10-fold) samples on tryptic soy agar with 0.6% yeast extract or xylose lysine tergitol agar and counting bacterial colonies after incubation (35°C, 24 h).

Results: LTS phase cells consistently exhibited the highest UV resistance ($P < 0.05$). In saline, D-values of exponential, stationary, and LTS *Salmonella* Typhimurium were 0.35, 0.40 and 0.53 min, respectively; in apple juice D-values were 8.69, 8.81, and 10.12 min, respectively ($P < 0.05$). UV radiation (80 μW/cm^2) of *Salmonella* Typhimurium for 2.5 min in saline reduced the number of exponential- and stationary-phase cells by 7.19 and 6.30 log CFU/ml, respectively. In contrast, LTS cells were reduced by only 5.08 log. Within the three physiological states, LTS phase cells had the least sub-lethal injury in the surviving population ($P < 0.05$).

Significance: The results of present study indicate that the LTS state cross-protects *Salmonella* Typhimurium against UV radiation and should be considered when determining the UV radiation D-value for this pathogen.

P2-178 Screening and Characterization of Antibiotic-resistant Plasmids in *Salmonella*

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Introduction: Due to the prolonged and extensive use of antibiotics, the drug resistance of *Salmonella* is more and more serious. Plasmid, as a self-replicated movable element, is widespread in *Salmonella*, and participating in horizontal transfer of antibiotic-resistant genes.

Purpose: The purpose of this study was to locate the antibiotic-resistant genes in antibiotic-resistant plasmids in *Salmonella* isolates, and to determine the prevalence of *Salmonella* with different antibiotic-resistant plasmid types and antibiotic-resistant genes.

Methods: The incompatibility groups (Inc) of 78 *Salmonella* isolates were typed by multiplex PCR. The plasmid elimination assay was conducted with 32 antibiotic-resistant *Salmonella* isolates in 5% SDS at 44.5°C.

Results: The types of the harboring plasmids in 78 *Salmonella* isolates were comprising of nine plasmid incompatibility groups (Inc P, Inc HI2, Inc N, Inc A/C, Inc FIIS, Inc FIA, Inc FIB, Inc FIC and Inc I1). Among them, the Inc P group and Inc HI2 group were the largest groups. Based on the carrying of antibiotic-resistant genes and the typing of plasmids, it was found that all the isolates which carrying bla_{OXA-1} gene and bla_{CMY-2} gene contained Inc HI2 plasmids; the isolates which carrying *qnrA* gene basically contained Inc A/C plasmids (4/5, 80.0%). After plasmid elimination assay, 15 isolates were changed from antibiotic-resistant to antibiotic-susceptible isolates, and the elimination efficiency was 46.9% (15/32). Later, the plasmids of 15 antibiotic-resistant mutants were extracted, and the plasmid profiles were significantly changed after the plasmid elimination assay. The lost plasmids were mainly Inc HI2 group, and the β-lactamases antibiotic-resistant genes (bla_{OXA-1}, bla_{TEM-1} and bla_{CMY-2}) were lost accordingly with the loss of Inc HI2 plasmids, as well as the antibiotic-resistance phenotypes.

Significance: These data suggest that the horizontal transfer of antibiotic-resistant genes in *Salmonella* was mainly related to Inc HI2 plasmids, but not Inc P plasmids.

P2-179 Antibiotic and Disinfectant Resistance of *Salmonella* from Retail Meats in China

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Introduction: *Salmonella* is a leading cause of foodborne illnesses and deaths worldwide. The frequent use of antibiotic and disinfectant in food production and processing may have imposed a selective pressure and contributed to the emergence of resistant microbes. However, little information is available regarding the antibiotic and disinfectant resistance of *Salmonella* isolates from retail meats in China.

Purpose: The objective of this study was to identify the antibiotic and disinfectant resistance phenotypes, genotypes, and genetic relatedness of *Salmonella* isolated from retail meats in China.

Methods: *Salmonella* isolates were recovered from chicken, beef, and pork purchased from selected supermarket. Antibiotic susceptibility was tested according to the standard disk diffusion method. The MICs of disinfectants were determined using the agar dilution method. All isolates were screened for the presence of disinfectant resistance genes and further analyzed for genetic relatedness by PFGE.

Results: Overall, 50.9% (n = 163) of 320 retail meat samples were contaminated with *Salmonella*, and 32 different serovars were identified. The predominant serotype was *Salmonella* Derby (34.8%), followed by *Salmonella* Enteritidis (12.3%) and *Salmonella* Rissen (9.0%). Overall, 82.8% isolates were resistant to OTC, 66.3% to TMP, 28.8% to AMX, 23.9% to AMP, 20.3% to LEV, 11.7% to GEN, 11.7% to EFT, 15.3% to CIP, and 3.7% to AMC, respectively. The MICs of the disinfectants cetyltrimethylammonium bromide and cetylpyridinium chloride were 8 - 128 mg/l and 8 - 256 mg/l. The *qac* and *sugE(p*) gene was found less prevalent (0.0% to 14.7%). Up to 71 distinct PFGE types were identified among the 163 *Salmonella* isolates. PFGE revealed that the resistant isolates were associated with the sampling supermarkets or groceries.

Significance: *Salmonella* can serve as a critical vector in spreading disinfectant and antibiotic resistance. The use of disinfectant in food processing environments may have played a role in the emergence of antibiotic and disinfectant resistant bacteria.

P2-180 Virulence Factor Profile of *Salmonella* Enteritidis Bacteriophage Isolates

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Introduction: The characteristics and virulence factor profile of *Salmonella* Enteritidis bacteriophages isolated from foods were not known much.

Purpose: This study aimed to isolate *Salmonella* Enteritidis bacteriophages from various sources and to identify the virulence factors profile of bacteriophage isolates.

Methods: Twelve bacteriophages against *Salmonella* Enteritidis from sewage were purchased from the bacteriophage bank in Korea. Forty-two bacteriophages were isolated from 60 fermented foods including kimchi, soy paste, and yogurt. Genomic DNA of host bacteria and bacteriophage isolates was prepared by genomic DNA kit and commercial genomic DNA extraction kit and phage DNA extraction kit, respectively. The presence of ten virulence factors (*avrA, ssaQ, mgtC, siiD, sopB, gipA, sodC1, sopE1, spvC, bcfC*) was examined by PCR.

Results: Although six bacteriophages were negative for 10 virulence factors, 48 out of 54 carried 1 or multiple virulence factors. Among 54 isolates, *avrA+ssaQ+mgtC+siiD+sopB+sodC1+sopE1* was the most prevalent virulence profile of *Salmonella* Enteritidis bacteriophage. *sopB* was present in 65% of 20 bacteriophage isolates from kimchi and 56.25% of 16 bacteriophages from soy paste. However, 12 bacteriophages from sewage did not carry *sopB*. *bcfC* was detected in bacteriophages only from soy paste and its detection rate was 62.5%. Interestingly, *sopE1*was detected in all bacteriophages and host bacteria used in this study.

Significance: *Salmonella* Enteritidis bacteriophage isolates from various sources showed typical virulence factor profile. Their role and safety should be evaluated in further research.

P2-181 Evaluation of 16S rRNA Sequencing for the Detection of *Salmonella* in Cilantro

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Introduction: *Salmonella enterica* is a known cause of foodborne gastroenteritis in the United States and has been associated with outbreaks in fresh produce such as cilantro. *Salmonella* detection methods are complex, time consuming, and efforts to decrease the time to detection are needed. Our previous work indicates that an initial 24-hour non-selective pre-enrichment in Modified Buffered Peptone Water (mBPW) favored the growth of Firmicutes, particularly the Peptostreptococcaceae, as opposed to Enterobacteriaceae.

Purpose: This study evaluates alterations to the FDA Bacteriological Analytical Manual (BAM) method for *Salmonella* detection to significantly increase the proportional abundance of Enterobacteriaceae; i.e., *Salmonella*, to that of non-target species, notably Peptostreptococcaceae, in cilantro using16S rRNA sequencing of enrichment cultures.

Methods: Genomic DNA was extracted from broth cultures of cilantro samples processed using the BAM method for the detection of *Salmonella* in leafy greens. Variable treatments were tested including; the use of two resuscitation broths, mBPW and universal pre-enrichment broth (UP), and shaking (165 rpm) or static incubation at 37°C during the initial 24-hour non-selective resuscitation. 16S rRNA amplicons were generated using primers specific to the V1 - V3 regions and sequenced on the Illumina MiSeq using a 600 cycle reagent kit.

Results: Both pre-enrichment broths performed equally for the enrichment of Enterobacteriaceae (P = 0.882, n = 15). However, shaking the 24-hour mBPW and UP pre-enrichments significantly increased the Enterobacteriaceae by 34.9% (P = 6.61E-06, n = 30) and decreased the Peptostreptococcaceae by 70% (P = 5.62E-04, n = 30). In spiked samples, the effect of shaking was significant for the enrichment of Enterobacteriaceae in general (P = 1.91E-04, n = 13) but significant changes in the abundance of *S. enterica* were not observed (P = 0.368, n = 13).

Significance: We have shown that aeration via shaking during the 24-hour pre-enrichment of cilantro samples significantly favors the enrichment of Enterobacteriaceae and depresses the growth of Peptostreptococcaceae using 16s rRNA sequencing.

P2-182 Can Corn Oil Serve as an Additive to Help Increase the Recovery of *Salmonella enterica* in Oregano?

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Introduction: In recent years, spices have increasingly been associated with outbreaks of *Salmonella*, underscoring the need for enhanced surveillance and an improved outbreak response. Spices, like vegetables, fruits, and medicinal herbs, are known to possess phenolic compounds that are associated with antimicrobial effects and properties. Subsequently, the presence of these antimicrobial compounds may impede the detection of *Salmonella* that persists in dried products.

Purpose: The goal of this study was to evaluate the effects of adding corn oil in growth media as a compound to sequester the antimicrobial compounds found in spice, while allowing *Salmonella* to grow during pre-enrichment culture.

Methods: Oregano samples were artificially contaminated with *Salmonella enterica*, pre-enriched in modified Buffered Peptone Water with and without 2% (vol/vol) corn oil and incubated overnight at 37°C. Samples were transferred to selective enrichment broth of Rappaport-Vassiliadis and

tetrathionate and plated on Xylose-Lysine-Tergitol 4 Agar and various other chromogenic agars. The positives plates were examined for *Salmonella* colonies as described in the FDA Bacteriological Analytical Manual, and suspect colonies were confirmed using the colorimetric Gram Negative card and Vitek® 2 Compact, software Version 5. In addition to the plating method, molecular approaches such as molecular serotyping PCR, and shot-gun metagenomics were used to assess the increase in recovery of *Salmonella.*

Results: From the 30 samples processed for each condition tested, an average 283 CFU/ml of *Salmonella* was recovered in samples artificially contaminated with *Salmonella* grown in mBPW with corn oil as compared to the 17 CFU/ml without the corn oil. The results demonstrated that addition of corn oil increased the recovery of *Salmonella* by ≥ 50% in oregano samples.

Significance: The addition of corn oil in the pre-enrichment broth may also enhance the recovery of *Salmonella* from other spices that contain antimicrobial compounds, a crucial step that may enhance detection using both traditional culture and molecular methods of contaminated spices.

P2-183 Role of Oxidative Stress Resistance in the Survival and Morphological Changes of *Campylobacter jejuni* under Aerobic Conditions

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Introduction: As a microaerophilic foodborne pathogen, *Campylobacter jejuni* inescapably encounters high oxygen tension during transmission to humans via foods; thus, the ability of *C. jejuni* to survive under oxygen-rich conditions significantly impacts food safety.

Purpose: The purpose of this study was to investigate the role of oxidative stress in the survival of *C. jejuni* under oxygen-rich conditions.

Methods: The effect of oxidative stress on aerotolerance was investigated in *C. jejuni* by using three mutants defective in key genes of oxidative stress defense, including *ahpC*, *katA*, and *sodB*. We measured the levels of viability and accumulation of reactive oxygen species (ROS) under aerobic conditions, and observed morphological changes with fluorescence microscopy.

Results: All the three mutants exhibited reduced viability under aerobic conditions compared to the wild type (WT), and the *ahpC* mutant showed most significant viability reductions. The levels of lipid peroxidation and protein oxidation were significantly increased in the mutants compared to WT. Under aerobic conditions, the *ahpC* and *katA* mutants developed coccoid forms by aeration, whereas the *sodB* mutant established elongated cellular morphology. Higher levels of ROS were accumulated in *C. jejuni* under aerobic conditions than microaerobic conditions, and supplementation of culture media with an antioxidant reduced the level of ROS accumulation and also increased *C. jejuni* viability under aerobic conditions. Compared to microaerobic conditions, interestingly, aerobic culture substantially induced the formation of coccoid cells, and antioxidant treatment reduced the emergence of coccoid cells, possibly viable-but-non-culturable cell (VBNC), under aerobic conditions. The ATP concentrations and PMA-qPCR analysis supported that oxidative stress is a factor that induces the development of a VBNC state in *C. jejuni*.

Significance: These findings in this study clearly demonstrated that oxidative stress resistance plays an important role in the survival and bacterial morphology of *C. jejuni* under aerobic conditions.

P2-184 Phenotype and Genotype Correlations of Antimicrobial Resistance in *Campylobacter* Using In Vitro Antimicrobial Susceptibility Testing and Whole Genome Sequencing

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Introduction: *Campylobacter* is a leading cause of foodborne illness worldwide. Antimicrobial resistance in *Campylobacter* spp. from food supply is a global public health concern. Whole-genome sequencing (WGS) potentially provides a single, comprehensive, and cost-effective approach to define the resistance mechanisms and predict antimicrobial resistance phenotypes.

Purpose: The objective of this study was to evaluate the correlation between resistance phenotype and genotype using in vitro antimicrobial susceptibility testing (AST) and WGS.

Methods: Seventy-four *Campylobacter* isolates recovered from the National Antimicrobial Resistance Monitoring System were selected in this study. Standard broth micro dilution was used to determine antimicrobial susceptibility profiles of nine antimicrobials, including ciprofloxacin (CIP), erythromycin (ERY), gentamicin (GEN), tetracycline (TET), azithromycin (AZI), clindamycin (CLI), florfenicol (FFN), nalidixic acid (NAL) and telithromycin (TEL). Resistance breakpoints of EUCAST epidemiological cut-off values were used to interpret the AST data. Genomic DNA was sequenced as paired-end reads using Illumina MiSeq. Genome sequences were assembled by using CLC genomics workbench 6.0.2. Previously reported antibiotic resistance genes were downloaded from GenBank to an in-house database. Resistance genotypes were determined using assembled WGS data through BLAST analysis.

Results: Eleven resistance genes, including *tetO, blaOXA-61, aph(3')-IIIa, aph(2")-Ic, aph(3')-Ic, aadE, Sat4, ant(6'), aad9, aph(2")-Ig, aph(2')-If* plus mutations in three house-keeping genes (GyrA at position 86, 23S rRNA at position 2074 and 2075) were identified by WGS. Overall, between resistant /susceptible phenotypes and genotype correlated well, with 100% for TET, CIP/NAL and ERY. A few discrepancies were observed for GEN, AZI, CLI and TEL and the correlation between phenotype and genotype for these drugs ranges from 95.9% to 98.6%. All isolates were susceptible to FFN and no genes associated with FFN resistance were detected.

Significance: WGS can provide comprehensive resistance genotypes, and is capable of accurately predicting resistance phenotypes, suggesting that WGS has the potential to be used as a routine method for antimicrobial resistance surveillance programs.

P2-185 The Role of Cell Membrane in the Survival of *Campylobacter jejuni* at a Low Temperature Used in Storage and Processing Environments

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Introduction: *Campylobacter jejuni* is a leading cause of foodborne gastroenteritis in humans. As it passes from host to human, *C. jejuni must survive* during transit on food products in stressful storage conditions which include different temperatures.

Purpose: To better understand cold stress response in *C. jejuni*, we tested the survival rate in different media, transcription levels of outer membrane proteins, fatty acid profiles, and morphological change at a refrigerated temperature.

Methods: Direct plate counting, quantitative real time-PCR (qRT-PCR), fatty acid methyl ester (FAME) analysis, and transmission electron microscopy (TEM) and field emission scanning electron microscopy (FE-SEM) were performed to examine the survival of *C. jejuni* at low temperature.

Results: Survival of *C. jejuni* human (11168) and poultry (A74O22) isolates were tested in chicken juice (CJ), brain heart infusion (BHI), *Mueller* Hinton (MH), and phosphate buffered saline (PBS) at 4°C. *C. jejuni* A74O22 remained culturable for a longer period than did *C. jejuni* 11168 and the colony was culturable onto MH agar in both CJ and PBS up to 63 days. The relative gene expression levels of membrane proteins (*mapA, omp50,*

pglF, peb4, and *htrB*) as revealed by qRT-PCR was decreased in both *C. jejuni* strains after 30 days. In addition, all tested genes were most significantly downregulated in *C. jejuni* A74O22 that was maintained in CJ compared to other media. Regardless of the types of suspended media, membrane fatty acid composition was found to be decreased slightly following exposure to cold stress. However, cis-9-octadecenoic acid (18:1) and cis-6,9,12,15-eicosatetraenoic acid were observed only in *C. jejuni* suspended in CJ. TEM and FE-SEM revealed that prolonged storage resulted in transformation of spiral cells to coccoid, condensed cytoplasm, and release of cellular contents.

Significance: This study provides useful information for understanding the role of cell membrane in *C. jejuni* survival mechanisms when exposed to low temperature.

P2-186 Inactivation of *Pseudomonas stutzeri* in Beef Using High Pressure Processing, Gamma Radiation, and Ultraviolet Light

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Introduction: Recent advances in microbiome research indicate that abnormal gut microflora may play a role in human disease processes. *Pseudomonas* species are typically regarded as nuisance spoilage microorganisms in foods, including beef. However, recent research on food and human gut microbiomes has indicated that *P. stutzeri*, may play a role in creation of abnormal gut microflora and inflammatory bowel disease in people with Autism Spectrum Disorder.

Purpose: The purpose of this study was to determine the inactivation kinetics of *P. stutzeri* in beef using High Pressure Processing (HPP), Gamma Radiation (GR) and Ultraviolet Light (UV-C).

Methods: A six-isolate cocktail of *P. stutzeri* was inoculated (10^9 CFU/g) into irradiated beef which was then treated with the non-thermal process interventions, and the *P. stutzeri* recovered using APC Petrifilms.

Results: When the *P. stutzeri* was inoculated into 80% lean ground beef and subjected to HPP (450 mPa, 0 - 15 min, 5°C) the D-10 was 2.09 min. When the *P. stutzeri* cocktail was suspended in the ground beef and irradiated (0-1.0 kGy, 5°C) the D-10 was 0.20 kGy. Approximately 1.6 log of *P. stutzeri* was inactivated on beef surfaces subjected to UV-C (5 mW/cm^2, 0-2.0 J/cm^2, 5°C). When the ability of UV-C to inactivate the *P. stutzeri* suspended in beef purge inoculated onto food grade high density polyethylene and stainless steel surfaces was examined, ca. 3.5 log (0.5 J/cm^2) and 6.5 log (1.0 J/cm^2) reductions were observed.

Significance: These results indicate that HPP, GR, and UV-C, which do not require the use of exogenous water or chemicals to inactivate microorganisms, can be used to control *P. stutzeri* in beef and beef purge. While still speculative, control of opportunistic pathogens such as *P. stutzeri* may yield benefits for people with certain genetic or metabolic conditions.

P2-187 Inactivation of Shiga Toxin-Producing *Escherichia coli* in Veal Using High Pressure Processing, Gamma Radiation, and Ultraviolet Light

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Introduction: Shiga Toxin-producing *Escherichia coli* (STEC) are common contaminants in meat and are responsible for numerous foodborne illness outbreaks and product recalls. Recently, veal has received attention from regulatory agencies due to possible contamination with STEC.

Purpose: The purpose of this study was to determine the inactivation kinetics of STEC in veal using non-thermal process interventions including high pressure processing (HPP), gamma radiation (GR), and ultraviolet light (UV-C).

Methods: An eight-isolate cocktail (Big Six, O104, O157:H7) STEC was inoculated (10^8 CFU/g) into veal, treated with the non-thermal process interventions, and the STEC recovered using *E. coli* Petrifilms.

Results: When the STEC cocktail was suspended in 95% lean ground veal and irradiated (0 - 1.8 kGy, 5°C) the D-10 was 0.39 kGy. When the STEC cocktail was inoculated in the ground veal and subjected to HPP (450 mPa, 0-30 min, 5°C) the D-10 was 5.69 min. The ability of the STEC cocktail to grow in ground veal following treatment with GR (1.8 kGy) and HPP (450 MPa, 30 min) during storage (10°C, 14 d) was examined. The STEC were able to grow ca. 2 log in the untreated veal, but were unable to grow in the HPP and GR treated veal. When the STEC cocktail was surface-inoculated onto veal cutlets and subjected to UV-C (5 mW/cm^2, 0 - 2.0 J/cm^2, 5°C), ca 1.5 log was inactivated. When the ability of UV-C to inactivate the STEC suspended in veal purge inoculated onto food grade high density polyethylene and stainless steel surfaces was examined, ca. 4 log (0.5 J/cm^2) and 6 log (1.0 J/cm^2) reductions were observed.

Significance: This indicates these non-thermal process interventions, which do not require the use of exogenous water or chemicals to inactivate microorganisms, can inactivate STEC in veal products, therefore reducing the risk of foodborne illness to consumers.

P2-188 Variability of Biofilm Formation, Quorum Sensing and Motility of *Vibrio parahaemolyticus* on Food Contact Surfaces

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Introduction: *Vibrio parahaemolyticus* is recognized as a marine seafood-borne pathogen that occurs globally and causes gastrointestinal and immunological disorders in humans. Like other *Vibrio*, it can form biofilms through bacterial attachment to surfaces, which is recognized as a vital characteristic of the microorganism, and it can attach on various sources of organic and inorganic matter and form biofilms on them.

Purpose: We investigated the correlation of variability of biofilm, quorum sensing and motility of 22 strains of *V. parahaemolyticus* on the stainless steel (SS) surface.

Methods: Biofilm formation index (BFI) assay, motility assay, autoinducer 2 (AI-2) assay, exoprotease assay and field emission scanning electron microscopy (FESEM) analysis were used as the indexes to determine any correlation between biofilm formation and motility/quorum sensing of *V. parahaemolyticus* on stainless steel surface. The student *t*-test was used to analyze for Pearson correlation between two indexes.

Results: The strains VP 4 (oyster isolates), VP 9 (warty squid isolates) and VP 13 (mussel isolates) were highest biofilm former among 22 strains of *V. parahaemolyticus* based on the BFI and FESEM. The swimming motility showed positive correlation (39.5%) with the formation of biofilm than swarming motility (-0.12%) in varieties of *V. parahaemolyticus* strains. The biofilm forming ability of *V. parahaemolyticus* strains was dependent on quorum sensing. We observed 72.8% positive correlation between SS biofilm and AI-2 and 60.6% positive correlation between SS biofilm and exoprotease production.

Significance: This study found that the biofilm forming ability of *V. parahaemolyticus* strains was dependent on quorum sensing and motility. These findings afford useful information in understanding of strain variability, besides in strain selection for exploitation in the context of food safety

challenge and to determine the ability to form biofilms. These insights in biofilm formation features will permit us to further optimize approaches to control the biofilm formation of *V. Parahaemolyticus.*

P2-189 Use of a Proteomic and Genotypic Approach to Understand Proteins Associated with Outer Membrane Vesicles Secreted by *Cronobacter* Species

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Introduction: Secretion of outer membrane (OM) vesicles (OMVs) by Gram-negative bacteria occurs under *in vitro* and *in vivo* growth conditions. OMVs are thought to contribute to pathogenesis by delivering LPS components, OM proteins (OMPs), and effector molecules such as toxins, enzymes, adhesins, and nucleic acids to target host cells. Known examples include the ompA protein and the heat-labile enterotoxin expressed by *Acinetobacter baumannii* and *Escherichia coli*, respectively.

Purpose: However, the secretion of OMVs by *Cronobacter* and their contribution to pathogenesis is unknown. In this study, OMVs were obtained from *C. sakazakii*, *C. malonaticus*, and *C. turicensis* and their proteome-associated contents were analyzed by SDS-PAGE, and protein sequencing.

Methods: OMVs were partially purified from cells grown on Trypticase soy agar with 1% NaCl at 37°C for 18 h by removing the cells by centrifugation (8,000 vg, 15 min.). OMVs in the supernatant were concentrated by ultracentrifugation, and the resulting pellets were analyzed by SDS-PAGE. Separated proteins were transferred to membranes, and stained with Coomassie blue. Protein bands were excised and sequenced. PCR primers targeting OMV genes were designed and used for determining their presence in a collection of 240 strains.

Results: Electron microscopy of negatively-stained cells showed that the OMVs are secreted as pleomorphic microvesicles (< 300 nm in size). Sequence analysis indicated that OMVs contained proteins with strong homologies to OmpA, OmpC, OmpX, MipA, conjugative plasmid transfer protein (CTP), GroEL, and an OM autotransporter/adhesin (OMATP) protein. PCR analysis revealed that these genes are common among *Cronobacter* species. However, in comparison to strains representing other phylogenetically-related species, gene targets such as the CTP gene and OMATP were found to be *Cronobacter*-specific.

Significance: The presence of OMPs and their genes suggests that OMVs in *Cronobacter* are involved in multiple functions including adherence and invasion, stress response, plasmid maintenance, and extracellular transport, alluding to their possible roles in pathogenesis.

P2-190 Evaluation of Genomic Diversity and Gene Content Differences among *Cronobacter* Isolated from Dairy Manufacturing Environments Using a Pan Genome Microarray

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Introduction: *Cronobacter sakazakii*, *C. malonaticus*, and *C. turicensis* have been implicated in neonatal infections and cause septicemia, meningitis, and necrotizing enterocolitis. It has been isolated from a wide range of sources such as, powdered infant formula, powdered milk, lactose, and starches and associated manufacturing environments.

Purpose: Despite a wealth of published genomes of these species, genomics-based epidemiology of the genus is not well established. A diverse group of 195 unknown *Cronobacter* and other taxonomically-related isolates obtained through an environmental surveillance sampling program of 55 milk powder and cheese manufacturing facilities were analyzed using a novel pan-genome microarray to annotate the gene content, highlight the genomic diversity and phylogenetic relationship among the strains.

Methods: A pan-genome DNA microarray with ~22,000 features from 15 published *Cronobacter* genomes was developed. Genomic DNA was isolated using a QIAcube workstation following the manufacturer's recommendations and hybridizations were performed according to the Affymetrix GeneChip Expression Analysis Technical Manual.

Results: The microarray was clearly able to distinguish the seven *Cronobacter* species from one another, and from related non-*Cronobacter* species. The interrogation of 115 milk powder *Cronobacter* isolates with the microarray identified three *C. turicensis* (Ct), 15 *C. dublinensis* (Cd), and 97 *C. sakazakii* (Cs) isolates. Strain-level differences showed that the Cd isolates grouped within two of three Cd clades, the Ct isolates within a single Ct clade, and the Cs isolates within seven of eight Cs clades.

Significance: The microarray showed that the Cd and Ct isolates were more clonal than the Cs isolates. The microarray also showed that all Cd and Ct isolates possessed the common virulence plasmid compared to 94 (97%) of Cs isolates. The current study establishes a powerful platform for further genomics research of this diverse genus, a prerequisite towards development of future countermeasures against this important foodborne pathogen.

P2-191 Ingested *Salmonella enterica*, *Cronobacter sakazakii*, *Listeria monocytogenes*, and *E. coli* O157:H7 by Houseflies (*Musca domestica*) are Transmitted to their Eggs and First Filial (F1) Generation Adults

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Introduction: Flies contaminate food and food-contact surfaces with their body surfaces or through regurgitation and/or defecation. The potential spread of foodborne pathogens by flies increases if they feed on contaminated food. However, it is uncertain if ingested pathogens are passed to fly eggs and to subsequent life stages or generations without re-acquiring bacteria.

Purpose: To estimate the probability of transmission of *S. enterica*, *C. sakazakii*, *L. monocytogenes*, and *E. coli* O157:H7 to housefly eggs and F1 generation adults after parental flies consumed a contaminated meal.

Methods: Adult flies were given liquid food containing low, medium, and high concentrations of each bacterium. Using a PCR detection method followed by isolation of the pathogen on specific media, the presence of pathogens was assessed on surface-disinfected eggs laid by parental flies and on body surfaces and alimentary canals of newly emerged F1 adults.

Results: The probability of transmission of pathogens to housefly eggs was higher when parental flies ingested food containing medium bacterial loads. *Cronobacter sakazakii* was 16, 6, and 3 times more likely to be transmitted to housefly eggs than *S. enterica*, *E. coli* O157:H7, and *L. monocytogenes*, respectively. Only *S. enterica* and *C. sakazakii* were transmitted to F1 adults and their presence was 2.4 times more likely on their body surfaces than in their alimentary canals. The highest probabilities of finding *S. enterica* (60%) and *C. sakazakii* (28%) were observed after parental houseflies ingested food containing medium and high concentrations of each pathogen, respectively.

Significance: Foodborne bacteria ingested by adult houseflies are transmitted to the housefly's progeny, giving another insight into the transmission capabilities of flies. To better protect public health, it is important to highlight the need for effective implementation of preventative pest management plans in food facilities to minimize the hazard posed by the presence of flies.

P2-192 Survival of *Listeria monocytogenes* ATCC 7644 in Fermented Milk and Mahewu

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Introduction: *Listeria monocytogenes* has been isolated from a wide range of food products including fermented products. While their growth and survival have been determined in popular fermented products, there is no reported work on locally fermented foods such as *mahewu* and locally fermented milk.

Purpose: The aim of this work is to evaluate the survival of *Listeria monocytogenes* in fermented milk and *mahewu*, and to also determine the physicochemical properties of these products under different storage conditions.

Methods: Milk and maize were fermented with a mixed starter culture. Fermented milk and maize were each inoculated with 10^8 CFU/ml of *Listeria monocytogenes* ATCC 7644 and stored at 37°C, room temperature (22°C) and 4°C for five days. After which *Listeria monocytogenes* counts as well as the physicochemical properties were determined after every 24 h.

Results: *L. monocytogenes* counts taken showed that fermented milk stored at 4°C after 120 h of storage had a 2.60-log reduction, while at 37°C there was a 0.70-log reduction and at room temperature (22°C) there was a 0.90-log reduction. *Mahewu* stored at 4°C stored for 120 h had a 2.99-log reduction, 37°C had 1.00-log reduction and at room temperature (22°C) there was a 1.50-log reduction. Statistical analysis showed that temperature had a significant effect on the survival of *L. monocytogenes*. However there was no significant effects of storage time or temperature on the physiochemical products of *mahewu* and fermented milk.

Significance: This research indicated that *L. monocytogenes* can survive in the two fermented foods however storage at 4°C reduced the numbers of the pathogens compared to storage at 37°C or room temperature (22°C). The importance of good manufacturing practices and good hygiene to reduce bacterial contamination can therefore not be overemphasized.

P2-193 Examination of Environmental Isolates of *Listeria monocytogenes* Indicates That Their *inlA* Genotypes are Intact and the Strains Potentially Virulent

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Introduction: *InlA* is an essential virulence gene involved in the uptake of the foodborne pathogen *Listeria monocytogenes* into host cells. It is needed for virulence and is intact in clinical strains and often truncated in isolates from processed foods and processing facilities. The genotypes found among environmental isolates are largely unknown.

Purpose: We sequenced the *inlA* alleles from 103 *L. monocytogenes* strains isolated over a 3-year period from naturally contaminated watersheds near a leafy green growing area in Central California to assess the ecology of this essential virulence gene in the environment.

Methods: The *inlA* genes from each strain were amplified by PCR and sequenced using standard techniques. The sequences and other strain information including (serotype, date of isolation, and the watershed) were analyzed.

Results: Twenty-six different *inlA* alleles were found, some of which were unique. Twenty-three of the alleles are predicted to encode intact copies of InlA, while two are predicted to encode inactive versions due to mutations resulting in Premature Stop Codons. Another allele encodes has a 9 nucleotide (3 amino acid) deletion, an allele that was previously described for a clinical strain, indicating that it is still functional. Of the 103 strains analyzed, 93 encode intact copies of *inlA*, 8 encode the allele with the 9 nucleotide deletion, and 2 encode shortened, truncated copies of *inlA*. The intact and 9-nucleotide shortened alleles were persistent in the watersheds. Further analysis of the strains is ongoing.

Significance: The finding that 90% of environmental *L. monocytogenes* isolates contain intact *inlA* alleles varies significantly from isolates found in processing plants. It indicates the potential virulence of the majority of *L. monocytogenes* strains near active produce fields. This information is important to public health labs and growers as to the varieties of *L. monocytogenes* that could potentially contaminate fresh produce in the field by various means.

P2-194 Persistent and Transient *Listeria* Strains Show Different Abilities to Form Biomass and Strains Isolated from Harborage Sites Vary in Accumulation of Genetic Changes

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❖ ***Developing Scientist Competitor***

Introduction: *Listeria monocytogenes* is a human foodborne pathogen that may cause a severe invasive disease known as Listeriosis in immune-compromised individuals. Previous studies have described the ability of *Listeria* strains to persist for years in the environment of food processing plants, while undergoing minimal genetic change. The ability of *Listeria* to form biofilms may contribute to environmental persistence.

Purpose: This study was conducted to (i) measure the ability of persistent and transient *Listeria* isolates to form biomass and (ii) determine the amount of genetic change persistent isolates from the same harborage site accumulated over time.

Methods: Thirty *Listeria* isolates were selected based on classification as persistent or transient from previous longitudinal studies that used combined testing and molecular subtyping. Isolates were characterized by ability to form biomass at 12°C and 30°C using a crystal violet assay; each isolate was assayed in triplicate in two biological replicates. Persistent isolates from the same harborage site were also characterized by whole genome sequencing and comparative genomic analyses.

Results: Significantly more biomass production was observed ($P < 0.05$) at 30°C versus 12°C. Transient *Listeria* isolates produced more biomass at 30°C compared to persistent isolates ($P < 0.05$), while no difference in biomass was observed between persistent and transient isolates at 12°C. Among the different *Listeria* species, *L. monocytogenes* showed the most ($P < 0.05$) biomass production across both temperatures. Paired *Listeria* isolates from the same harborage site in three facilities accumulated a range of genetic change over time as evidenced by 16, 18, and 252 single nucleotide polymorphisms.

Significance: Our results show that *Listeria* forms more biomass at ambient temperature as compared to food processing temperature and transient *Listeria* isolates form more biomass at ambient temperature as compared to persistent isolates. Paired *Listeria* isolates from the same harborage site within a facility accumulated a range of genetic change over time.

Poster

P2-195 Phenotypic Characterization of *Listeria* Prophages Present in Lysogenic *Listeria* Isolates from Foods, Environments, Animals and Humans

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Introduction: *Listeria monocytogenes* is a foodborne pathogen that can cause a serious fatal infection. *Listeria* prophages have been shown the association with improving the survival and fitness of *Listeria* hosts. Characterization of *Listeria* prophages from different sources will provide useful information for reducing *Listeria* contamination.

Purpose: In this study, we investigated the effect of UV and Mitomycin C inductions on lysogenic *Listeria* isolates from foods, environments, animals and humans. Host ranges of the prophages were performed for the phenotypic characterization.

Methods: Total of 25 isolates of *Listeria* spp. and 34 isolates of *L. monocytogenes* were tested for the presence of *Listeria* prophages by UV and Mitomycin C inductions using Mack as a host strain. Each purified phage (10^8-10^9 PFU/ml) was used for the host range characterization by spotting (5 µl) onto 19 hosts including 15 *L. monocytogenes* and 4 *Listeria* spp. (in 3 replicates). Host range data were analyzed by clustering analysis.

Results: Of 59 *Listeria* isolates tested, the presence of prophages was observed in four isolates (6.78%) by UV induction, eight isolates (13.56%) by Mitomycin C induction, and four isolates by both methods. Host range determination of 19 prophages (8 from UV and 11 from Mitomycin C induction) showed five clusters based on the lysis patterns. While 13 (68.42%) prophages could lyse 18/19 of host strains, including five common species, 5 (26.32%) prophages could not lyse two *L. monocytogenes* isolates. The only one prophage induced from *L. seeligeri* could lyse all hosts, suggesting the widest host range prophage in this collection.

Significance: Our data suggests that Mitomycin C shows better effect on *Listeria* prophage induction. The prophage collection will be used for further research to better understand the diversity of *Listeria* prophages and the linkage between persistence of *Listeria* and *Listeria* prophages.

Poster

P2-196 Functional Characterization of the *Listeria* Genomic Island 1

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Introduction: A predominant clone of *Listeria monocytogenes* (*Lm*) has been responsible for the majority of Listeriosis cases in Canada since 1988. Within this clonal complex, the majority of strains possessed an uncharacterized genomic island (LGI1). Encoded on the island are a combination of putative stress response, and virulence genes, possibly enhancing the ability of *Lm* to survive in the food chain and cause Listeriosis.

Purpose: To elucidate the contribution of LGI1 to *Lm* survival in the food chain and virulence.

Methods: LGI1 function was studied using gene deletions, and real-time reverse-transcription polymerase chain reaction (16 coding regions). LGI1 genes with putative efflux (*emrE*), regulatory (*lmo1851*), and adhesion (*sel1*) functions were deleted in a clinical *Lm* 08-5578 strain using allelic exchange and pKSV7 vector. Isolates were exposed to acid (HCl, pH 2.5 - 4.5), cold (4°C), salt (10 - 20% NaCl), and quaternary ammonium compounds (QACs) in either brain-heart infusion or tryptic soy broth, and their survival and growth were measured. Adhesion and invasion of Caco-2 and HeLa cells of Δ*sel1* was compared to the parent strain, and other controls (EGD-SmR BUG5; 10403S). Differences in growth, and adhesion/invasion efficiencies were assessed using ANOVA with Dunnett's test, at $P < 0.05$.

Results: *Lm* 08-5578 was highly tolerant to the tested stresses, with 14/16 LGI1 coding sequences induced in the presence of QAC benzalkonium chloride (5 µg/ml). The *lmo1861* gene was constitutively expressed under all tested conditions (4°C, 37°C, 52°C, QACs, 0 - 30 UV). Deletion of *lmo1851* and *sel1* had no effect on the stress response and adherence/invasion, respectively, whereas deletion of the *emrE* gene resulted in the increased susceptibility to QACs ($P < 0.05$).

Significance: LGI1 appears tightly regulated. It was induced specifically in the presence of QACs, and increased *Lm* tolerance to these compounds. As such, this island improves survival and persistence of *Lm* in the food chain.

P2-197 *emrE* Gene Located on the *Listeria* Genomic Island 1 Encodes for an Efflux Pump That Contributes to *Listeria monocytogenes* Tolerance to Quaternary Ammonium Compounds

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Introduction: Contamination of deli meats with *Listeria monocytogenes* (*Lm*) resulted in 57 cases of Listeriosis and 24 deaths in 2008 in Canada. Sequencing of the strains implicated in the outbreak (08-5578 and 08-5923) revealed the presence of a novel putative efflux pump ($EmrE_{Lm}$) located on an uncharacterized 50 kb island, LGI1.

Purpose: To investigate the role of LGI1 encoded *emrE* in *Lm* tolerance to antimicrobials.

Methods: Non-polar $emrE_{Lm}$ deletion mutant (Δ$emrE_{Lm}$) was created in *Lm* 08-5578 using allelic exchange and pKSV7 vector. Isolates were exposed to antimicrobials [benzalkonium chloride (BAC), E-San, ciprofloxacin, chloramphenicol, erythromycin, gentamicin, tetracycline, triclosan] and acriflavine dye, and their minimum inhibitory concentrations (MICs) were assessed with agar and microbroth dilution methods. Real-time reverse transcription PCR was used to measure transcript levels of $emrE_{Lm}$, *lde*, *mdrL*, *sigB,* and putative LGI1 regulators (*lmo1851*, *lmo1861*) in *Lm* 08-5578 following 1 h exposure to BAC (10 µg/ml). Differences in the lag phase (LP; h), growth rate (GR; ΔOD_{600}/h), and maximum optical density (MOD; OD_{600}) of the parent and Δ$emrE_{Lm}$ (*t*-test), and genetically similar strains with (n = 8) and without LGI1 (n = 8; Mann-Whitney) were assessed at sub-lethal QACs concentrations (24 h, 30°C).

Results: Increased transcription of *lmo1861* (82.4-fold), *emrE* (49.6), *sigB* (4.1), and *lmo1851* (2.3) was observed in the presence of BAC. Deletion of the *emrE* gene resulted in longer LP ($P < 0.0001$), and slower GR ($P < 0.05$) at sub-lethal QACs concentrations, and 2 - 3 times lower MICs; no change in MICs to other tested antimicrobials was observed. Shorter LP ($P < 0.05$), faster GR ($P < 0.001$), and higher MOD ($P < 0.05$) were seen for strains possessing LGI1 than those without LGI1.

Significance: These data confirm the role of a novel *Lm* efflux pump, EmrE, in *Lm* tolerance to QACs. Since QAC sanitizers are commonly used in the food chain, there is a concern *Lm* strains possessing $emrE_{Lm}$ will have a survival advantage in food processing environments.

P2-198 *Listeria monocytogenes* Isolated from Ready-to-Eat Food Processing Environments in British Columbia Possess Traits Associated with Increased Risk

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❖ ***Developing Scientist Competitor***

Introduction: The foodborne pathogen *Listeria monocytogenes (Lm)* causes Listeriosis, a rare but severe disease affecting at-risk populations. Genotypic characterization of virulence-associated genes and assessment of antimicrobial resistance can provide a useful approximation of the risk associated with particular strains.

Purpose: Assess *Lm* isolated from Ready-to-Eat (RTE) food processing environments (FPE) in British Columbia (BC) for characteristics associated with either increased risk of causing disease or treatment failure.

Methods: *Lm* (n = 36) isolates from BC RTE FPE were serotyped and virulence gene *inlA* was profiled. Isolates were also screened for the 50-kbp *Listeria* genomic island 1 (LGI1) associated with epidemic clone V strains that have caused illness across Canada. Conventional PCR was used to amplify *inlA* and LGI1. *InlA* amplicons were subjected to Sanger sequencing and these data were examined for the presence of virulence attenuating mutations, while LGI1 amplicons were visualized using agarose gel electrophoresis. Antimicrobial resistance was assessed using a disc diffusion assay with a panel of 18 antimicrobials.

Results: Most isolates (97%) belonged to serotypes commonly linked to listeriosis and possessed full-length *inlA* (81%) or a 3-codon deletion at aa741-743 (17%) that is not thought to result in loss of virulence. One isolate contained a novel mutation in *inlA* resulting in a premature stop codon at aa760. LGI1 was absent in all isolates. Resistance was observed in all isolates for cefoxitin and nalidixic acid. Most isolates were resistant to clindamycin (94%) and 92% and 67% showed intermediate resistance to ciprofloxacin and vancomycin, respectively. All isolates were susceptible to the remaining antibiotics.

Significance: These results show that *Lm* strains recovered from BC FPE may be capable of causing Listeriosis if transferred to RTE foods. While these results may seem concerning, the high-risk strains identified here did not show resistance to antibiotics commonly used to treat Listeriosis.

P2-199 Effects of Ultra-high Pressure Processing (UHP) on Biofilm Formation of *Listeria monocytogenes*

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Introduction: Ultra-high pressure processing (UHP) provides a valuable alternative to thermal pasteurization during food processing, especially for Ready-to-Eat products. Among pathogenic microorganisms, *Listeria monocytogenes* is a major concern of Ready-to-eat products. Although previous studies proved that the inactivation of *L. monocytogenes* is effective after ultra-high pressure processing, a small amount of the *L. monocytogenes* may still live and the formation of its biofilm on the surface induces serious food safety issues.

Purpose: The aim of the work was to investigate the effect of ultra-high pressure processing (UHP) on the biofilm formation of *L. monocytogenes*.

Methods: *L. monocytogenes* (initial count of 10^9 CFU/ml) was subjected to ultra-high pressure treatment at 100 to 500 MPa for 15 min at room temperature (20°C), and then was incubated in BHI for 24 h, 48 h and 72 h at 37°C. Microbial survival curves and growth curves were measured and compared with the amount of the biofilm after the ultra-high pressure treatment. Crystal violet staining assay was used to measure the biofilm production of *L. monocytogenes* in different time periods.

Results: After ultra-high pressure treatment, *L. monocytogenes* counts reduced from 9 log CFU/ml to 2.4 log CFU/ml at 500 Mpa. During subsequent culturing, although *L. monocytogenes* grew fast and the count increased to 8.4 log CFU/ml in 24 h as the same count as the control group (without ultra-high pressure treatment), the biofilm formation was still low after ultra-high pressure treatment. The absorbance value ($OD_{595\ nm}$) of biofilm from the treated samples was 0.24, 0.15, and 0.12 after 24 h, 48 h and 72 h, respectively, while the control group was 0.27, 0.29, and 0.37, respectively.

Significance: This study suggests that ultra-high pressure has effects on the biofilm formation of *L. monocytogenes*, and it may weaken the biofilm formation capacity of *L. monocytogenes* and reduce the food safety problems.

P2-200 Comparison of Oxidative Stress Response and Biofilm Formation of *Listeria monocytogenes* Serotypes 4b and 1/2a

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❖ ***Developing Scientist Competitor***

Introduction: Among 13 serotypes of *Listeria monocytogenes*, serotypes 4b and 1/2a account for most of the sporadic Listeriosis cases and environmental isolations.

Purpose: To understand the mechanism of stress response in these serotypes, six strains of each serotype were selected in this study to differentiate *L. monocytogenes* under oxidative stress from physiological and genetic response.

Methods: Oxidative stress tolerance was examined by plate count during exponential phase, long-term-survival phase and biofilm state. The capability of forming biofilm in two serotypes was also compared with crystal violet assay after 3 days of incubation. Three oxidation resistance genes (*kat, sod, fri*), two stress-regulator encoding genes (*sigB, perR*), and one DNA repair gene (*recA*) were selected for transcription analysis by quantitative RT-PCR.

Results: Most serotype 4b strains exhibited a stronger resistance to 0.6% H_2O_2 than did serotype 1/2a during exponential phase and long-term-survival phase, while the later demonstrated a relatively hyper-biofilm in BHI broth at 37°C. At the genetic level, there were significant differences in the expression of three genes (*sod*, *fri* and *perR*) under oxidative stress between two serotypes, which suggests their importance of serotype variation in stress resistance. In addition, *recA* appeared to be critical for survival under stress in *L. monocytogenes* but not important for the difference among serotypes.

Significance: Our data gave a primary conclusion on the mechanism of two common serotype strains under oxidative stress. Further investigation is required to focus on *sod*, *fri*, and in particular *perR,* to elucidate the factors caused the different resistance in two common serotypes to stress.

P2-201 Proteins of the Cold Shock Domain Family (Csps) Contribute to Nisin and Benzalkonium Chloride Stress Tolerance in *Listeria monocytogenes*

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Introduction: *Listeria monocytogenes* causes rare but serious foodborne illness (Listeriosis) and high mortality in those with diminished immunity. Cold shock domain family proteins (Csps) are global gene expression regulators that promote stress adaptation responses in bacteria. Nisin and Benzalkonium chloride (BC) are widely used antimicrobials in preservation of food and disinfection of food processing environments, respectively.

Purpose: To investigate the functional contribution of Csps to the innate resistance of *L. monocytogenes* against stress associated with nisin and BC.

Methods: *L. monocytogenes* EGDe wild type and *csp* deletion mutant strains were phenotypically compared under nisin and BC stress using growth assays and gene expression analysis by quantitative reverse transcription PCR (RT-qPCR).

Results: In absence of Csp functions the sensitivity of *L. monocytogenes* EGDe to nisin and BC stress exposure was increased. Growth and survival capacity of the *L. monocytogenes* EGDe under nisin and BC stress is significantly ($P < 0.05$) diminished without Csps compared to the wild type strain. In addition, Csp loss also increases the susceptibility of *L. monocytogenes* to cell envelope-targeting antibiotics including ampicillin and polymixin B. The induction of *csp* gene transcripts in response to nisin but not BC exposure was detected. Finally a comparative gene expression analysis revealed an altered expression in genes encoding peptidoglycan-binding proteins (PBPs) in absence of Csp functions.

Significance: Our results suggest that Csp mediated gene expression regulation events contribute to the optimal execution of intrinsic nisin and BC resistance mechanisms in *L. monocytogenes*.

P2-202 Comparative Analysis of *Clostridium sporogenes* PA3679 Strains from Different Sources Using Pulsed-field Gel Electrophoresis (PFGE) and Whole Genome Sequencing (WGS)

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Poster

Introduction: *Clostridium sporogenes* PA3679 has historically been used as a surrogate for proteolytic *Clostridium botulinum* strains to validate thermal processing. It has been suggested that *C. sporogenes* PA3679 is a proteolytic *C. botulinum* strain that has lost the neurotoxin gene cluster, but the true taxonomic classification of PA3679 remains unclear.

Purpose: The objective was to obtain cultures of *C. sporogenes* PA3679 from various sources and perform comparative genomic analysis to assess the genetic variability among the strains.

Methods: Pulsed-field gel electrophoresis (PFGE) was performed on *C. sporogenes* PA3679 strains collected from five different sources. Whole genome sequencing was conducted using an Illumina MiSeq. The phylogenetic relatedness and genetic variability was assessed based on the 16S rDNA sequencing and whole genome single nucleotide polymorphism (SNP) analysis.

Results: All *C. sporogenes* PA3679 strains from five sources were categorized into 2 groups (group I: ATCC7955 NCA3679 1961, 1961-2, 1990, and 2007; group II: NFL, UW, FDA, Campbell, and ATCC7955 NCA3679 1961-4). The 16S ML tree illustrated that both groups of isolates clustered with proteolytic *C. botulinum*, but group I strains formed a distinct cluster with other non-PA3679 *C. sporogenes* strains. SNP analysis revealed that group I strains were more similar to the reference PA3679 genome (GenBank accession: AGAH00000000) than group II isolates.

Significance: Based on the genetic characterization of the *C. sporogenes* strains in this study we conclude that the reference *C. sporogenes* PA3679 genome and the group I *C. sporogenes* strains were genetically distinct from strains obtained from four other sources (UW, NFL, FDA and Campbell Soup Company). Considering the widespread use of *C. sporogenes* PA3679 and its genetic information in numerous studies aimed at understanding the taxonomy of *C. botulinum* and related species and validating thermal processing technologies, the accurate identification and thorough genetic characterization of *C. sporogenes* PA3679 is warranted.

P2-203 Foodborne Botulism: Two Italian Cases Associated with Home-canned Vegetables in Oil from the South of Italy

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Introduction: Foodborne botulism is a severe neuroparalytic disease related to the consumption of foods contaminated with preformed neurotoxin. In Italy the disease is mainly associated with the consumption of home-preserved foods.

Purpose: Two cases of foodborne botulism occurred in 2013 and 2014 associated with the consumption of home-preserved turnip tops in oil, typical canned food prepared in the south of Italy, were described.

Methods: Both cases involved young students who arrived at the hospital with typical symptoms of foodborne botulism and referred the consumption of home-canned vegetables in oil. In the first case, analyses from serum, stool, and leftover food were forwarded to the National Reference Centre for Botulism and other cans to the Istituto Zooprofilattico Sperimentale of Turin (IZSTO). In the second case, serum, stool swab, gut wash and leftover food was analyzed by IZSTO. Multiplex Real-time PCR assay for toxins gene, mouse bioassay for toxins and classical microbiological culture were performed.

Results: In the first case, type B toxin was detected in serum by mouse test; stool sample was negative for toxins and *Clostridium* strains and leftover food was positive for type B toxin gene. *C. botulinum* type B strain was isolated from food and type B toxin and its gene was detected. Moreover, the mouse bioassay confirmed these data. In the second case, serum was positive for type B toxin; gut wash and rectal swab were positive for type B toxin gene and *Clostridium* isolates; the leftover food was positive for type B toxin, its gene and *Clostridium* strains. Moreover, the mouse bioassay confirmed these data.

Significance: Common features of both cases were that patients from the south of Italy moved to the north for studying and received home-preserved food from their mothers. For that reason, risk communication measures are important to reduce or avoid number of cases.

P2-204 Pressure-assisted Thermal D-values of Nonproteolytic *Clostridium botulinum* Types B and F

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Introduction: The impact of High Pressure Processing (HPP) on the inactivation of non-proteolytic spores of *Clostridium botulinum* is important in extended shelf life chilled low-acid foods. Three most resistant strains (Ham-B, Kap 9-B, and 610-F) were selected after screening 17 non-proteolytic of *C. botulinum* strains (8 type B, 7 type E, and 2 type F) for inactivation kinetics.

Purpose: Study the inactivation kinetics of resistant non-proteolytic *C. botulinum* type B and F strains (Ham-B, Kap 9-B, and 610-F) spores suspended ACES buffer (0.05 M, pH 7.0) using high pressure processing.

Methods: Spores of non-proteolytic *C. botulinum* strains, Ham-B, Kap 9-B and 610-F were prepared using biphasic media and diluted in ACES buffer (0.05 M, pH 7) to 10^5-10^6 CFU/ml and placed into a modified sterile transfer pipette, heat-sealed and subjected to a combination of temperatures (80-91°C) and high pressures (600-750 MPa) in a laboratory- scale high pressure test system. Survivors in the processed samples were determined by 5-tube MPN method using TPGY broth after incubation for 3 months.

Results: Pressure-assisted thermal D-values (min) of Ham-B, Kap 9-B, and 610-F decreased as the process temperature increased from 80 to 91°C with any pressure combination. Highest log reductions (> 5.0) of spores of Ham-B, Kap 9-B, and 610-F occurred at the highest temperature and pressure combination (91°C and 750 MPa) tested. Pressure-assisted thermal D-values at 91°C and 600 MPa for Ham-B, Kap 9-B, and 610-F were 2.39, 2.96, and 2.45 min, respectively. D-values of Ham-B, Kap 9-B, and 610-F decreased to < 1.0 min as the pressure increased from 600 to 750 MPa at this temperature.

Significance: The results indicate that high pressure processing in combination with high temperature can be used to inactivate *C. botulinum* spores in less time than thermal processing alone.

P2-205 Differential Gene Expression Profiles in *Bacillus anthracis* Sterne-infected Human Primary Cells of Pulmonary, Gastrointestinal, and Cutaneous Origin

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Introduction: *Bacillus anthracis* is a Gram-positive spore forming bacteria that is classified as a bioterror agent. It causes three types of anthrax infections in humans and many species of animals with mortality rates of > 90%, > 25%, and < 1% in pulmonary, gastrointestinal, and cutaneous anthrax, respectively. Infection could be acquired via contact with farm animals, contaminated food supply, or bioterrorist attack.

Purpose: The purpose of this study was to identify biomarkers that could be utilized in diagnostic and medical counter-measures from the transcriptomic gene expression profiles of human primary cutaneous (NHEK), gastrointestinal (InMyoFibs), and pulmonary (SAEC) cell lines at 1, 3, 5 and 24 h after infection with the spores of an avirulent strain of *B. anthracis.*

Methods: Three independent RNA samples from spore-challenged and control groups and Agilent human 4x44k whole genome arrays were used for microarray analysis. The raw data were normalized using 75% scaling and Student *t*-test to compute the significant gene list ($P < 0.05$ and fold change > 1.5). Common and cell-specific genes and biomarkers were identified by using Ingenuity Pathway Analysis Software.

Results: An up-regulation of 1729, 4981, 1539 genes, a down-regulation of 2178, 5558, 698 genes along with 36, 489, and 52 cell-specific genes was observed in NHEK, InMyoFibs, and SAEC cells, respectively. Nine biomarker genes and 68 miRNAs were commonly and significantly affected among three cell lines. Some of the key biomarkers included ITGB1, SGK1, DAB2, and ARAP3; immunological mediators such as CCL3/5/7, IL1A, IL6/8, LIF, TLR2 and transcriptional regulators including c-FOS, c-JUN, and NFκB.

Significance: Identification of target genes involved in the germination of spores and progression of anthrax, and establishing methods to silence them would help in developing novel medical counter-measure targets and reducing infection rates due to handling/consuming infected foods or bioterrorism.

P2-206 The Microbial Diversity and Characterization of *Bacillus* Species for the Enhanced Fermentation of Bambara Groundnut in the Production of African Food Condiments

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❖ ***Developing Scientist Competitor***

Introduction: African food condiments are products from alkaline fermentation (pH 6 – 9) of several legumes native to Africa and they serve as a low-cost meat substitute. Typically, the fermentation is spontaneous without a starter culture and no standard process control; thus, food safety and product quality consistency is a concern. Production from bambara groundnut is limited, despite the legume's high potential as substrate because it is high in carbohydrate and protein contents. Current identification methods of the microbial diversity of alkaline fermentation of African condiments have been less accurate phenotypic and biochemical tests with *Bacillus* spp. identified as the predominant genera.

Purpose: This study endeavors to identify the microbial diversity of African food condiments using phenotypic and genotypic methods. Furthermore, determine the genetic diversity of *Bacillus* spp. involved in alkaline fermentation of bambara groundnuts.

Methods: Microbial enumeration of viable aerobic count, Enterobacteriaceae, fungi and aerobic endospore-forming bacteria (AEFB). Direct Bacterial Profiling by MALDI-TOF Mass Spectrometry of the *Bacillus* isolates. Further $(GTG)_5$-Rep-PCR fingerprinting and DNA sequencing of the 16S rRNA and *gyr*A genes.

Results: The microbial diversity comprised mostly of aerobic endospore-forming bacteria (3.0×10^9 CFU/g), Enterobacteriaceae (2.0×10^3 CFU/g) and molds (4.0×10^2 CFU/g). *B. subtilis* groups dominated the alkaline fermentation; however, *B. cereus* was also identified. This was shown in the microbial clustering of AEFB based on MALDI-TOF MS and rep-PCR (GTG_5) fingerprinting. The phylogenetic inferences obtained from sequences of 16S rRNA and *gyr*A gene showed a strong correlation with *B. subtilis* group. Typed strains *B. subtilis* subsp. *subtilis* SFBA3, *B. amyloliquefaciens* SFBA2, *B. pumilus* P25 and *B. licheniformis* O16 were suggested as starter cultures for fermentation with bambara groundnut.

Significance: Starter cultures from *Bacillus* strains identified by genotypic methods will enhance food safety and commercial production of alkaline fermented African condiments in developing countries.

P2-207 Crtical Parameters for the Safe Production of Tempeh

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Introduction: Tempeh is a traditional Indonesian food made from mold fermentation of a number of cereal grains. The most common mold for tempeh production is *Rhizopus oligosporus,* however other *Rhizopus* spp. can be used. Due to close proximity to soil, cereal grains commonly have low

levels of *Bacillus* spp. *Bacillus cereus* is a Gram positive, endospore-forming pathogenic bacterium that is ubiquitous in soil. *B. cereus* is the source of "fried rice syndrome," a form of food poisoning associated with toxin formation in improperly cooled, starchy foods.

Purpose: The purpose of this study was to establish critical processing parameters for soybean tempeh that allow confluent growth of mold while inhibiting bacterial pathogen growth.

Methods: Prior to inoculation acetic or lactic acids added to the cooking water lowered the bean pH to 5.9, 5.7, and 5.5 for acetic and 4.8, 4.6, and 4.4 for lactic. *Bacillus cereus* was used as the target pathogen, since it is the most likely bacterial pathogen to be present in the soybeans. Both *R. oligosporus* and *R. oryzae* were independently used as starter cultures. Tempeh was incubated at 35°C until confluent mold growth occurred.

Results: *R. oligosporus* fermented consistently within 24 hours. However, depending on pH and inoculum level, *R. oryzae* growth was slowed, or completely inhibited. *B. cereus* growth was inhibited at all tested pHs for lactic acid but uninhibited for all tested pHs of acetic acid.

Significance: The data suggest that *B. cereus* growth is independent of the fermentative mold used. If proper acidification of the beans is not reached, *B. cereus* growth is possible during the tempeh incubation period.

P2-208 Survival of *Salmonella* Species and *L. monocytogenes* during Pancake Cooking Process

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Introduction: Recent recalls and outbreaks due to foodborne pathogens in thermally processed foods highlight the need for food industries to validate their thermal process as a part of their food safety system.

Purpose: The purpose of this study was to evaluate the thermal inactivation kinetics of *Salmonella* spp. and *L. monocytogenes* during pancake cooking process.

Methods: Whirlpak bags containing pancake batter samples were individually inoculated with cocktail strains of *Salmonella* spp. or *L. monocytogenes* cocktail to achieve target level of 10^8 CFU/g. Following inoculation, Whirlpak samples containing inoculated samples were treated at 160, 165, 170 or 175°F in pre-heated water bath. Treatment times varied with the temperature between 15 to 60 s. Product temperatures and come-up times were recorded. The study constituted 3 replicates. Samples inoculated with *Salmonella* spp. and *Listeria monocytogenes* were enumerated using scientifically valid methods. Water activity analysis was also performed. Log-transformed data was analyzed using ANOVA. The level of significance used was 5%.

Results: Overall, *Salmonella* spp. and *L. monocytogenes* populations significantly decreased ($P < 0.05$) with increase in treatment temperature and time. *L. monocytogenes* was relatively heat resistant ($P < 0.05$) when compared to *Salmonella* spp. A minimum 4-log reduction in *Salmonella* spp. and *L. monocytogenes* was observed when batter samples were treated at 160°F for 30 s and 160°F for 60 s, respectively. Water activity did not significantly differ ($P > 0.05$) between batter and cooked samples.

Significance: The study findings indicate that properly cooked pancakes (that achieve an internal temperature of 165°F or greater therefore, no longer a slurry and are suitable for sale) achieve acceptable lethality of vegetative pathogens from a food safety standpoint. The data in this study provides scientific basis for the ConAgra Foods frozen manufacturing facility's food safety plan and support the use of an operational pre-requisite program for finished product quality as opposed to a CCP.

P2-209 Microbiological Growth Profile of *Staphylococcus aureus* and *Bacillus cereus* in Artisan Bread Dough Systems during Routine Manufacturing Conditions

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Introduction: Artisan bread dough systems ($a_w > 0.93$) used for bakery products may support the growth of *S. aureus* and/or *B. cereus* and, given favorable conditions, may produce heat stable enterotoxin during routine manufacturing.

Purpose: The purpose of this study was to evaluate the microbiological growth profile of *S. aureus* and *B. cereus* in dough systems.

Methods: Dough samples were individually inoculated in triplicate with strains of *S. aureus* or *B. cereus* to achieve a target level of 10^2 to10^3 CFU/g. Following inoculation, samples were stored at 25 or 30 or 35°C and analyzed at several time points between 0 and 24 h. Dough samples were analyzed for *S. aureus* or *B. cereus*, lactic acid bacteria (LAB), yeast, pH and water activity using scientifically valid methods. In accordance with FDA published guidance, the limit of food safety was defined as 10^5 CFU/g for *S. aureus* and 10^6 CFU/g for *B. cereus*.

Results: At all of the evaluated temperatures, neither *S. aureus* nor *B. cereus* reached the food safety limit. *S. aureus* populations significantly ($P < 0.05$) decreased during the storage time while *B. cereus* populations declined and remained stable following 18 h of storage. The observed trends at all the temperatures may be attributed to the growth of LAB and yeast populations added as a part of the dough formulation. Water activity did not change ($P > 0.05$) during storage. pH significantly ($P < 0.05$) decreased with increase in LAB populations.

Significance: Data for *S. aureus* and *B. cereus* suggest the important role of 'competitive inhibition' from LAB and yeasts at 25, 30 or 35°C in dough based systems. The study findings indicate competitive inhibition resulted in no significant food safety risk at all of the evaluated temperatures and storage times. The data generated in this study provide scientific basis to support manufacturing processes for artisan bread dough manufacturing systems.

P2-210 Modeling the Risk of Salmonellosis Associated with Dry Roasted Sunflower Seeds

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Introduction: Quantitative microbial risk assessment (QMRA) models are increasingly viewed as a means to help food processors make scientific decisions in support of their food safety systems.

Purpose: The purpose of this study was to assess the risk of salmonellosis associated with the consumption of dry roasted sunflower seeds using both laboratory data and QMRA mathematical models.

Methods: Sunflower seed samples were individually inoculated with cocktails of *Salmonella* spp. to achieve ~10^8 CFU/g. Dry roasting parameters included: Oven settings of 225°F or 275°F and roasting times of 5 to 45 min. The dry roasting study was repeated 3 times and *Salmonella* spp. were enumerated using scientifically valid methods. Prediction intervals (95%) were used to estimate the range of expected log reduction during dry roasting. Data on sunflower seed consumption and *Salmonella* prevalence and concentration on sunflower seeds were based on data from published sources, internal company data or expert opinion. A QMRA model was developed using SAS software. A variety of what-if scenarios were evaluated.

Results: Applying minimum 4-log reduction to sunflower seeds reduced the average number of *Salmonella* positive servings significantly ($P < 0.05$) compared to consumption of raw sunflower seeds. Predicted salmonellosis cases from dry roasted sunflower seeds were less than one per year, and based on a worse-case scenario (99^{th} percentile), given the assumed parameters, the model estimates that a single salmonellosis case would occur

about every 5 years. When CDC under-reporting factors are considered, the model estimates that one salmonellosis case from dry roasted sunflower seeds would be detected every 100 years.

Significance: The quantitative risk analysis estimations indicate that the risk of salmonellosis based from properly dry roasted sunflower seeds is extremely low. The techniques used in this study can provide a scientific basis for food safety plans in the sunflower dry roasting industry.

P2-211 Distribution of Antibiotic Resistance and Virulence Factors in *Enterococcus* Species Isolated from Horses in Korea

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Introduction: Enterococci are Gram-positive oval cocci that are present as the part of the normal microflora of the intestinal tracts of animals and humans. Their ability to acquire resistance against numerous antibiotics and harbor putative virulence traits are considered as one of the reasons for the rise of their opportunistic infections. As the growth rate of horse industry for leisure and food increases in Korea, the surveillance for *Enterococcus* spp. obtained from horses should be needed.

Purpose: In the present study, we investigated the occurrence of antibiotic resistance and virulence factors among enterococci collected from horses in Korea.

Methods: A total of 3,078 swab samples were obtained from horses and horse-associated environments in Korea and *Enterococcus* spp. were speciated using specific PCR and VITEK II. After antibiotic susceptibility tests were performed by using disc diffusion method according to CLSI guideline, presence of the six antibiotic resistance genes and five virulence genes were determined by PCR. The biofilm formation ability was evaluated and PFGE was performed to analyze for clonal relatedness among the isolates.

Results: Overall, 265 samples of all examined contained *Enterococcus* isolates and *E. faecalis* (49.8%) and *E. facium* (22.3%) were the major species in all samples. Antibiotic resistance rates were very low but the biofilm production was detected from 134 (50.6%) enterococcal isolates, especially in *E. faecalis.* The PFGE results revealed that horse isolates were closely related to horse-associated environmental isolates in same places.

Significance: In this study the horses were well taken care of and antibiotics were rarely prescribed. However, continuous monitoring was needed to prevent transmission to human by food and direct contact, since the spread of *Enterococcus* spp. between horses and environments was possible and nearly half of enterococcal isolates had ability to product biofilm.

P2-212 Safety and Quality Evaluation of Some African Traditional Fermented Foods Produced Using Selected Starter Culture

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Introduction: *Fufu* and *ogi* are two traditional African foods produced from spontaneous submerged fermentation of cassava roots and maize grains. These foods are produced in homes at small scale levels and widely consumed by millions of people. Safety and quality of these foods are doubtful because of general lack of standards in their production.

Purpose: The purpose of this work was to investigate the effect of controlled starter culture fermentation on the safety and quality of cassava *fufu* and maize *ogi*.

Methods: Cassava and maize were fermented separately by submerging inside water for 72 h and 48 h, respectively, using selected starter cultures isolated from previous spontaneous fermentations. The starter cultures were determined for their rate of acid production, amylase activity, bacteriocin and hydrogen peroxide production as well as their level of antimicrobial activity. The fermented foods were investigated for the levels of cyanide reduction by the cultures, proximate and sensory properties.

Results: The starter cultures *L. plantarum* strain INAVEM-P1, *L. plantarum* strain OP, *L. fermentum* strain KLDS 10733 and *L. fermentum* strain 1 for cassava fermentation and *L. plantarum* strain 6225118, *L. plantarum* strain OP and *L. fermentum* strain IMAU82114 for maize fermentation were used. They demonstrated high antimicrobial activities indicated by their zones of inhibitions against test organisms. Initial cyanide content of the cassava was 25.4 ppm. Mixed culture fermentation significantly ($P < 0.5$) detoxified cyanide content by 92% and reduced pH from 4.16 - 3.75 in *ogi* and from 4.00 - 3.63 in *fufu* as well as improved proximate composition and sensory properties.

Significance: Controlled fermentation of cassava for *fufu* and maize for *ogi* using mixed LAB starter cultures with suitable technological properties ensured rapid pH reduction and proper cyanide reduction hence improved the safety and quality of the products.

P2-213 Protective Biofilms Using Lactic Acid Bacteria

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Introduction: Biofilms formed by "friendly" lactic acid bacteria (LAB), present in foods and agricultural products or used as starter cultures, may be a promising tool for the control of pathogen biofilm formation.

Purpose: To evaluate the potential application of *Lactococcus* spp and *Lactobacillus* spp strains to inhibit formation of biofilms by pathogenic bacteria.

Methods: Two *Lactococcus (Lc. lactis* 69 and 94*)* and three *Lactobacillus (Lb. helveticus* 352*, Lb.* spp 13 and *Lb.* spp 40), isolated from Brazilian foods, were tested for their ability to produce biofilms capable to inhibit formation of biofilms by *Listeria monocytogenes* ATTC 7644, *Salmonella* Typhimurium ATTC 14028 *and Escherichia coli* O157: H7 ATCC 35150*.* Tests were carried out with each LAB strain individually or in combinations of one *Lactococcus* and one *Lactobacillus* strain*.* Cultures of LAB (7 log CFU/ml) were transferred to 24-well polystyrene microtiter plates containing MRS broth and incubated at 30ºC. After 48 h, the liquid was removed and each pathogen, grown in TSB (7 log CFU/ml), was added to the wells. After 24, 48 and 72h at 30ºC, the liquids were removed and the cells attached to the wells (biofilms) were suspended by vigorous shaking and enumerated. The statistical significance of results was determined using a *t*-test (95% confidence interval).

Results: *Both Lactococcus strains inhibited the formation of biofilms by all tested pathogens in 24 h, when tested alone or combined with Lactobacillus. Afterwards, the inhibition was time-dependent and varied according to the Lactococcus strain, target pathogen and combination of LAB. After 24 h, the most effective control was against L. monocytogenes, caused by the combination of Lb. helveticus 352 with both Lc. lactis* strains*.*

Significance: Protective biofilms based on these bacteria may be an interesting alternative to control formation of biofilms by bacterial pathogens in the food industry environment.

Poster

P2-214 *Lactobacillus bulgaricus* ATCC 11842 as a Potential Surrogate for Inactivation Studies at ≥ 70°C

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❖ ***Developing Scientist Competitor***

Introduction: Pasteurization is an effective method of inactivating bacterial pathogens in a variety of products. However, it has been shown that hepatitis A virus (HAV) has greater thermal resistance than bacterial pathogens with $D_{72°C}$ of ca. 0.9 min in buffer. Thus, the most common surrogate used for pasteurization studies, *Listeria innocua*, is not resistant enough for validation studies.

Purpose: The purpose of this study was to determine the thermal inactivation kinetics (D- and z-values) of *Lactobacillus bulgaricus* in milk as a potential surrogate for HAV. Additionally, the effect of sublethal heat on increasing thermotolerance of *L. bulgaricus* was also investigated.

Methods: Thermal inactivation of *L. bulgaricus* ATCC 11842 was performed in 2% fat ultrahigh temperature treated milk at 65, 67, and 70°C for 0 - 60 min. Milk was inoculated by with 1.5 x 10^7 CFU/ml of *L. bulgaricus* and placed in 2 ml vials. For the effect of sublethal heating, cells were treated at 45 or 50°C for 30 min prior to inoculation of milk. Following heating for appropriate times, vials were placed in an ice bath, serially diluted and spread-plated onto MRS agar plates. Plates were incubated anaerobically for 48 h at 37°C and colonies enumerated. Each treatment was replicated thrice and D- and z-values were determined using a first-order model.

Results: D-values for *L. bulgaricus* in UHT milk were 9.98 ± 1.0, 2.68 ± 0.16 and 0.45 ± 0.08 min, at 65, 67, and 70°C, respectively, with a z-value of 3.7°C. After sublethal heating, $D_{70°C}$for the 45 or 50°C treatment were 0.34 ± 0.05 or 0.48 ± 0.1 min, respectively.

Significance: Thus, while the heat resistance of *L. bulgaricus* is lower than HAV, it has potential value in validation studies for inactivation of HAV at high cell concentrations. Sublethal heating did not cause the D-value to increase significantly.

P2-215 Degradation of Histamine by *Lactobacillus plantarum* D103 Isolated from Miso, a Fermented Soybean Food

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Poster

Introduction: Histamine is heat stable and the causative agent of scombroid poisoning. Histamine is physiologically degraded through the oxidative deamination process catalyzed by either histamine oxidase or histamine dehydrogenase of certain microorganisms.

Purpose: To isolate histamine-degrading bacteria in fermentation foods, the Miso products and mustard pickle products sold in supermarkets were purchased from southern Taiwan.

Methods: This research was to isolate histamine-degrading bacteria from Miso and mustard pickle products, and evaluate the influence of environmental condition such as pH, temperature and salt concentration on histamine-degradation activity of histamine-degrading isolate.

Results: Four and two histamine-degrading bacteria isolated from Miso and mustard pickle samples were identified as *Lactobacillus plantarum* (4 isolates) and *Lactobacillus brevis* (2 isolates), respectively. Among them, *L. plantarum* D103 degraded histamine up to 100% in MRSH broth. In general, the higher bacterial growth of *L. plantarum* D103 could accompany the higher histamine oxidase activity and histamine-degrading capability. Therefore, the optimal growth, histamine oxidase activity and histamine degrading capability of *L. plantarum* D103 were at 30°C, pH 5 and 3.0% NaCl for 24 h incubation.

Significance: The histamine-degrading isolate, *L. plantarum* D103, will be as a starter culture in inhibiting histamine or biogenic amines accumulation during Miso food fermentation.

P2-216 Norovirus Contamination on Hands of Infected Individuals during Norovirus Outbreaks in Long-term Care Facilities

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Introduction: The majority of the norovirus outbreaks in the U.S. are reported in long-term care facilities (LTCFs). Although direct person-to-person transmission is regarded as the primary transmission route for norovirus, outbreak investigations suggest that the hands of infected individuals play an important role in norovirus transmission.

Purpose: The aim of the study was to collect and test hand rinse samples from infected individuals for norovirus.

Methods: As part of a prospective study on norovirus outbreaks in LTCF, hand rinse samples and stool samples were collected from 22 infected residents and staff between days 0 - 4 after onset of norovirus symptoms. Hand rinse samples (25 ml) were collected using a glove juice method and concentrated using for 18 h and norovirus was lysed by guanidinium isothiocyanate. Viral RNA was then purified and further concentrated using Qiagen midi-columns Zymo-spin columns. Norovirus was extracted from stool using guanidinium isothiocyanate-silica based extraction kit on an automatic magnetic bead-beating platform. GI and GII norovirus was detected and quantified by real time RT-PCR and further positive samples were further typed by conventional RT-PCR and sequencing of the positive products.

Results: Of the 22 hand rinse samples, 10 (45.5%) tested positive for norovirus with viral loads ranging from 3.4 to 7.9 log RNA copy numbers per hand rinse sample. Genotypes of viruses detected in hand rinse samples were identical to the genotypes detected in the stool samples from the individuals.

Significance: These findings demonstrate that almost half of the symptomatic people from which a hand sample was collected tested positive for norovirus with significant higher viral loads than was reported previously in controlled human volunteer studies. These data support the need for better hand hygiene strategies to prevent norovirus transmission.

P2-217 Norovirus Attachment on Solid Surfaces According to pH, Ionic Strength and Temperature: Surface Free Energy Contribution

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Introduction: Norovirus increasing incidences around the world are supported by their ability to bind agri-food surfaces. Furthermore, proteins and adsorbents surface free energies are reportedly important factors in adsorption phenomena.

Purpose: We aimed to estimate the surface free energies of noroviruses, fresh foods and agri-food surface, calculate their interfacial free energies of interaction and evaluate adsorption.

Methods: Virus-like particles (VLPs) of GI.1 and GII.4 noroviruses and feline calicivirus have been produced, purified and used. Surface free energies of those viral particles and of surfaces such as lettuce, strawberry, polyethylene and stainless steel have been estimated using contact angle method. Norovirus adsorption was also tested according to pH, ionic strength and temperature.

Results: Almost all surface free energies and all total interfacial free energies of interaction predicted spontaneous adsorption for the VLPs tested. As expected, GII.4 VLPs adsorbed clearly on stainless steel, polyethylene and lettuce and adsorption percentages on all surfaces were in the same order with maxima (~94% of 0.35 µg, n = 2) at pH 4 near the isoelectric point, and decreased with higher pH. Adsorption increased with increasing ionic strength, as well as increasing temperature at pH far from pH 4.

Significance: Physicochemical conditions are likely more important than surface free energies in adsorption amplitude. Acidic pH, high ionic strength and temperatures in agri-food sector should matter during processing as they can contribute in norovirus adsorption on surfaces and foods.

P2-218 Human Gut-on-a-Chip as a Model for Enteric Virus Culture

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Introduction: The successful cultivation of foodborne viruses is a high-priority research outcome with respect to food safety. A reproducible cell culture model for human noroviruses (NoV) has been unattainable despite numerous attempts in many cell lines. The human gut-on-a-chip technology developed by the Wyss Institute offers a highly differentiated model that reconstitutes many features of normal small intestine physiology; namely, villi formation, establishment of tight junctions, and formation of basal proliferative crypts, and therefore provides a good platform in which to study enteric virus replication.

Purpose: To date, the gut-on-a-chip has never been tested for virus infection. The objective of these studies was to establish whether the gut-on-a-chip environment could support enteric virus replication and be used as a model for human norovirus culture.

Methods: The enteric picornavirus Coxsackievirus B1 (CVB1) was tested as a positive control in the system. CVB1 was adsorbed on the apical or basal side of 5-day differentiated Caco-2 cells in the gut-on-a-chip for 2 h without flow, at which time the cells were rinsed with medium, and continuous flow was resumed. Apical and basal effluents were collected at 6, 24, and 48 h post-infection (hpi) and assayed for CVB1 production by qRT-PCR and plaque assay. The cells on the chips were fixed and analyzed by immunofluorescence.

Results: CVB1 infection of Caco-2 cells in the gut-on-a-chip resulted in complete destruction of villi and production of infectious virus within 48 hpi, thus demonstrating its utility as a model for enteric virus replication.

Significance: As the efforts for identifying an appropriate cell culture system that will support NoV replication continue, we have identified and present preliminary data that show the human gut-on-a-chip system is capable of supporting virus replication using CVB1 as a prototype. Ongoing studies are examining replication of human norovirus in this model.

P2-219 Binding of Human Norovirus to Naturally Occurring Human Fecal Bacterial Isolates

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❖ ***Developing Scientist Competitor***

Introduction: Study of human noroviruses (HuNoVs) is hindered by lack of a cultivable strain. Recent studies suggest *in vitro* cultivation of HuNoV might depend upon bacterial cofactor(s), although their importance remains unknown.

Purpose: To characterize the binding affinity of select bacterial isolates to representative HuNoV strains.

Methods: Sixteen bacterial isolates (5 ATCC strains; 11 fecal isolates obtained from HuNoV-positive stool samples) were used in this study. Isolates were grown anaerobically, exposed to HuNoV GII.4 New Orleans, GI.6, or the Tulane virus surrogate, and then pelleted. To characterize binding affinity, the pellet and supernatant were separately subjected to RNA extraction and RT-qPCR. Turnip Crinkle Virus (TCV), a plant virus with similar size and structure to HuNoV, was used as a negative control. The three bacterial strains showing the highest binding affinity to HuNoV GII.4 were chosen for additional ELISA-based studies to determine evidence of histo-blood group antigen (HBGA)-like molecules corresponding to ABH, Lewis A, Lewis B, Lewis Y and H type 1.

Results: When cultured in tryptic soy broth (TSB), all bacteria tested exhibited a high level of binding to GII.4 New Orleans (89.62 ± 4.95% capture efficiency). Only bacteria incapable of growing in TSB, including *Bacteroides thetaiotaomicron* (chopped meat media), *Lactobacillus gasseri* and *L. plantarum* (MRS broth), showed a significantly lower level of binding (45.47 ± 12.0% capture efficiency). This interaction was specific, as there were different bacterial binding patterns for GI.6 and Tulane viruses; no binding was observed for TCV. Only one fecal isolate showed HBGA activity, with possible Lewis A and ABH-like motifs present.

Significance: Preliminary data suggests HuNoV bind a range of bacteria in a strain specific manner, and the ligand responsible for this interaction may not be exclusively HBGA-like moieties. These data have relevance in efforts to cultivate HuNoV and for methods to concentrate and purify HuNoV for downstream detection.

P2-220 Effect of Seasonal Variation on Microbial Safety of Lettuce in Lagos State, Nigeria

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Introduction: Lettuce is consumed raw in salads and is susceptible to microbial contamination through environment, agricultural practices and its morphology, thus a potential vehicle for foodborne illness.

Purpose: This study examines the effect of seasonal variations on the microbial safety of lettuce in Lagos State.

Methods: Samples of field source lettuce (n = 90), irrigation water (n = 30) and manure (n = 30) used on farm were collected monthly for five months (August to December 2013) for microbial analysis using standard bacteriological techniques; Total Plate Count (TPC), Total Coliform Count (TCC), *Escherichia coli, Listeria* spp., *Salmonella* spp., and *Shigella* spp. counts. Farmers whose farms were sampled were assessed on adoption of Good Agricultural Practices (GAP) pertaining to food safety through questionnaires. Relative humidity (RH), temperature and rainfall data were obtained from Nigeria Meteorological Station. Risk hazard map was constructed.

Results: TPC, TCC, *Listeria* ranges were: 5.87 - 8.14, 4.62 - 6.71, 4.15 - 6.19 log CFU/g while *Shigella, E. coli* and *Salmonella* spp. counts were 3.16 - 4.09, 3.16 - 4.09 and 3.00 - 3.26 log CFU/25g of lettuce samples, respectively. Counts of isolated pathogens exceeded the international safety limits.

Salmonella, Shigella, E. coli counts and TPC had a negative correlation with RH and a positive correlation with rainfall. Irrigation water and rainfall had a positive correlation with the microbial count except *Salmonella* spp. count. Manure had a positive correlation with all the microbial counts. Of the respondents 26.7% were GAP adopters; 40% moderately adopted GAP and 33.3% were non-GAP adopters – highest microbial load was obtained from these category.

Significance: This study suggested that seasonal variation influences lettuce contamination, although sources other than irrigation water and manure initiated contamination. The institution of GAP on farm to improve microbial safety of lettuce produced for public consumption is emphasized.

P2-221 Public Health Consequences of Microbial Spoilage and Nutritional Quality of *Dacryodes edulis* Fruits

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Introduction: The consumption of microbiologically deteriorated fruit pose serious health implications as this may become a route of infections. The nutritional quality of the deteriorated fruit is also in doubt.

Purpose: This study was therefore aimed at assessing the effects of spoilage on nutritional quality and microbial loads of *Dacryodes edulis* fruits.

Methods: The methods adopted were standard microbiological procedures and AOAC approved techniques.

Results: Bacterial species observed were *Bacillus, Staphylococcus, Xanthomonas, Erwinia, Pseudomonas, Escherichia* and *Lactobacillus species.* The fungal species isolated were *Alternaria, Trichoderma, Geotrichum, Aspergillus* and *Fusarium* species. Others were *Clodosporium, Penicillium* and *Saccharomyces* species. Moisture content ranged from 14.7% to 19.2% from 0 to 7th day, crude fiber (13.7% to 7.9%). Ash content (2.4% to 1.7%) and fat content was from 55.2 to 32.2% within the same period. Carbohydrate value reduced from 14.1% to 7.1% from 0 to 7th day. However, crude protein content showed a different trend as it increased from 4.41% in the 0 day to 7.93% on Day 3 and then began to decrease till 4.02% on Day 7. Dipping in NaCl and sodium metabisulphate reduced spoilage and increased shelf life. Pathogenicity tests showed that *Xanthomonas, Erwinia and Pseudomonas* species were the actual pathogens with *Fusarium, Alternaria and Cladoposium* species.

Significance: The microbial counts increased with storage time. *Pseudomonas* and *Escherichia* species showed signs of being pathogenic in man by being hemolytic and agglutinating human blood components. The results obtained therefore indicated adverse effects of consuming microbially deteriorated *D. edulis* fruits.

P2-222 Effect of Peak Wavelength of Light Emitting Diodes and Illumination Temperature on Photodynamic Inactivation of Selected Foodborne Bacteria

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❖ ***Developing Scientist Competitor***

Introduction: While previous studies have demonstrated the antibacterial efficacy of LEDs in the blue region at room temperature, studies reporting the antibacterial effect of LEDs outside this region are limited. Furthermore, a direct comparison of the antibacterial efficacy of LEDs at different temperature is necessary for food application.

Purpose: The objective of this study was to investigate the effect of LEDs of three different wavelengths and temperatures on the photodynamic inactivation of *Campylobacter jejuni*, *Lactobacillus plantarum*, *Staphylococcus aureus* and *Vibrio parahaemolyticus*.

Methods: A spectrometer and an irradiance meter were used to characterize the three (405, 460 and 520 nm) LEDs. Four bacterial strains in phosphate buffer saline were illuminated with each LED at 4, 10 and 25°C for 7 h. Inactivation curves were modeled using the modified Gompertz Model and the decimal reduction times (D-values) corresponding to the three LEDs were compared.

Results: The intensity of the three LEDs was approximately 120 mW/cm^2. Illumination with 405 and 460 nm LEDs produced significant inactivation ($P < 0.05$) in the population of *C. jejuni* and *V. parahaemolyticus* (> 4 log), while *L. plantarum* and *S. aureus* showed relatively less susceptibility. The 520 nm LED brought about negligible inactivation. Of the three LEDs, 405 nm LED proved most effective as reflected by the D-values, which was generally 3 - 6 fold lower for the 405 nm LED compared to the 460 nm LED. Temperature had no significant effect ($P > 0.05$) on the photodynamic inactivation of *C. jejuni*. However, a significant inactivation was observed in *L. plantarum* with 460 nm LED at 25°C (D = 1121 J/cm^2), and not at 4 and 10°C.

Significance: The results of this study demonstrate the antibacterial efficacy of LEDs were highly influenced by wavelengths and illumination temperatures, indicating that 405 and 460 nm LEDs would be suitable LEDs for food application.

P2-223 The Complete Mitochondrial Genome of the Foodborne Parasitic Pathogen *Cyclospora cayetanensis*

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Introduction: *Cyclospora cayetanensis* is a coccidian parasite responsible for waterborne and large scale foodborne outbreaks worldwide. In the U.S., the two most recent diarrhea outbreaks caused by *C. cayetanensis* were solely associated with the consumption of fresh produce and sickened 631 and 304 persons in 2013 and 2014, respectively.

Purpose: In different apicomplexan parasites multicopy organellar DNA such as mitochondrion genomes have been used for detection and genetic traceback analysis. A complete *C. cayetanensis* mitochondrial genome sequence was obtained in this study and it will be used to design alternative molecular methods for enhanced detection and characterization of the parasite.

Methods: We sequenced the *C. cayetanensis* genomic DNA obtained from stool samples from patients infected with *Cyclospora* in Nepal using the Illumina MiSeq platform. By the use of bioinfiormatics the metagenomic reads of non-coccidian origin sequences were filtered out and by targeted alignment, we were able to obtain contigs containing *Eimeria*-like mitochondrial, apicoplastic and chromosomal genomic fragments.

Results: A complete *Cyclospora* mitochondrial genome was assembled and confirmed by sequencing of cloned PCR amplicons obtained with primers designed on the basis of the draft assembly sequence. The results show that the *C. cayetanensis* mitochondrion genome is 6274 bp long, with a 33% GC content and appears to exist in concatemeric arrays, similar to *Eimeria* spp. mitochondrial genomes. Phylogenetic analysis of the *C. cayetanensis* mitochondrial genome placed this organism in closely related cluster with *Eimeria* species. The mitochondrial genome of *C. cayetanensis* contains coding genes for *Cytochrome B (cytb), Cytochrome C oxidase subunit 1(cox1),* and *Cytochrome C oxidase subunit 3 (cox3)* in addition to 14 large subunit (LSU) and nine small subunit (SSU) fragmented rRNA genes.

Significance: The complete mitochondrial genome sequence presented here will provide novel molecular targets for detection and characterization of *C. cayetanensis* during foodborne outbreaks.

P2-224 Sporulation in *Paenibacillus* Biofilms

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❖ ***Developing Scientist Competitor***

Introduction: *Paenibacillus* species are Gram-positive bacteria that cause food spoilage. They form spores when the environmental condition is unfavorable and are the main cause of food contamination because their spores are highly resistant to heat and disinfectants. In the environment, most bacteria form biofilms, which also cause food spoilage.

Purpose: The purpose of this study is to examine the relationship between biofilm formation and sporulation of *Paenibacillus* species isolated from the environment.

Methods: Thirteen *Paenibacillus* strains isolated from various sources such as decayed foods or raw materials were cultured in 96-well microtiter plates at 30°C for 96 h and biofilm formation was examined by crystal violet staining. To determine the sporulation efficiency, the cells in each of the biofilm and planktonic cell fractions were heated at 80°C for 10 min, and then heat-resistant spores were counted by plating method. To purify the spores derived from biofilms and planktonic cells, the spores were precipitated by centrifugation of the culture medium, and the remaining vegetative cells and the mother cells were removed by washing the precipitates with distilled water.

Results: Most of the *Paenibacillus* species formed biofilms. Moreover, most of the spores formed were found in the biofilms of *Paenibacillus glucanolyticus* and *Paenibacillus polymyxa.* Lastly, the spores derived from these biofilms were longer than those derived from planktonic cells, as observed by phase contrast microscopy.

Significance: These findings suggest that biofilms are reservoirs of spores. Therefore, the understanding and control of biofilms as well as sporulation are fundamental to the prevention of food spoilage by *Paenibacillus* species.

P2-225 Characterization of bla_{shv18} Gene Transfer between *Klebsiella pneumoniae* on Shredded Lettuce

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Introduction: Lettuce is one of most commonly consumed fresh vegetables in the world. Research has demonstrated that high populations of antibiotic resistant bacteria are associated with fresh produce. Only a limited number of studies of have focused on transfer of antibiotic resistance genes on fresh produce.

Purpose: The purpose of this work was to evaluate the potential transfer of extended-spectrum β-lactamase gene (bla_{shv18}) on shredded lettuce and characterize its transconjugants.

Methods: *Klebsiella pneumoniae* strain ATCC 700603 served as the donor and 3 recipient (KP342, KP4, KP8) were used for the mating experiments. Shredded lettuce was dipped in the sterile tap water containing 7 - 8 log CFU ml^{-1} mixture of a donor and a recipient for 1 min. Inoculated lettuce (25 g) was placed into a sterile sampling bag and stored at 15°C and 24°C. After 1 and 2 days of storage the lettuce samples were plated out on TSA containing appropriate antibiotics. Presumptive transconjugants were verified by detection of transferred gene as well as comparing by random amplified polymorphic DNA (RAPD) patterns. Transfer frequency and antimicrobial susceptibility of transconjugants were compared.

Results: At 15°C, transconjugants were only detected on day 2 post-mating. At 24°C, the transfer of bla_{SHV18} gene occurred at a frequency of 1.12E-05 to 2.98E-04 at day 1 and 2 post-mating. In particular, the transfer rate on day 2 at 24°C was significantly greater than under other conditions and days ($P < 0.05$). The bla_{shv18} gene carried by transconjugants was detected by PCR. According to the RAPD profile, transconjugant and recipient exhibited similar band patterns that were different from the donor. Resistances to trimenthoprim/sulfamethoxazole, ceftazidime and cefotaxime were co-transferred with bla_{shv18} gene.

Significance: These results suggest that transfer of antibiotic resistance genes on fresh produce may permit the spread of antibiotic resistance genes into the community having a negative impact on human health.

P2-226 Characterization of Bacterial Contamination in Soaps Collected from Commercial Soap Dispensers

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✦ ***Undergraduate Student Award Competitor***

Introduction: Hand washing with soap is typically used to prevent the spread of enteric disease and the transfer of harmful pathogens in the environment and to foods. However, recent studies have found that soap dispensers themselves, and hence commercial soap, may become contaminated with bacteria. This contamination can impact the efficiency or utility of hand washing.

Purpose: To examine the types and levels of bacteria present in 100 commercial soaps dispensed in containers.

Methods: One hundred soap samples were collected from restrooms at various businesses, restaurants, schools, and public parks around North Carolina. Data were collected on the type of soap and dispenser (including refillable vs. disposable) and sampling location. Samples were plated for enumeration on Reasoner's 2A (R2A) and Brain Heart Infusion (BHI) agars in triplicate, and 72 colonies that showed different physical characteristics were then isolated for later sequencing.

Results: Bacterial contamination was observed in 28/100 (28%) of the soap samples when plated on BHI agar, and 19/100 (19%) on R2A agar. Based on container type, 47% of the soap solutions obtained from refillable soap containers showed bacterial growth, compared to 39% of the soaps obtained from disposable containers. The range on agar plates was 4 CFU/ml to >1200 CFU/ml, with the majority of samples having very low numbers of bacterial colonies, and only ~2% having very high numbers. Sequences for isolate typing are pending.

Significance: The results suggest that there is a low level of bacterial contamination in public soap dispensers in North Carolina and that the dispenser type makes little difference in the likelihood or levels of bacteria. This contamination may be related to a failure to properly clean the dispenser, and/or associated bacterial growth although studies are merited to better understand the sources and significance of bacterial contamination in commercially dispensed soaps in public restrooms.

P2-227 Incidence of Pathogens and High Aluminium Concentrations Raise Safety Concerns about Street Vended Soya *Wara* in Nigeria

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Introduction: Soy *wara* is a common Ready-to-Eat food that is currently produced and sold without regulation. There is the need to assess the microbiological safety as well as aluminum content due to the use of alum as a coagulant by some producers.

Purpose: This study encompassed microbiological safety assessment as well as determination of aluminum contents of samples processed with different coagulants including alum.

Methods: A total of 90 samples comprising of samples produced using different coagulants were purchased from vendors at six different locations. Samples of soy *wara* were inoculated on plate count agar for aerobic mesophile counts, MacConkey agar for coliforms, Oxford *Listeria* agar for Listeriae, Baird Parker agar for staphylococci, *Bacillus cereus* agar for *Bacillus cereus* and Sabouraud dextrose agar for fungi, respectively. In addition samples were tested for the presence of *E. coli* O157:H7 and *Salmonella* using enrichment procedures and plating on Sorbitol MacConkey agar and *Salmonella Shigella* agar, respectively.

Results: Overall, 21% of the samples had standard plate counts exceeding 100,000 CFU/g and 14% had *Staphylococcus aureus* counts higher than100, 000 CFU/g. *Listeria monocytogenes* was isolated in 14.4% of samples and *Escherichia coli* and *Salmonella enterica* were detected in 5.6% and 2.2% of all samples, respectively. Aluminum concentration was highest in Soy *wara* processed with alum with a maximum value of 0.776 mg Al/g.

Significance: This study shows the potential hazards associated with soy *wara* and also that Gram positive pathogens and fungi can easily grow on this product whilst low water activity appears to retard the growth of Gram negatives. These have direct implications for health and future upgrade and industrialization of soy *wara.*

P2-228 Microbiological Enumeration of Dietary Supplements and the Creation of a Sequence Database for the Microbiota Found in Functional Foods

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Poster

Introduction: Understanding the health effects of the microbiome is at the forefront of many current scientific studies. Modulating the microbiome has the potential to improve the health of both the intestinal tract and the immune system, as well as, enhance the bioavailability of nutrients. Probiotics are "live microorganisms which, when administered in adequate amounts, confer health benefits on the host." The popularity of these products has grown exponentially over the past few years.

Purpose: Recent studies demonstrated that a daily intake of at least 10^7 to 10^9 viable probiotic cells is necessary to obtain sufficient colonization within the gut. The viability of microbes within dietary supplements is equally important as the current review criteria for a given product. This process consists of strain taxonomy, antibiotic resistance, extent of use in foods, and source of that particular strain.

Methods: We have tested ten supplements. Each product was plated and grown in both aerobic and anaerobic conditions using several different types of media. Colonies are counted using a colony counter and expressed in CFU/ml. Colonies isolated from these plates were identified using custom designed primers and whole genome sequencing.

Results: Nine of the ten products tested support the manufacturer's viability claims. We have also isolated a contaminant, *Enterococcus faecium*, from one of the products. We are being proactive in testing probiotic viability and creating a whole genome database for microbial ingredients to substantiate the manufacturer's labeling claims.

Significance: The effectiveness of probiotics is not regulated; however, proper labeling will help ensure consumer safety. These data help FDA monitor these supplements to ensure accuracy of manufacturer labeling and product safety in an effort to protect the consumer.

P2-229 Sequencing and Analysis of the Metagenomes from Oregano, White Pepper, and Cloves

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Introduction: Recently, spices have been implicated in at least fourteen illness outbreaks worldwide, including the United States, resulting in 1946 human illnesses, 128 hospitalizations, and two deaths. It is necessary to identify potential foodborne pathogens associated with spices such as oregano, white pepper, and cloves and improve methods for mitigation and rapid outbreak response. Shotgun metagenomic sequencing is a useful tool for meeting these goals by: (1) simultaneously identifying pathogens of interest present in each spice, (2) evaluating the efficacy of enrichment methods and microbiome shifts throughout the process, and (3) establishing variations between the natural flora found in spices. Spices are difficult to analyze because of their essential oils and chromophores; therefore, specific method optimization is often necessary.

Purpose: The purpose of this study was to optimize a protocol for shotgun metagenomic sequencing and assess its utility to identify the microbiome of spices.

Methods: Oregano, white pepper and cloves were enriched following a modification of the BAM method using Tryptic Soy Broth (TSB), and modified Buffered Peptone Water (mBPW). Samples were collected throughout the enrichment process. DNA was extracted using the Qiacube and shotgun metagenomic sequencing was performed on the MiSeq using the Nextera kit with modifications. The data was analyzed using Metaphlan.

Results: Analysis shows that the three spices tested have different metagenomic profiles; however, they contain many similar bacteria, including members of *Salmonella*, *Cronobacter*, *Shigella*, *Yersinia*, *Escherichia coli* and *Bacillus cereus*. Results also show that profiles shift throughout the enrichment process and differ depending on the growth media.

Significance: Shotgun metagenomic sequencing revealed the microbiomes of oregano, white pepper, and cloves and the presence of potential foodborne pathogens. This and the ability to trace shifts in microbial communities during enrichment can help the FDA improve our pathogen identification methods during outbreaks.

P2-230 A Rapid and Innovative Test System for *Legionella*, Especially *Legionella pneumophila,* in Water

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Introduction: The current ISO method (ISO 11731:1998; 11731-2 2004) used to detect and identify the *Legionella* species takes at least 10 days and requires several steps with different media. Therefore, new rapid methods for the detection of *Legionella* are of great interest.

HybriScan® *Legionella* kit and HybriScan *Legionella pneumophila* kit use ribosomal RNA as a modern detection target. The rRNA is greater in number than DNA (no PCR is needed), and is only present in living cells. It, therefore, perfectly suits specific and sensitive detection. Within 2.5 h, a direct quantitative detection of 10,000 colony forming units (CFU) per liter is easily possible and 1 - 10 CFU/l can be detected with an enrichment step in BCYE Medium.

Purpose: An accurate and efficient rapid test method as an alternate to PCR

Methods: Specificity is achieved by targeting conserved or unique rRNA sequences. A biotin-labeled capture probe is used to immobilize the target sequence on a solid support plate (streptavidin-coated microtiter plate). A digoxigenin labeled detection probe provides an enzyme linked optical signal read-out. Detection results from application of anti-DIG-horseradish peroxidase Fab fragments. The bound complex is visualized by horseradish peroxidase substrate TMB. Photometric data are measured at 450 nm and compared with standard solutions.

Results: The quantitative analysis of 39 *Legionella* positive samples with HybriScan were compared with ISO 11731 (GVPC and BCYE Agar) and no significant difference could be found. HybriScan®*D Legionella* even showed to be more sensitive on low concentrations.

Significance: Fast, cost-effective analysis, and high sensitivity and specificity

P2-231 Quantitative Risk Assessment for *Listeria monocytogenes* in Cantaloupe

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❖ ***Developing Scientist Competitor***

Introduction: The multistate listeriosis outbreak associated with cantaloupe in 2011 has drawn increasing concern and scrutiny on the melon production chain. Recent advances of quantitative microbial risk assessment (QMRA) provide a systematic and objective way to evaluate the food safety risk within the "farm-to-fork" continuum. However, no QMRA has been developed for melons, limiting the ability to provide quantitative guidelines.

Purpose: Based on data from the published scientific literature and industry practice, a QMRA model was developed for *Listeria monocytogenes* associated with either fresh-cut or whole cantaloupe consumption.

Methods: A simulation model describing exposure and public health assessment from cantaloupe consumption was developed using the @ RISK software. Relevant steps in the farm-to-fork chain were included. The baseline model assumed a prevalence of 1% and an initial level of *L. monocytogenes* contamination of -1 log CFU/cm^2 of. Variability and uncertainty in variables and parameters using a Monte Carlo framework with Latin Hypercube resampling and 10^5 iterations.

Results: The model predicted the risk per serving of fresh-cut cantaloupe consumption to be approximately 10-fold higher than that of whole cantaloupe consumption. The estimated annual Listeriosis cases associated with fresh-cuts cantaloupe consumption in the U.S. among susceptible and general healthy population were 0.3 and 0.001 cases per year, respectively. The sensitivity analysis highlighted that temperature at retail and at household storage are the two most important factors affecting risk associated with fresh-cuts consumption, while household storage after cutting is the main factor for whole melon consumption.

Significance: This study provides a first quantitative framework to evaluate the impact of contamination by *L. monocytogenes* in the farm-to-fork cantaloupe supply chain, and to assess potential risk reduction strategies.

P2-232 Integrated Analysis Pipelines for Whole Genome MLST and Whole Genome SNP in BioNumerics® 7.6 Applied to a *Listeria monocytogenes* Outbreak Linked to Caramelized Apples

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Introduction: *Listeria monocytogenes* is a ubiquitous organism in the environment and a rare cause of human disease. Though Listeriosis occurs infrequently, it is characterized by a high case-fatality rate which can exceed 30% percent. In December 2014 and January 2015, an outbreak of Listeriosis was linked to prepackaged caramelized apples. Most of the *Listeria monocytogenes* isolates obtained during this outbreak have been whole genome sequenced. A key challenge is the need to rapidly compute and interpret the relevant information from these large data files.

Purpose: We apply two pipelines for high resolution WGS-based molecular typing to the data from this outbreak: whole genome multilocus sequence typing (wgMLST) and whole genome single-nucleotide polymorphism analysis (wgSNP).

Methods: A wgMLST scheme is created from a set of reference sequences, from which all coding regions are extracted and used to create a set of discernible loci. Two independent approaches, an assembly-free and a BLAST-based allele calling algorithm, are used to determine locus presence and detect the allelic variants. The wgSNP algorithm detects SNP variants by mapping the WGS reads to a reference sequence, which can be internal or external to the data set. For both methods, all calculation intensive data processing steps are performed on the BioNumerics® Calculation Engine, which can be deployed locally or in the cloud.

Results: The BioNumerics® 7.6 software and its integrated calculation engine offer a powerful platform where both wgMLST and wgSNP can be performed and validated against traditional data such as MLST or PFGE, rapidly providing a robust, portable and high resolution picture of molecular typing data.

Significance: Rapid and automatic processing of WGS data ensures a reliable and easy to follow workflow in routine molecular surveillance, reducing the time needed to detect and contain an outbreak, eventually reducing the cost on public health and food safety.

P2-233 Risk Assessment of Pesticide Residues Using Food Commodity Intakes Calculated by KFCIC Software

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Introduction: Estimation of the food commodity intake is important for risk assessment of hazardous substance in foods such as pesticide residues. However, estimation of the food commodity intake is not easy since normal diets are composed of not only commodity-type foods but also mixed and processed foods. Herein, the Korea Food Commodity Intake Calculation (KFCIC) software to estimate the food commodity intake using database of the Korea National Health and Nutrition Examination Survey (KNHANES) has been developed. The KFCIC software has been designed to segregate food consumption data from the KNHANES further into food commodity levels.

Purpose: The purpose of this study was to evaluate risk assessment of pesticide residues using the food commodity intake data calculated by the KFCIC software.

Methods: Several representative food commodities such as rice, potato, apple, peach, cucumber, tomato and perilla leaf were selected to determine their exposure to pesticide residues. The calculated items were the average values in the database of KNHANES in 2008~2012.

Results: The average daily intakes of rice, potato, apple, peach, cucumber, tomato and perilla leaf were 217.72 g, 19.74 g, 32.05 g, 9.28 g, 11.80 g, 16.80 g and 3.20 g, respectively, which were utilized to determine their exposure to several pesticide residues. The average amount of pesticide residues was 0.101 mg/kg which was evaluated by monitoring the pesticide residues of commercial agricultural products. As a result, the amounts of pesticide residues were ranged between 0.021 and 1.898% of hazard index (% ADI). Further assessment proved that the risks of the pesticide residues were in very low level.

Significance: This study will prove that the KFCIC software can be an excellent tool to estimate food commodity intakes which are the basic resources to access human exposures to hazardous contaminants such as pesticide residues and their potential risks.

P2-234 A Semi Quantitative Risk Assessment of *Campylobacter jejuni* on Various Types of Processed Meat Products Using FDA-iRISK

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Introduction: Processed meat products are commonly implicated products associated with *Campylobacter jejuni*. Since refrigeration temperatures and vacuum packaging cause the high risk for *C. jejuni* survival, processed meat products such as ham and sausage can be a good medium for *C. jejuni* survival.

Purpose: This study conducted semi quantitative risk assessment of *C. jejuni* on various types of processed meat products using FDA-iRISK to compare their risk at retail market.

Methods: Dry-cured ham, round ham with sodium nitrite, garlic seasoning ham with sodium nitrite, round ham without sodium nitrite, garlic seasoning ham without sodium nitrite, and sausage without sodium nitrite were inoculated with a cocktail mixture of two *C. jejuni* strains (ATCC 33560, NCTC 11168) at a concentration of 6.0 ± 0.5 log CFU/g. The inoculated samples were vacuum-packed and incubated at 4 and 10°C. The experimental data and data in the literature were used as input data for FDA-iRISK.

Results: *C. jejuni* survived better in processed meat products at 4°C than at 10°C in all samples. The value of total DALYs per year was higher at 4°C. The lowest risk was observed in dry-cured ham at 10°C, followed by sausage without sodium nitrite, round ham with sodium nitrite, garlic seasoning ham with sodium nitrite, garlic seasoning ham without sodium nitrite, and round ham without sodium nitrite. Overall, the risk of ham with sodium nitrite was lower than the risk of ham without sodium nitrite. However, the antimicrobial effects of the garlic seasoning were not observed.

Significance: The greater risk of *C. jejuni* in various processed meat products was observed at 4°C than at 10°C. However, risk of *C. jejuni* in processed meat products even at 10°C should not be overlooked since the *C. jejuni* has a low infectious dose. Therefore, care must be taken to avoid contamination of *C. jejuni* on processed meat products.

P2-236 Quantitative Microbial Risk Assessment for *Campylobacter* in Ham in Korea

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❖ ***Developing Scientist Competitor***

Introduction: In recent, *Campylobacter* foodborne outbreaks have been increased in Korea, and the pathogen has been isolated from poultry, meat and processed meat products, especially for ham. Moreover, the consumption of processed meat products has been increased in Korea, which may increase the probability of *Campylobacter* infection.

Purpose: The objective of this study was to evaluate the risk of foodborne illness for *Campylobacter* in ham.

Methods: To identify the hazard, the general characteristics of *Campylobacter* and campylobacteriosis outbreaks were searched. In exposure assessment, the prevalence of *Campylobacter* in ham (press ham and fermented ham), and conditions for distribution and storage of ham were surveyed, followed by fitting the data to @RISK Fitting program to find appropriate data distribution. Also, a predictive model of *Campylobacter*, and consumption amount and frequency for ham were investigated. Appropriate dose-response models for *Campylobacter*, which were utilized for hazard characterization, were searched though literatures. With the collected data, a simulation model with @RISK was developed to estimate the risk of *Campylobacter* in ham.

Results: The *Campylobacter* cell counts in 200 ham samples were below detection limit (0.7 log CFU/g). An equation [-Ln(1-Beta(p+1, n-p+1))/V] thus showed that the mean value of initial contamination level of *Campylobacter* on ham was -4.1 ± 0.6 log CFU/g. The probabilities of *Campylobacter* infection per person per day by intake of ham were 1.28×10^{-11} for mean and 1.10×10^{-9} for maximum value, which can be considered low risk. Also, the time and temperature for distribution and storage were important factors, influencing *Campylobacter* foodborne disease through ham consumption.

Significance: The result indicates that the risk of *Campylobacter* in ham is considered low in Korea, and the microbial risk assessment from this study can provide scientific evidences for risk management and risk communication.

P2-237 A MatLab-Based App to Optimize Food Formulation and Recipes Taking into Account the Impact of pH and Storage Tmperature on Growth, Survival and Inactivation of Foodborne Pathogens

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Introduction: Nowadays, predictive microbiology or the use of recognized mathematical models to describe microbial behavior is commonly used in food research applications. Indeed growth, survival or death of specific microorganisms in food may be evaluated for specific properties of food (water activity and pH) and the storage conditions (temperature, relative humidity and atmosphere). Coroller *et al.* reported in 2012 a single modelling approach to apprehend food features yielding inactivation, survival and growth of *L. monocytogenes* that was validated on more than thousand datasets.

Purpose: This study aims at extending this approach to other pathogenic strains using a MatLab-based app to further improve industrial outreach.

Methods: Boundaries were determined in BHI broth to quantify growth abilities and destruction upon temperature and pH stress exposure for selected strains, i.e., *Listeria monocytogenes* SOR100, *Salmonella* Typhimurium ADQP305, *Escherichia coli* SOR200 and *Pseudomonas aeruginosa* ATCC15442. Shortly, growth kinetics and kill-curves were fitted, respectively by Rosso logistic and Weibullian models. Secondary models enabled to

quantify the impact of environmental conditions on bacterial behavior, i.e., growth, survival and inactivation. A MatLab-based app was developed to represent the variation of population for given conditions of formulation and storage.

Results: This app estimates the difference of population (ΔlogN) for given conditions of pH, temperature and storage time. The GUI graphical user interface indicates iso-contour plots which appear in "warm colors" for an increase of population or in "cold colors" for a decrease or death of population. Even though expected, this representation offers the advantage to easily visualize safe combination for food formulation.

Significance: Taking into account the impact of temperature and pH on microbial behaviour is crucial to ensure food safety throughout shelf life. This app enables visual representation of environmental conditions ensuring death of major pathogens, to further evaluate and optimise food recipe and storage conditions.

P2-238 Monte Carlo Simulation of Microwave-assisted Pasteurization Processes

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Introduction: Non-proteolytic *Clostridium botulinum* is a major safety hazard for extended shelf life refrigerated foods.

Purpose: The objective of this study was to evaluate the thermal lethality of products (10 oz. beef meatball trays and 16 oz. salmon fillet trays) processed in a microwave-assisted pasteurization system (MAPS).

Methods: Thermal processing parameters were selected to inactivate the spores of *C. botulinum* Types B and E in beef meatball trays and Type E in salmon filet trays. After a mathematical method was developed to accurately simulate the internal temperature of products, Monte Carlo simulation was used to analyze the lethalities delivered at different processing steps of the pasteurization process.

Results: The simulation results showed that the majority of the lethality (63 - 70%) was accumulated in the microwave-assisted heating (MAH) section, while the remaining lethality was completed in the cooling section, suggesting that the cooling section can contribute a significant portion of the lethality. Monte Carlo simulation was also used to analyze the effect of different processing parameters on the total lethality of products. With a target lethality of 6 log-reductions in the spores, more than 98.8% of the processes will achieve a minimum of a 5-log reduction of the spores of *C. botulinum* Type B in 10 oz. beef meatball trays, and more 98.5% of the processes achieve > 5 log-reductions in the spores of *C. botulinum* Type E in 16 oz. salmon fillet trays. The results of sensitivity analysis showed that the MAH section is critical to the accumulation of lethality in the products, with the heating temperature being the most sensitive parameter influencing the total lethality, followed by the heating rate and time in this section and the temperature in the pre-heating section.

Significance: The results of this study may be used to improve the design and operation of the MAPS.

P2-239 Identifying and Modeling Multi-scale Risk Factors for Contamination by Foodborne Pathogens in Mixed Farms

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❖ ***Developing Scientist Competitor***

Introduction: An increasing number of foodborne outbreaks attributed to produce has led to the recognition of this class of products as vehicle for foodborne pathogens. The production of vegetable crops in a mixed farming environment (produce grown in the same premises with farm animals) is also gradually increasing in the U.S.

Purpose: The objectives of this study were to: 1) identify possible risk factors for pathogen contamination in produce at pre-harvest level, and 2) compare different modelling tools that can be used to analyze and identify risk factors in order to control and manage the pathogen contamination risk at farm level.

Methods: A broad literature search was carried out, and studies that investigated possible risk factors for contamination from *Listeria*, *Salmonella*, and pathogenic *E. coli* in a variety of produce at pre-harvest level were summarized and discussed. Potential pre-harvest risk factors were identified and divided into three categories: farm management factors, weather factors, and environmental factors.

Results: Presence and survival of pathogens in wild and domestic animals, water, soil, and manure are well documented. Weather factors such as temperature, freeze-thaw cycle, and rainfall have been investigated as possible risk factors, although consistent evidence is lacking to conclusively support the association between these factors and contamination on produce, especially in mixed farms. Classification trees and logistic regression are the primary statistical modelling tools that have been used to identify potential risk factors for contamination.

Significance: This study analyzed possible risk factors of microbial contamination in mixed produce farms and discussed statistical tools that can be used to evaluate and determine those risk factors. The information provided in this study could serve as a useful resource to evaluation and rank risk factors in produce production.

P2-240 Secure Turkey Supply Plan-enhancing Food System Protection through Public-private Partnerships

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Introduction: One of the keys to food system protection is to mitigate the risks and vulnerabilities to a given food system in the face of an adverse event. The Secure Turkey Supply Plan (STS) is meant to reduce the unintended consequences that a standard response to an outbreak of highly pathogenic avian influenza (HPAI), involving quarantine and stop movement orders, would have on the turkey industry.

Purpose: Through collaboration, form accurate risk- and science-based plans and guidelines to inform risk management decisions associated with a HPAI outbreak.

Methods: A public-private partnership approach is utilized to enhance the data collection and accuracy ensuring that all recommendations within the STS are feasible for the food system and reasonable within the regulators' judgment. The working group partnership includes representatives from government, industry and academia.

Results: Normal daily mortality data collected from 74 turkey houses showed an industry representative number of 3 dead birds per 1,000 in a house per day for meat turkeys (3/1,000). The original protocols called for increased surveillance and restrictions if the daily mortality in a house was above 2/1,000. At the rate of 2/1,000, there is a predicted rRT-PCR false positive rate of 4.9%. Over a two week period, a farm with 3 houses has an 89% chance of at least 1 false positive test. At a rate of 3/1,000 the false positive rate is 1.1%. Other enhancements to the STS include the biosecurity recommendation of a pre-movement isolation period of 5 days before movement of meat turkeys.

Significance: The pro-active risk assessment work has resulted in science based and industry viable recommendations to the STS in both biosecurity and flock health monitoring. These ensure infected but undetected commodity and live bird movements do not perpetuate the spread of HPAI and increase the impact of an outbreak.

P2-241 Evaluation of Microbial Risks for Certified Agricultural Standards-Compliant Ready-to-Eat Food Products in Taiwan (2010–2014)

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Introduction: Preventing food poisoning outbreaks is a critical task for the government. Due to lifestyle changes and increased need for convenience among consumers, Ready-to-Eat (RTE) food products have become more popular in Taiwan, especially foods compliant with the Certified Agricultural Standards (CAS), an indicator of quality. Microbial risk evaluation can help ensure the safety of RTE products. Also, products which are exposed to minimal processing would benefit from additional microbial risk testing.

Purpose: The objective of this study is to identify microbial risks for certified agricultural standards-compliant Ready-to-Eat food products in Taiwan.

Methods: We collected microbial analysis data of outbreak-associated food samples, food survey samples, and RTE products compliant with CAS in Taiwan from 2010-2014. Samples were evaluated for sanitary indicators (total count, coliforms, and *E. coli*) and pathogenic bacteria (*Salmonella*, *Staphylococcus aureus*, and *Bacillus cereus*).

Results: We analyzed data from approximately 1,000 samples/year tested by the government; the pathogenic bacteria positive ratio was 10.6% (532/4,997). Among *Bacillus cereus, Staphylococcus aureus,* pathogenic *E. coli, Vibrio parahaemolyticus,* and *Salmonella*, the positive samples were 345, 161, 83, 21 and 15, respectively. The pathogenic bacteria positive ratio of CAS-compliant samples was 1.1% (4/359); only 4 samples, 2 rice ball (3 & 4 MPN/g) and 2 glutinous oil rice (4 & 11 MPN/g), were *Bacillus cereus* positive. No other pathogens were found. The results showed *Bacillus cereus* was detected more frequently than other foodborne pathogens in all samples. The pathogenic bacteria were identified on CAS-compliant RTE samples collected from factories.

Significance: Evaluations of samples collected from factories indicate CAS-compliant RTE products are of high quality and present low microbial hazard risk. The 4 samples identified as contaminated with *Bacillus cereus*, though in low amounts, may contain emetic toxin-producing strains. CAS-compliant RTE data from products in the market remains unknown; these products may pose potential risk.

P2-242 Toxicology and Risk Assessment of Chemical Mixtures

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Introduction: Evaluating potential health risks posed by exposures to multiple chemicals is challenging for toxicology research and risk assessment (RA). Problem formulation is complex and mixtures RA frameworks vary greatly among agencies.

Purpose: This study 1) describes RA approaches for chemical mixtures; 2) characterizes differences in RA frameworks; and 3) assesses regulatory acceptance of these frameworks with a focus on food mixtures.

Methods: Reviews were completed for mixture RA paradigms used by US federal agencies, nonprofit organizations (WHO, IPCS) and international agencies (EU). There are significant overlaps and differences between paradigms and there appears to be no single unified approach. Considering the current challenges in food mixtures, a hypothetical case study was conducted using a tiered screening approach and hazard index method to evaluate the effects of mixtures in foods. A hypothetical new bean product is being considered to replace pinto beans for a food program. Component levels of the following differ between old beans (OB) and new beans (NB) (assuming a 10% decrease): cadmium, deltamethrin, cyfluthrin; and bisphenol A levels are the same in OB and NB. Non-cancer health effects following chronic oral exposure were evaluated.

Results: First tier provided a crude filter using the most recent and conservative health reference values for mixture components. Some assumptions in the first tier were refined in the second tier. Components were grouped based on health effects and biomonitoring data was used to estimate exposure in the second tier.

Significance: Results indicate that tiered approach is a useful way of rapidly screening the effects of chemical mixtures.

P2-243 Geospatially Explicit Synthetic Poultry Data: Filling in Data Gaps with Synthetic Populations

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Introduction: Agent-based models are an important tool for analyzing spread of animal infectious disease outbreaks and evaluating potential control interventions. However, geospatial information – often unavailable in the public domain – is needed to implement these models. Synthetic populations are an increasingly powerful source of data that can fill this data gap.

Purpose: A synthetic poultry farm database was created for use in infectious disease models and evaluated for accuracy. Results for the synthetic poultry farm database are described here.

Methods: The database was developed using Census of Agriculture county data along with spatial data on landuse/landcover, transportation, elevation/slope, and hydrography to generate farm probability maps and to create spatial features representing farms with appropriate characteristics such as farm type and size. Farm locations are generated in places where the suitability layer indicates areas of high probability for a poultry farm. Counts, sizes, and types are derived from the Census of Agriculture counts by county.

Results: The U.S. synthetic poultry database contains 145,903 commercial poultry farms. The dataset is geospatial, containing an estimated latitude/longitude coordinate for each farm as well as farm characteristics. Locations of the synthetic farm suitability layer (i.e., places deemed to be suitable locations for poultry farms) were compared to random placement of farms in suitable places using an agreement matrix statistical methodology. Total accuracy is 78.8 (95% CI: 74.8, 82.2), suggesting that the synthetic location model does a good job of matching likely poultry farm locations.

Significance: Realistic, geospatially explicit, statistically accurate synthetic data provide a source of open, readily available, de-identified data for use in a wide variety analyses relevant to food safety. These datasets are more reliable than those created using random placement.

P2-244 Global Burden of Disease Initiative: Food Source Attribution Estimates for 14 WHO Subregions

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Introduction: The WHO Global Burden of Foodborne Disease Initiative provides the first worldwide estimates of incidence and burden of foodborne disease and attributes this burden to specific food exposures. These estimates provide a stronger information foundation for enhancing the effectiveness of efforts to reduce foodborne disease around the globe.

Purpose: Results on WHO regional incidence of foodborne illnesses will be presented in a symposium on the GBFD Initiative. This presentation presents estimates of the food sources of illnesses for all WHO subregions.

Methods: The WHO initiative used expert judgments to develop source attribution estimates where other data and research were inadequate. An expert elicitation using Cooke's Classical Model was designed to attribute overall exposure to different major pathways, including food, and to attribute foodborne exposure to specific classes of foods. A fundamental methodological challenge in such elicitations is assessing whether subject experts can provide dependable and meaningful uncertainty judgments. Cooke's method addresses this challenge by using responses to a set of calibration questions to optimize the accuracy and informativeness of aggregate estimates across experts.

Results: This elicitation provides food source attribution estimates for 11 major pathogens for each of 14 WHO subregions. For each pathogen, the study estimates the percentage of foodborne illness in the subregion due to exposure via specific food categories. This presentation presents food source attribution results by WHO subregion and comparisons across WHO subregions. Source attribution estimates are expressed as median values and 90% credible intervals.

Significance: New WHO food source attribution estimates strengthen understanding of the causes and importance of foodborne diseases around the world. This understanding will enhance capacity both to improve public health in low income countries and the ability to assure the safety of food traded internationally.

P2-245 Assessment of the Dose-Response Relationship of *Toxoplasma gondii* Infection in Mice Experimentally Infected with Type II Bradyzoites

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❖ ***Developing Scientist Competitor***

Introduction: *Toxoplasma gondii* is a protozoan parasite that is responsible for approximately 24% of all estimated deaths attributed to foodborne pathogens in the United States. Substantial proportions of human *T. gondii* infection are acquired through consumption of raw or undercooked meat. Currently the response of human population to exposures to *T. gondii* contaminated meat is not clear.

Purpose: Since no human data is available, the goal of this study was to select the optimal animal study and develop a dose-response relationship to predict the infectivity to humans of ingestion of *T. gondii* in the meat.

Methods: Relevant studies in literature were searched in the database and eligible studies should meet two criteria: i) contain ≥ 3 different doses to challenge the intermediate hosts, ii) at least 5 animals were challenged in each dose. Optimal study was selected based on the animal species, stage and genotype of *T. gondii* used in the experiment, and route of administration. Data from the optimal study was pooled and fitted into 4 common sigmoidal-shaped mathematic models (Exponential, Beta-poisson, Weibull and Logistic) in OriginPro 9.

Results: A mice experiment of infection with type II bradyzoites was selected to predict the dose-response curve for the following reasons: i) type II is a predominate strain in the United States, ii) bradyzoites stage is presented in the meat, and iii) oral administration in mice mimic the route of meat consumption in humans. Among 4 developed models, a Beta-poisson dose-response function ($P(d) = 1-(1+d/189.484)^{-0.737}$) was selected based on its simplicity, popularity as well as the best fit ($R^2 = 0.993$).

Significance: This study developed a dose-response model that predict the human response of consuming *T. gondii* contaminated meats, and could be used as an important input in a quantitative risk assessment to further estimate the public health burden.

P2-246 Effects of Cultured Sugar/Vinegar Blend and Nisin with Modified Atmosphere Packaging on the Growth Kinetics of *Listeria monocytogenes* in Ravioli at Various Temperatures

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Introduction: As the risk of *L. monocytogenes* was well-known, esp. at RTE ravioli, the control of *L. monocytogenes* was highly needed with natural antimicrobial agents such as cultured sugar/vinegar (CSV) blend and nisin.

Purpose: To control the risk of *L. monocytogenes* at RTE ravioli, the antimicrobial effects of CSV blend and nisin were investigated.

Methods: Ravioli dough was prepared with 0, 0.1, 0.3, 0.5, and 1% CSV blend and 0, 0.1, 0.2, and 0.3% nisin. Spinach filled raviolis were inoculated with *L. monocytogenes* for a target population of approximately 2.0 ± 0.5 log CFU/g. Inoculated raviolis were modified atmosphere packaged (MAP) and stored at 4, 10, 17, and 24°C. At selected times after inoculation, the diluted samples were plated onto PALCAM agar in duplicate, and incubated at 35°C for 48 h. The colonies on duplicated plates of each sample were counted with an automated colony counter.

Results: Growth kinetic parameters of the observed data were fit well to a modified Gompertz equation ($R^2 > 0.95$). Generally, lag times (LTs) of *L. monocytogenes* on spinach raviolis under MAP was longer than those of *L. monocytogenes* under aerobic-packaged raviolis, regardless of the storage temperature. At the temperatures below 10°C with MAP, the growth of *L. monocytogenes* was significantly inhibited in raviolis which made with 1% CSV blend or 0.3% nisin during 60 days of storage. The growth rate of *L. monocytogenes* in raviolis with nisin was slower than that with CSV blend.

Significance: The addition of 1% CSV blend or 0.3% nisin in spinach ravioli with combination of MAP effectively controlled the growth of *L. monocytogenes* at refrigerated temperature. Thus, very low level of the CSV blend can be applied in manufacturing of natural RTE ravioli to improve the microbial safety and quality of the products.

P2-247 Development of a Predictive Model for *Vibrio parahaemolyticus* in Raw White Shrimps

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Introduction: *Vibrio parahaemolyticus* is a halophilic bacterium that occurs naturally in estuarine environments and transmitted to humans primarily through the consumption of contaminated raw or undercooked seafood. This pathogen is a major causative agent of foodborne infection in Taiwan. However, information is limited about the growth and survival of naturally-occurring *V. parahaemolyticus* in raw shrimps under commercially relevant storage conditions in Taiwan.

Purpose: This study aimed to develop a mathematical model to describe the effect of storage temperature on the growth rate of naturally-occurring *V. parahaemolyticus* in raw white shrimps harvested in Taiwan.

Methods: Raw shrimps were placed in sterile containers and stored at 5, 10, 15, 20, 25, 30, and 35°C. At each sampling time, shrimps were analyzed for *V. parahaemolyticus* counts by a plate count method. The populations of *V. parahaemolyticus* in shrimps during storage were used to estimate the growth rates and correlated to the storage temperature to develop a mathematical model.

Results: The growth of *V. parahaemolyticus* in shrimps at 5 and 10°C were not observed. The average growth rates of *V. parahaemolyticus* at 15, 20, 25, 30, and 35°C were 0.021, 0.054, 0.101, 0.297, and 0.60 log CFU/h, respectively. In general, the observed growth rates were higher ($P < 0.05$) than the predicted rates from post-harvest shrimps described in the literature. A square-root model was developed for the growth rates of *V. parahaemolyticus* in shrimps as a function of storage temperature.

Significance: The findings may assist the seafood industry and risk managers in designing more effective temperature control systems for seafood production and distribution to reduce the risk associated with *V. parahaemolyticus*.

P2-248 Growth/No Growth Model to Predict *L. monocytogenes* Growth Response at Low Concentrations of Sodium Nitrite and Sodium Chloride

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Introduction: Recently, processed meat products formulated with low concentrations of sodium nitrite and sodium chloride have been produced because of health concern, but low concentrations of the additives may allow *Listeria monocytogenes* growth in the products.

Purpose: The objective of this study was to develop growth/no growth (G/NG) models to predict *L. monocytogenes* growth responses in processed meat products formulated with low concentrations of sodium nitrite and sodium chloride.

Methods: A five-strain mixture of *L. monocytogenes* was inoculated in NBYE (nutrient broth plus 0.6% yeast extract) supplemented with sodium chloride (0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, and 1.75%) and sodium nitrite (0, 15, 30, 45, 60, 75, 90, 105, 120, and 141 ppm). The inoculated samples were stored aerobically and anaerobically at 4, 7, 10, 12, and 15°C for up to 1,680 h. Growth (G; value of 1) or no growth (NG; value of 0) for each combination was determined by turbidity for each storage temperature. The growth response data were analyzed by a logistic regression to predict the growth probability of *L. monocytogenes* as a function of sodium nitrite and sodium chloride. To validate the model performance, the predicted growth response from developed G/NG models were compared to the observed growth responses from frankfurters.

Results: Although a single application of sodium nitrite or sodium chloride significantly ($P < 0.05$) suppressed *L. monocytogenes* growth at 4 - 15°C, the combination of sodium nitrite and sodium chloride more effectively ($P < 0.05$) inhibited *L. monocytogenes* growth than single application of sodium nitrite or sodium chloride under both aerobic and anaerobic storage condition. Validation of the developed G/NG models with frankfurters showed 92% of agreement between predicted and observed growth responses.

Significance: The probabilistic models developed should be useful in determining appropriate concentrations of sodium nitrite and sodium chloride at low concentrations to inhibit *L. monocytogenes* growth in processed meat products.

P2-249 NaCl Influences Cell Morphology and Gene Expression of *Escherichia coli* O157:H7 and Non-O157 Strains

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❖ ***Developing Scientist Competitor***

Introduction: NaCl has been formulated in various foods as a flavor enhancer, and it is known that NaCl may influence the physiological and genetic responses of bacteria in foods.

Purpose: Therefore, the purpose of this study was to elucidate the effect of NaCl on cell morphology and gene expression of *Escherichia coli* O157:H7 and non-O157 strains.

Methods: *E. coli* O157:H7 NCCP11142 and non-O157 strains (*E. coli* O111 ATCC12795 and *E. coli* O26 ATCC43887) were exposed to 0%, 2%, and 4% NaCl at 35°C until bacterial populations reached stationary phase, and cell pellets from the culture were used to analyze gene expression levels of osmotic stress-related genes (*betA*, *ompC*, *proV*, and *proW*) and heat stress-related genes (*clpB*, *dksA*, *dnaJ*, *dnaK*, *grpE*, *msbB*, and *rpoE*) by quantitative real-time (qRT)-PCR. In addition, *E. coli* O157:H7 and non-O157 strains were attached on cover glasses in tryptic soy broth at 35°C for 24 h. The attached *E. coli* cells on the cover glasses were exposed to 0%, 2%, and 4% NaCl for 1 h, and cell morphology of *E. coli* strains was then observed under Field Emission Scanning Electron Microscopy (SEM).

Results: Of 11 tested genes, the levels of *proV* and *proW* (osmotic stress-related gene), and *rpoE* gene (heat stress-related gene) expressions were significantly increased ($P < 0.05$), as NaCl concentration increased, regardless of *E. coli* strain. The expression levels of *proV* were higher ($P < 0.05$) in *E. coli* O157:H7 than in non-O157 strains. In addition, SEM images showed that size of *E. coli* O157:H7 cells became smaller after exposure to NaCl, but cell sizes of non-O157 strains were not influenced by NaCl.

Significance: These results indicate that NaCl may affect stress-related gene expressions of *E. coli* O157:H7 and non-O157 strains and decrease the cell size of *E. coli* O157:H7.

P2-250 Effect of L-Monosodium Glutamate and Disodium 5'-Inosinate on Antibiotic Resistance of Foodborne Pathogens

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❖ ***Developing Scientist Competitor***

Introduction: L- monosodium glutamate (MSG) and disodium 5'-inosinate (DSI) have been used in various processed foods, and they may increase stress resistance of bacteria.

Purpose: This study elucidated the effect of MSG and DSI on antibiotic resistance of *Listeria monocytogenes*, *Escherichia coli*, and *Staphylococcus aureus*.

Methods: *L. monocytogenes* (NCCP10805, NCCP10806, NCCP10807, NCCP10808, NCCP10809, NCCP10810, NCCP10811, NCCP10920, and NCCP10943), *E. coli* (NCCP10439, NCCP14037, NCCP14038, NCCP14039, and NCCP15661), and *S. aureus* (ATCC13565, ATCC14458, ATCC23235, ATCC27664, and NCCP10826) were subjected to tryptic soy broth formulated with 0, 0.3, and 1.0% MSG and DSI at 35°C for 24 h, and 0.1 ml aliquots of each culture were subcultured in same media at 35°C for 24 h. Subculture was repeated ten times. Bacterial cells (OD_{600} = 0.2) were spread-

plated on Mueller Hinton agar. Ten antibiotic disks (ampicillin, bacitracin, penicillin G, tigecycline, rifampicin, streptomycin, vancomycin, gentamicin, erythromycin, and lincomycin) for *L. monocytogenes* and *S. aureus*, and ten disks (tigecycline, rifampicin, streptomycin, gentamicin, florfenicol, neomycin, tetracycline, amoxicillin, ceftiofur, chloramphenicol) for *E. coli* were placed on the media, followed by incubation at 30°C (*L. monocytogenes*) and 35°C (*S. aureus* and *E. coli*) for 24 h. Clear zones were measured to evaluate antibiotic susceptibility.

Results: Clear zones of *S. aureus* and *E. coli* strains by MSG and DSI were not decreased. However, clear zone sizes of *L. monocytogenes* decreased ($P < 0.05$) from 34 to 14 mm (NCCP10811), from 28 to 18 mm (NCCP10920), and from 36 to 14 mm (NCCP10943) against penicillin G (10 µg) after exposure to 1.0% MSG. Exposure of *L. monocytogenes* to 1.0% MSG decreased ($P < 0.05$) clear zone sizes on vancomycin (30 µg) from 25 to 21 mm (NCCP10920) and from 25 to 20 mm (NCCP10943). DSI did not affect antibiotic susceptibility of *L. monocytogenes*.

Significance: These results indicate that MSG may increase the antibiotic susceptibility of *L. monocytogenes*, depending on strain and antibiotic.

P2-251 G/NG Model to Predict Minimum Concentrations of NaCl and $NaNO_2$ to Inhibit *S. aureus* Growth in Low-Sodium Conditions

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Introduction: Although *Staphylococcus aureus* can grow in low-sodium processed meats, minimum concentrations of NaCl and $NaNO_2$ have not been determined to inhibit *S. aureus* growth in the products.

Purpose: The objective of this study was to develop growth/no growth (G/NG) model to predict minimum concentrations of NaCl and $NaNO_2$ to control *S. aureus* in low-sodium conditions.

Methods: A mixture of *S. aureus* strains NCCP10826, ATCC13565, ATCC14458, ATCC23235 and ATCC27664 was inoculated in nutrient broth formulated with NaCl (0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, and 1.75%) and $NaNO_2$ (0, 15, 30, 45, 60, 75, 90, 105, and 120 ppm). The plates were then incubated at 4, 7, 10, 12, and 15°C for up to 80 days under aerobic and anaerobic conditions. Growth responses [growth (*1*) or no growth (*0*)] were then determined every 24 h by turbidity. The growth response data were analyzed to select significant parameters ($P< 0.05$) by a stepwise selection method, which were used to calculate minimum concentrations of NaCl and $NaNO_2$ to inhibit *S. aureus* growth. The performance of developed G/NG models were eventually validated with observed growth responses.

Results: Under anaerobic storage, *S. aureus* growth was not observed. However, *S. aureus* growth was observed only at 10 - 15°C in aerobic storage. For single application of $NaNO_2$, *S. aureus* growth increased at higher $NaNO_2$ concentration at 10 - 15°C. Minimum concentrations of NaCl in combination of $NaNO_2$ decreased ($P < 0.05$) to inhibit *S. aureus* growth at 10 - 15°C, as $NaNO_2$ concentration increased. A concordance percentage between observed and predicted growth responses was about 88%.

Significance: This result indicates that the developed models should be useful in calculating minimum concentrations of NaCl and $NaNO_2$ combinations to inhibit *S. aureus* growth in processed meat products.

P2-252 Predictive Models and Software for *Listeria monocytogenes* in Ready-to-Eat Meats with Varying Antimicrobial Use

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Introduction: Antimicrobials are used to control the growth of microorganisms in foods under different product formulations, however assessing the safety of a new formulation requires extensive challenge testing which is time consuming and costly. To address this issue, predictive models derived from experimental data can be used to estimate microbial growth.

Purpose: To develop a predictive model and software application that will estimate the growth of *Listeria monocytogenes* in Ready-to-Eat meats with different formulations and anti-microbial concentrations.

Methods: 18 experimental data sets describing microbial growth with different levels of moisture, NaCl, pH, and the antimicrobial e(Lm)inate LAD were used to develop the model, including controls. In experiments where growth was observed the Baranyi Roberts model was used, and a linear model was used where inactivation due to the use of antimicrobial was observed. Secondary modelling to examine the influence of formulation parameters involved the use of Locally Weighted Polynomial Regression (LOESS), and the final results were integrated into a web-based software application.

Results: The LOESS method enabled the growth rate and lag time to be modelled simultaneously as functions of moisture, NaCl, pH and e(Lm) inate LAD concentration. Percentage accuracy and bias factors to assess model performance ranged from 0.01 - 4% for the growth rate and lag time, indicating good agreement with experimental data. The full range of experimental conditions was used as options in the final software application.

Significance: The work demonstrates the utility of predictive microbiology specifically for antimicrobial use in foods. By housing the model in a web-based software application, a food manufacturer can quickly assess the impact of different product formulations before carrying out any experimental work, saving on time and money and increasing product safety.

P2-253 Growth Kinetics of *Listeria monocytogenes* in Chopped Produce Using Predictive Modeling

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Introduction: The FDA Food Code defines potentially hazardous food as requiring time/temperature control for safety (TCS) to limit growth of pathogens, such as *Listeria monocytogenes*, and covers the storage and handling practices for fresh produce including leafy greens, tomatoes, and cut melons. However, no recommendations have been made for other chopped produce items.

Purpose: To study the growth kinetics of three *L. monocytogenes* foodborne outbreak strains on chopped produce using predictive modeling.

Methods: Mushroom, broccoli, onion, green and black olives, celery, cantaloupe, and avocado were chopped and inoculated with ca. 10^3 - 10^4 CFU/g of three antibiotic resistant *L. monocytogenes* strains (1/2a, 1/2b, 4b) and stored at 5°C for 14 days, 10°C for 12 days, or 25°C for 6 days. At different time intervals, samples were stomached and plated onto PCA with appropriate antibiotics for enumeration. Growth kinetics were analyzed using Prism GraphPad and modeled using DMFit. A *P*-value of less than 0.05 was considered significant.

Results: The growth rates ((log CFU/g)/h) of *L. monocytogenes* in all produce items at 25°C were significantly higher than at 5°C and 10°C. Using the Baranyi/Roberts growth model, the overall highest rates were seen for serotype LS-(1/2a) at 5°C (0.023 ± 0.007 in avocado), 10°C (0.067 ± 0.004 in cantaloupe), and 25°C (0.179 ± 0.008 in avocado). The longest lag phases (h) were observed at 5°C by LS-(1/2b) in celery (308.2 ± 65.7) and LS-(1/2a) in

onion (311.6 ± 2.1). The highest overall maximum populations (log CFU/g) were attained by LS-(1/2a) in cantaloupe at 5°C (7.92 ± 0.011), 10°C (9.16 ± 0.09), and 25°C (8.79 ± 0.08).

Significance: The modeling results obtained can be used to provide guidance to the retail food industry, will help educate consumers on safe handling practices for cut produce, and will aid in determining whether TCS designation should be applied to other produce items.

P2-254 Development of Survival, Growth, and Death Models for *Salmonella* during Non-isothermal Time-temperature Profiles in Leafy Green Supply Chain

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❖ ***Developing Scientist Competitor***

Introduction: *Salmonella* is a pathogen of concern for leafy greens contamination, and has been attributed to two leafy green related outbreaks and over 100 illnesses in the US and Canada in the last six years. Most recently, organic baby spinach was recalled by several U.S. spinach producers in 2012 after safety regulators found the presence of *Salmonella*.

Purpose: The objective of this study was to develop a survival, growth, and death model for *Salmonella* in leafy greens in order to predict the growth and decline behaviors of the pathogen under non-isothermal time-temperature profiles.

Methods: Experimental data for the growth of *Salmonella* under different conditions and storage temperatures were retrieved from published studies, followed by development of three-phase linear model as primary growth model and square-root model as secondary model to calculate growth rate (μ) at different temperatures. Death data was also collected for storage temperature below 5°C. A total of 35 growth and 18 death curves were available from 16 published studies.

Results: The square-root model was fitted as $\mu = (0.20*(\text{Temperature}+0.57))^2$. Mean death rate at the temperature below 5°C was 0.014 log CFU/h. These models were validated using several dynamic time-temperature profiles for the supply chain of leafy greens. Variability was taken into consideration by calculating 95% confidence intervals. Based on statistical parameters, such as accuracy factor and bias factor, and acceptable prediction zone (APZ) analysis, the model predictions were found to be within acceptable range.

Significance: The results from this study will be useful for future microbial risk analyses and predictions of behavior of *Salmonella* in leafy green supply chain.

Poster

P2-255 Modeling the Effect of Storage Temperature on the Growth, Survival, and Inactivation of *Vibrio cholerae* Pacini and Total Viable Bacteria in Post-harvested Pacific White Shrimps *(Litopenaeus vannamei* Boone)

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Introduction: *Vibrio cholerae* are natural flora of marine environment. They are commonly associated with seafood and may reach levels that cause human disease when postharvest temperatures are not properly controlled and are consumed raw or undercooked.

Purpose: Predictive models were generated by inoculating pacific white shrimps with *V. cholerae*, measuring viability rates at storage temperatures from 0 to 35°C, and fitting the data to a model to estimate growth parameters.

Methods: The models were evaluated with pacific white shrimps containing natural populations of *V. cholerae.* Viable *V. cholerae* was measured by pour plate method on thiosulfate-citrate-bile salts-sucrose agar for both inoculated and naturally contaminated shrimp samples. In parallel, total viable bacterial counts were measured by pour plate method on plate count agar.

Results: *G*rowth/inactivation rates for *V. cholerae* were -0.008, -0.005, -0.002, 0.065, 0.125, and 0.252 log CFU/h at 0, 5, 10, 20, 30, and 35°C, respectively. The growth/inactivation rates for total viable bacteria were 0.007, 0.011, 0.024, 0.078, 0.130, and 0.322 log CFU/h at 0, 5, 10, 20, 30 and 35°C. Square root and Arrhenius models were generated for *V. cholerae* and total viable bacterial growth and inactivation kinetic data, respectively.

Significance: Evaluation studies showed that predictive growth for *V. cholerae* and total viable bacterial count were "fail-safe." The models can be used by aquaculture establishments and regulators in implementing management strategies to minimize *V. cholerae* risk and enhancing product quality in supply chains.

P2-256 Modeling Foodborne Pathogen Transfer and Exposure in the Household Environment

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Introduction: Bacterial pathogens can enter into households through food, people, pets and pet food, and environmental media such as air or water. Once in the house, it is not quantitatively known which factors and behaviors are most responsible for exposure as a result of bacterial pathogen spread, persistence, or growth. The role of pets as pathogen vehicles is also poorly understood. While numerous outbreaks have been traced back to human or pet food consumed in the household, actual routes of exposure beyond direct ingestion are unclear. Consequently, existing guidelines to reduce consumer risk are not based on quantitative estimates.

Purpose: This study aimed to: 1) model the spread of *Salmonella,* introduced via human or pet food, through household environments, and 2) assess exposure risk and the impact of mitigation strategies.

Methods: Data on *Salmonella* ecology on different foods and surfaces, and transfer by direct contact were derived from literature and experiments. A probabilistic Monte Carlo simulation model was developed to estimate *Salmonella* spread in the kitchen, living/dining room, bathroom, and on floors. Two main scenarios were considered: (a) a pet feeding or food preparation event, and short-term subsequent actions, (b) an entire day including multiple instances of pet feeding, food preparation, and interactions with household surfaces.

Results: Outcomes were expressed as *Salmonella* concentration on surfaces, and as doses associated with touching contaminated surfaces or ingesting cross-contaminated food. Results highlight that human food preparation and kitchen hygiene protocols are the actions most affecting pathogen spread and secondary exposure. Contamination entering the house via human food can lead to exposure up to 3 log CFU higher than equal contamination levels in pet food. Handwashing and avoiding conditions favorable to *Salmonella* growth during meal preparation can effectively mitigate risk.

Significance: This model provides a tool for communicating risk and developing risk-based hygiene guidelines.

P2-257 A Stochastic Single Cell Survival Model of *Escherichia coli* and *Salmonella enterica* in a Desiccation Environment

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Introduction: A very small number of viable *Salmonella enterica* and pathogenic *Escherichia coli* cells are required for these pathogens to infect humans. The behavior and/or survival probability of a small number (in particular a single cell) of these pathogenic bacteria can be used to assess their risk of causing foodborne illness. We focused on low water activity (a_w) and/or dried foods as critical pathogen carriers.

Purpose: We developed a predictive model that can be used to estimate the probability of the survival of a single cell as well as between serotypes.

Methods: The survival of the *Salmonella enterica* serotypes Stanley, Typhimurium, Chester and Oranienburg as well as *E. coli* O111, O26 and O157 were assessed. Single cells of each type were placed in microdroplets (2 µl) of distilled water on a 96-well microplate and exposed to temperatures ranging from 5°C to 25°C. The single cells of each pathogenic bacteria were prepared via a 10-fold dilution of the bacterial culture. The prepared microplates were then dried in a drying chamber (ca. 9% RH). We confirmed the survival of the cells in each well by adding nutrient broth (100 µl) at arbitrary intervals to promote cell recovery.

Results: The number of cells in each well followed a Poisson distribution. The mean number of cells was two. Survival/death data obtained using logistic regression as a function of time and temperature indicated that a higher drying temperature reduced cell survival. The changes in survival probability showed that more than 90% of the cells died during the drying process; however, a small fraction survived.

Significance: This predictive model for estimating the survival probability of a single cell after drying could play an important role in assessing the risk of low aw and/or dried foods.

P2-258 Quantifying and Modeling the Influence of Weather Conditions on the Survival of *Escherichia coli* on Oranges Following Application of Low Microbial Quality Water

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Introduction: The microbial quality of water used in agricultural applications can affect fresh produce safety. The influence of weather conditions on microbes deposited onto the surface fresh produce by water is poorly characterized.

Purpose: This study quantifies and models the effect of key weather attributes on the fate of *E. coli* introduced onto the surface of grove oranges through foliar spraying.

Methods: Orange trees were sprayed with low microbial quality water (~10^6 CFU *E. coli* /ml) in 19 separate monthly trials over 3 harvest seasons. Three replicates of 10 oranges each were harvested from each of three trees at appropriate intervals until *E. coli* could not be detected by enrichment. *E. coli* were enumerated by plate count and most probable number techniques. Solar radiation (SR), temperature, relative humidity (RH) and rainfall data were also obtained from the Florida automated weather network and plotted against log change in *E. coli* concentration between sampling points for each month.

Results: *E. coli* populations declined more rapidly during dry weather compared to humid weather. Change in RH, SR, rainfall, temperature and time interval between sampling points did not show strong linear correlations with log change in *E. coli* between sampling points ($R^2 < 0.4$). However, SR and temperature were significantly ($P < 0.001$) negatively correlated with log change in *E. coli*/h and RH was significantly ($P < 0.001$) positively correlated with log change in *E. coli*/h. Logistic regression analysis showed that log reductions in *E. coli* concentration were higher and more likely to occur due to dry weather (high SR and low RH) compared to log increases due to wet weather (low SR and high RH).

Significance: The assumptions in the FDA produce rule regarding a 0.5 log CFU/day reduction of *E. coli* on the surface of fruit may be an oversimplification.

P2-259 Thermal Inactivation of Non-O157 Shiga Toxin-Producing *Escherichia coli* in Laboratory Media

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Introduction: In recent times, a total of 46 outbreaks of non-O157 Shiga Toxin-producing *Escherichia coli* (STEC) have been reported. It has caused 1,727 illnesses and 144 hospitalizations in the United States as a result of which the Food Safety and Inspection services (FSIS) has declared non-O157 as an adulterant in ground or cooked beef and enforced a zero tolerance policy.

Purpose: The objective of this research was to determine thermal inactivation of non-O157 STECs in order to develop and validate mathematical models that can accurately predict elimination of these pathogens from ground beef containing varying fat levels and salt contents.

Methods: Decimal reduction time (D-values) for non-O157 strains were compared to O157:H7 strain of STEC in Tryptic Soy Broth (TSB) supplemented with 50-ppm nalidixic acid (NAL). Six strains of STECs; O26:H1, O45:H2, O103:H2, O111:H8, O121:H9, O145:Non-motile and O157:H7 (ATCC 43895) were used in this study. NAL resistance was developed in each strain and used for the experiments. D-values for O157 and non-O157 STEC strains were calculated at 55, 60, and 65°C.

Results: At 55°C, D-value of O157:H7 strain was not different ($P > 0.05$) from O111:H8 but it was lower ($P < 0.05$) than other non-O157 strains. Among the non-O157 group, O45:H2 had higher ($P < 0.05$) D-value than O26:H1 and O111:H8. At 60°C, there was no difference ($P > 0.05$) among the non-O157 group, while the D-value of O157:H7 was lower ($P < 0.05$) than all the non-O157 strains used in our study. Similarly, at 65°C no differences ($P > 0.05$) among the non-O157 strains were observed. D-values of O157:H7 were similar ($P > 0.05$) to that of O26:H1, O45:H2 and O145:Non-motile and lower ($P < 0.05$) than other strains of non-O157 group.

Significance: Results from this study will help the food industry in understanding and addressing problems associated with non-O157 STEC strains.

P2-260 Development of a Predictive Model for *Salmonella* in Beef Jerky Product with Temperature, pH, Potassium Sorbate and Water Activity as Controlling Factors

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Introduction: Humidity level is one of the important factors to inactivate *Salmonella* spp. during jerky processing. However, other conditions during the manufacture of beef jerky can also ensure microbiological safety of these products. Factors such as processing temperature (T), water activity (a_w), pH and chemical preservatives influence the survival of *Salmonella* spp. during jerky processing.

Purpose: The objective of this study was to develop a predictive model for the inactivation of *Salmonella* spp. in ground beef jerky, as a function of T, pH, potassium sorbate (PS), and final a_w.

Methods: Following a central composite design, ground beef was combined with PS (0 to 0.3%, w/w), adjusted from pH 5 - 7 and inoculated with a cocktail of 6 serotypes of *Salmonella* spp. Then, each combination was processed in an oven between 65 to 85°C until a final a_w ranging from 0.65 to 0.85 was achieved. Surviving *Salmonella* cells were enumerated on tryptic soy agar overlaid with xylose lysine deoxycholate agar (pre-tempered to 47°C) after petri dishes were incubated for 48 h at 30°C. Bacterial inactivation was quantified in terms of logarithmic reductions of *Salmonella* counts (log CFU/g) and inactivation rate (log (CFU/g)/h).

Results: The results indicated that pH, PS and T interacted to inactivate *Salmonella* in beef jerky. Decreasing pH of the meat significantly increased ($P < 0.05$) the efficacy of PS and T in reducing levels of *Salmonella* spp. Processing jerky at pH 5.5 with 0.25% PS, heated at 82°C achieved final a_w of 0.7 which resulted in a maximum *Salmonella* logarithmic reduction of 5.0 CFU/g and an inactivation rate of 1.3 log (CFU/g)/h.

Significance: The predictive model developed will be useful in designing effective drying processes for beef jerky under low humidity conditions and thereby, ensuring adequate degree of protection against risks associated with *Salmonella*.

P2-261 Correlation between Resistance to Ciprofloxacin and Roxarsone Found in *Campylobacter* Species

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Introduction: As bacteria develop resistance to many available antibiotic treatments, cross-resistance becomes a major concern. *Campylobacter* is known to possess a multidrug efflux pump that contributes to resistance of unrelated antibiotics. Some *Campylobacter* strains carry arsenic resistance genes that encode an efflux pump and a membrane permease, which could potentially act as alternate pathways for antibiotic resistance.

Purpose: To examine the prevalence of antibiotic and arsenic resistance in *Campylobacter* isolated from retail conventional and organic chicken and to determine any correlation between arsenic resistance and antibiotic resistance.

Methods: Sixty-five *Campylobacter* isolates from retail chickens, organic and conventional, were screened for resistance to arsenic and six antibiotics. Arsenic resistance was determined by minimum inhibitory concentrations for arsenate, arsenite, and roxarsone via agar dilution method and antibiotic resistance was determined using the disc diffusion method (following CLSI standard procedures).

Results: It was determined that all isolates were resistant to cephalothin and susceptible to gentamicin, erythromycin, and chloramphenicol. Isolates varied in resistance to tetracycline and ciprofloxacin, 34% and 26%, respectively, were resistant. Ciprofloxacin resistance was significantly correlated with increased roxarsone resistance ($P = 0.0022$), while tetracycline resistance was not significantly correlated with any arsenic resistance.

Significance: Cross-resistance is a major concern for the growing threat of antibiotic resistance. The correlation between ciprofloxacin resistance and roxarsone resistance could be an indication of a cross-resistance mechanism within *Campylobacter*.

P3-01 Whole Genome Sequencing Provides Rapid Traceback of Clinical to Food Sources during a Foodborne Outbreak of Salmonellosis

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Introduction: *Salmonella* serovar Bareilly is responsible for numerous outbreaks among humans. In 2012 a widespread foodborne outbreak associated with scraped tuna imported from India occurred in the United States.

Purpose: A comparative genomic analysis within the serovar was performed to explore, on a global scale, how effectively whole-genome sequencing (WGS) can differentiate outbreak isolates of *Salmonella* Bareilly from non-outbreak isolates sharing the same *Xba*I PFGE pattern.

Methods: We sequenced, on different platforms, 100 *Salmonella* Bareilly isolates including 41 isolates from the 2012 outbreak. A single isolate was sequenced on the Pacific Biosciences *RS* II Sequencer to determine the first complete genome sequence of *Salmonella* Bareilly that served as the reference genome. Subsequent raw reads were mapped to this reference genome to build a single-nucleotide polymorphism (SNP) matrix and construct a phylogenetic tree. Pathogen genomes were linked with geography by projecting the phylogeny on a virtual globe. Using the phylogenetic tree and the pathogen metadata a transmission network was reconstructed for *Salmonella* Bareilly.

Results: Using SNP analyses, we were able to distinguish and separate highly clonal *Salmonella* Bareilly strains sharing the same *Xba*I PFGE pattern. The isolates from the recent 2012 outbreak clustered together, sharing only a few SNPs differences between them. Our results revealed a common origin for the outbreak strains, indicating that the patients in the U.S. were infected from sources originating at the India facility. In addition, we identified a unique arsenic resistance operon carried by many of these strains.

Significance: Our data strongly suggests that WGS, combined with geographic mapping and the novel use of transmission networks for genetic data, vastly improves the rapid source tracking and surveillance of a bacterial outbreak that are critically important for characterizing outbreak dynamics and, ultimately, protection of the public health.

P3-02 Differential Gene Expression of *Lactobacillus animalis* NP51 at Different Temperatures

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Introduction: *Lactobacillus animalis* NP51 is a bacterium of interest in food industry because of its ability to inhibit foodborne pathogens and spoilage. The addition of this microorganism as probiotic to animal feed is generally recognized as safe (GRAS) for use in cattle. A consumption of 10^6 - 10^{10} CFU/g viable cells per day has been found to reduce pathogens in the live animal.

Purpose: This study aimed to analyze the amount of expression of various genes of *Lactobacillus animalis* NP51 at different temperatures 25°C (environment temperature), 39°C (host temperature-bovine), and 45°C (animal feed temperature).

Methods: RNA-Seq was performed to characterize differences in the transcriptome of *Lactobacillus animalis* NP51. The bacterial strain was grown overnight at 37°C for 18 h; overnight cultures were diluted into fresh medium and incubated at 25, 39, and 45°C until mid-logarithmic phase was reached. Total RNA extracted from two biological replicates of each temperature was rRNA depleted; individually bar-coded RNA-Seq libraries were prepared and sequenced on a MiSeq instrument. Raw data sets were assembled using *de novo* assembly. Gene expression was analyzed using DNAStar Array Star.

Results: To obtain the level of expression, host temperature was set up as control. By comparing 25°C to the control, 572 genes were identified as differentially expressed, 488 genes showed reduced expression while 84 increased expression. At 45°C, 54 genes were differentially expressed, 23 showed reduced expression and 31 showed increased expression. These include the downregulation of PTS gene expression, used for the uptake of carbohydrates; the late competence protein ComGA required for the competence-related block in chromosome replication, and the upregulation of the EmrE multidrug resistance protein.

Significance: This study provides important information about the transcriptional differences of *Lactobacillus animalis* NP51 at low and high temperatures providing the basis for the characterization of genes with a potential role in the inhibition of foodborne pathogens.

P3-03 Streamlining a DNA-based Biosensor Detection System for the Detection of Non-PCR Amplified Genomic Pathogenic DNA Targets in Food

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✦ ***Undergraduate Student Award Competitor***

Introduction: Our DNA-based biosensor, which utilizes gold nanoparticles (AuNPs) for signal amplification and magnetic nanoparticles (MNPs) for easy and clean separation from samples, has been shown to detect non-PCR amplified genomic DNA targets (DNAt) from bacterial pathogens. While this detection system can provide detection within an eight-hour window, we hypothesize it could be further streamlined to reduce the overall time to detection, as this would further reduce the overall cost of using this system.

Purpose: The purpose of this study is to reduce the amount of time it takes go from sample preparation to detection, from seven hours to four hours, without reducing the quality or sensitivity of detection.

Methods: Genomic DNA was extracted from *Salmonella* Enteritidis and *E. coli* cultures using either Trizol® or phenol/alcohol method. For extraction, all incubation/centrifugation periods were reduced by 50%, while controls used the standard protocols. For detection, all incubation periods of the biosensor were reduced by 50%, while the controls used the published method. DNA yields were calculated using a nanophotometer. DNAt (50 ng/ml) were detected by hybridizing the DNAt into a sandwich-like structure consisting of MNPs/DNAt/AuNPs, which were then placed onto screen-printed carbon electrodes to detect the voltammetric peaks of gold using differential pulse voltammetry. All samples were run in triplicate.

Results: DNA yields were similar ($P \geq 0.05$) between the normal and reduced incubation/centrifugation protocols. Equally, reducing the incubation periods of the biosensor system did not impact its ability to detect non-PCR amplified genomic DNAt compared to the established method ($P \geq 0.05$); peaks ranging from 2.5×10^{-5} A to 4.8×10^{-5} A were achieved using both methods.

Significance: These findings suggest that reducing the incubation periods within the DNA extraction method and biosensor system did not impact the overall sensitivity of this detection method, and that it can effectively detect pathogenic DNAt within a four-hour window.

P3-04 *Shigella* and Enteroinvasive *Escherichia coli* Phylogenomics and the Development of Markers for Rapid Detection

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Introduction: Bacillary dysentery, or Shigellosis, can be caused by closely related *Shigella* and enteroinvasive *Escherichia coli* (EIEC). These foodborne pathogens are human-specific with a similar mechanism of pathogenesis, which may be evolutionarily conserved.

Purpose: Improving the rapid identification of these pathogens is critical to outbreak investigation and response. Traditional methods involve labor-intensive biochemical and serological testing which are not always able to distinguish between *Shigella* and EIEC.

Methods: Phylogenetic analyses of 96 *Shigella* and EIEC isolates (capturing the diversity in the species), 18 *Escherichia* and 2 *Salmonella* isolates were conducted from whole genome sequence (WGS) data produced by an Illumina MiSeq or obtained from public databases. SNP analyses were used to construct a phylogenetic tree and to identify cluster specific genetic markers. Additional markers were obtained from clustering analysis of presence/absence of genes in annotated genomes.

Results: Based on 2,863 core SNPs, the *Shigella* and EIEC formed 10 phylogenetic clusters rendering those groups polyphyletic, which suggests that those bacteria have evolved independently multiple times and are closely related to each other and other pathogenic *E. coli*. Clustering of gene functions in metabolism, physiology and antibiotic resistance supports the SNP phylogeny. Multiple cluster specific genetic markers are presented here to assist rapid identification.

Significance: This comprehensive examination of *Shigella*, EIEC and other *E. coli* illustrates the very close relationships between these groups and underscores that the current *Shigella* nomenclature should be moved back to the *E. coli* group to reflect the phylogeny. In the meantime, the availability of markers will assist quicker and more discriminatory detection for improved food safety and public health.

P3-05 Sensitivity and Specificity of Conventional and Chromogenic Selective Agars for the Recovery of *Shigella* and Their Potential Growth Competitors

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Introduction: While most diagnostic tools rely today on molecular methods for pathogen detection and/or identification, regulatory agencies prefer to isolate the adulterant before taking action. Isolation of *Shigella* from food is challenging, partly because MacConkey agar (MAC), a low-selective-medium, is used in the FDA *Bacteriological Analytical Manual's* (BAM) method for this pathogen.

Purpose: To improve the method, we compared the performance of MAC with three more selective conventional agar media [Hektoen-Enteric (HEA), Xylose-Lysine-Desoxycholate (XLD) and *Salmonella-Shigella* (SSA)], and with three chromogenic media designed to enhance the differentiation of *Shigella* [Biolog *Shigella*/*Aeromonas* rainbow (RBW), R&F *Shigella* sp. (R&F) and Hardy diagnostic HardyCHROM SS (HSS)].

Methods: Eighty-one *Shigella* isolates, representing the diversity within the genus, were tested along with 26 non-*Shigella* strains as potential growth competitors of *Shigella.* We compared bacterial counts obtained on the non-selective tryptic soy agar and on the 8 selective agars, after 24 h incubation at 35°C. Colony size and color were also used in the performance comparison.

Results: Sensitivity, which is a measure of the agar's ability to recover and correctly identify *Shigella*, was highest on MAC agar followed by HEA, SSA, HSS and R&F. R&F recovered *S. sonnei* and most *S. flexneri* isolates but SSA was too inhibitory (< 10% recovery) for several *S. sonnei* isolates although it performed well for the other strains. All agars had good specificity (agar's ability to exclude *Shigella* correctly) except for MAC, which had a low specificity of 36%.

Significance: Adding SSA and R&F to MAC in the post-enrichment section of the BAM method for *Shigella* should improve our ability to recover and identify *Shigella* from contaminated foods. We will use R&F, MAC and SSA in future enrichment studies for this pathogen.

P3-06 Detection of Shiga Toxin-producing *Escherichia coli* on Fresh Produce Utilizing a Metagenomic Approach

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Introduction: Shiga Toxin-producing *Escherichia coli* (STEC) is a major cause of foodborne illness and has been associated with contaminated fresh produce. Assessing the detection sensitivity of STEC on fresh produce using a metagenomic approach will aid in developing a next-generation sequencing method to screen for contamination. Additionally, metagenomic analysis provides information about the overall microbial diversity on fresh produce and how the enrichment broth shifts the indigenous microbial population with the possible outcome of optimizing the broth to enhance detection of STEC with a reduction in enrichment time.

Purpose: The objective of this study was to evaluate a metagenomic approach to the detection and strain level identification of STEC on produce in both pre-enriched samples and enriched cultures of contaminated produce.

Methods: STEC-spiked bagged spinach samples and cultures from samples processed according to the BAM method were used to prepare sequencing libraries. Sequencing data from an Illumina MiSeq platform was analyzed using an in-house k-mer signature database to determine the microbial population. The sequencing reads were examined for STEC-specific molecular markers and, when STEC reads were present in sufficient abundance, the contaminating STEC genome was assembled from the complete sequencing data set.

Results: The indigenous bacterial species on unspiked spinach was determined as well as the shifts in the microbial population at several time points during enrichment for both spiked and unspiked samples. Even for low levels of STEC contamination (10 CFU/100 g spinach), it was not necessary to complete the overnight enrichment to determine the serotype, detect STEC-specific molecular markers, and assemble the STEC genome.

Significance: Utilizing a metagenomic approach may provide a more rapid method to detect and identify STEC contamination of fresh produce, thereby enhancing food safety and expediting response time during disease outbreaks.

P3-07 Application of an Array for Rapid Molecular Serotyping, Virulence Determination and Health Risk Assessment of Shiga Toxin-producing *Escherichia coli* Isolated from Foods

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Introduction: Consumption of foods contaminated with Shiga Toxin-producing *Escherichia coli* (STEC) have caused several outbreaks worldwide in the last few decades. There are several subtypes of Shiga Toxins but not all affect humans. Also, there are several hundred known STEC serotypes but many have not caused infections. Therefore, it is crucial to rapidly identify the pathogenic STEC strains that pose health risks.

Purpose: As part of the FDA's effort to ensure food safety, the FDA-ECID DNA microarray for molecular serotyping and virulence determination of pathogenic *E. coli* was evaluated for characterization of various STEC strains isolated from raw milk cheeses and fresh produce samples collected in the last half of 2014.

Methods: The FDA-ECID array has been designed as a rapid, affordable and high-throughput tool for molecular characterization of pathogenic *E. coli*. Molecular serotyping for the O and H antigens are based on the alleles from the *fliC*, *wzx*, and *wzy* genes that are represented on the array. For virulence determination probe sets are designed for the detection of multiple *eae*, *stx1* and *stx2* alleles, as well as other virulence factors.

Results: Thirty-two strains isolated from regulatory food samples were identified as STEC by preliminary PCR and microbiological testing. The array determined the serotype, Shiga Toxin type, and presence/absence of *eae* alleles of each STEC strain. One strain isolated from cheese was identified as potential pathogen of concern (O111:H8, *stx1*, *eae γ2*) and was recommended for recall. The molecular characteristics of the remaining thirty-one strains provided a basis for their regulatory exclusion.

Significance: The FDA-ECID microarray is a rapid and accurate tool for molecular serotyping and virulence assessment of STEC. Compared to conventional serological analysis that may take up to two weeks and often yields partial data, the FDA-ECID microarray accurately provided the results in two days.

P3-08 Multi-locus Genetic Characterization of *Cronobacter sakazakii* and Related Species

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Introduction: *Cronobacter sakazakii* is an opportunistic Gram negative human pathogenic bacterium known to cause fatal meningitis and necrotizing enterocolitis in infants and immunocompromised individuals. It can withstand severe dry conditions, and has been linked to powdered infant formula. Nevertheless, it has also been isolated from a wide variety of food and environmental samples. In this study, we have modified the PCR conditions and assessed the robustness of seven published multi-locus PCR protocols, and used for *Cronobacter* species identification.

Purpose: The major objective of this study was rapid species identification of *Cronobacter* species isolated from food and other sources by multi-locus sequence characterization.

Methods: In this study, a total of 185 *Cronobacter* isolates from food and other sources were isolated in our laboratory, initially by performing two-step enrichment followed by streaking on a selective agar. The recovered *Cronobacter* isolates were subsequently sequenced at seven different loci (*atp*D, *fus*A, *gln*S, *glt*B, *gyr*B, *inf*B, and *pps*) using ABI 3500 XL Genetic Analyzer**.** The sequencing data was analyzed using BioEdit and GENEIOUS programs.

Results: Multi-locus sequence characterization was done at seven loci including *atp*D, *fus*A, *gln*S, *glt*B, *gyr*B, *inf*B, and *pps* for the 185 isolates of *Cronobacter* recovered from food environment, surveillance, outbreak, sporadic cases and the ATCC reference cultures. The bi-directional DNA sequencing resulted high quality bases (> 98% HQ - 100% HQ) at all seven loci examined. Multiple alignments of the generated sequences revealed

inter- as well as intra-specific genetic polymorphism at all seven loci for all isolates of three *Cronobacter* species (including *C. dublinensis*, *C. muytjensii*, and *C. sakazakii*) sequenced.

Significance: The results suggest that multi-locus genetic characterization can be accomplished at seven different loci by modifying PCR conditions for the species-identification of *Cronobacter* isolated from food, outbreak, sporadic cases, environmental monitoring program, routine surveillance, and consumer complain of public health importance.

P3-09 Whole Genome Sequencing of Human-Pathogenic *Cyclospora cayetanensis* Parasites

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Introduction: *Cyclospora cayetanensis* is a human-pathogenic apicocomplexan parasite that infects the gastrointestinal track and causes acute gastrointestinal illness. In recent years, this protozoan parasite has led to several foodborne outbreaks in the United States and Canada associated with imported produce. To date, a large number of mammalian and avian species have been examined over the years for its presence. Nevertheless, humans are recognized as the host of this coccidian parasite. In our previous studies, we characterized the 70 kDa heat shock protein (HSP70) and the ribosomal RNA (rRNA) loci of *C. cayetanensis* for their rapid detection and understanding genetic diversity. In this study, we have performed the Whole Genome sequencing for twelve isolates of *C. cayetanensis* collected from three endemic regions.

Purpose: The major objective of this study was to obtain whole genome sequence of the *C. cayetanensis* parasite.

Methods: In this study, whole genome sequencing was performed on a total of twelve isolates of *C. cayetanensis* using Illumina MiSeq Next-Generation Sequencer in our laboratory. The data was analyzed using MiSeq, DNASTAR, and GENEIOUS software.

Results: Whole genome sequences were generated for twelve *C. cayetanensis* isolates from Nepal, Mexico and Peru using MiSeq Nextera XT DNA Sample Preparation Kit and MiSeq Reagent Kit V2, and *de novo* assembled into sequence contigs. Using MiSeq sequencer, whole genome sequencing was performed that resulted bases with high quality scores (Q ≥ 30: > 75% bases higher than Q30 at 2 x 250 bp), and with high base call accuracy. This novel tool can be used in understanding the transmission dynamics of *C. cayetanensis* parasite.

Significance: Our results suggest that the *C. cayetanensis* is a genetically distinct species. Whole genome sequencing can be used in understanding the species structure of this human-pathogenic parasite of public health importance.

P3-10 Optimization of Next Generation Sequencing Using the Illumina MiSeq for Detection of Human Norovirus from Stool Samples

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Introduction: Next generation sequencing (NGS) is a promising approach for detection and identification of norovirus (NoV), the leading cause of gastroenteritis in the U.S. This has been challenging by the relative low or gapped target mapping due to nucleic acid contamination, effect of sample preparation and biased viral genomic coverage.

Purpose: The purpose of this study was to optimize the sequencing protocol for NoV detection on the Illumina MiSeq platform.

Methods: Norovirus-containing stool samples were suspended in 10% phosphate-buffered saline (suspension), centrifuged at 9,000 x *g* for 3 min (supernatant), and the supernatant was filtered through a 0.22 µM membrane filter (filtrate). Viral RNA was isolated from each of these three different preparations (suspension, supernatant and filtrate) with three different kits available in our lab: QIAamp viral RNA kit, RNAqueous kit and RNeasy mini kit. NoV RNA was quantified with real time RT-PCR and libraries were generated with same amount of RNA for each sample with and without oligo(dT) selection. Sequencing was performed on MiSeq and Genomic Workbench was employed for the data analysis.

Results: (1) The viral RNA yield with the RNAqueous kit was significantly ($P < 0.05$, n = 3) lower than from the other two kits; the highest viral RNA yield was obtained with the QIAamp viral RNA kit. (2) The filtrate, supernatant and suspension of the same stool sample showed similar sequencing outcomes, according to the total reads number, viral reads number, and percentage of viral specific reads. (3) The same mapping pattern - with more reads mapping towards the 3'-end, was shown for all oligo(dT) selected samples, but not for non-oligo(dT) selected samples.

Significance: These results show the importance of sample preparation optimization, RNA isolation and library generation for NGS using Illumina MiSeq for detection of Norovirus from stool samples.

P3-11 A Custom DNA Tiling Microarray for Detection and Genotyping of Norovirus from Mixed Foodborne Virus Samples

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Introduction: The detection and identification of virus contaminants in food and environmental samples are essential for investigation and prevention of foodborne outbreaks. Norovirus (NoV) is one of the most important causative agents of foodborne gastroenteritis. Without a cell culture system, molecular methods such as microarray analysis have been applied to detect and genotype norovirus.

Purpose: The purpose of this study was to assess the effectiveness and reliability of a custom DNA tiling microarray for detecting and identifying norovirus from mixed common foodborne virus samples.

Methods: Hepatitis A virus (HAV) and coxsackievirus (CV) RNAs were extracted from virus-infected cell culture supernatants. A GII.4 NoV genome representing an exact match for a selected probeset on the array was commercially synthesized and cloned into an expression vector. NoV RNA transcripts were produced from in vitro transcription. Tiling Microarray was custom designed and manufactured by Affymetrix. Microarray analysis was performed following the modified Affymetrix GeneChip protocol.

Results: RNAs from three unrelated viruses, CV-B1, HAV-HM175/18f, and NoV-GII.4, were individually, or as a mixture, reverse transcribed into cDNA and hybridized to the microarray. Simultaneous detection of all three viruses was achievable at the input level of 10^4 copies/virus. Surprisingly, array signal intensities for a given virus was higher as a function of input when detected as a mixture rather than when examined individually. Importantly, the hybridization signal pattern specific for each virus was highly preserved when obtained as a mixture, and there was no noticeable cross-hybridization among the three viruses. As a result, successful detection and identification of three differing virus species was attained at lower than previously published input quantities.

Significance: We demonstrate the application of a tiling microarray for detection of norovirus in mixed foodborne virus samples. This method has the potential to address the ever increasing needs in surveillance and outbreak investigations.

P3-12 Molecular Serotyping of Shiga Toxin-producing *Escherichia coli* from Fresh Produce Enrichments Using FDA-ECID Microarray

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Introduction: Fresh produce contaminated with Shiga Toxin-producing *E. coli* (STEC) has been implicated in a number of foodborne outbreaks. There are over two hundred STEC serotypes, but not all cause human illness, therefore, it is important to accurately identify the serotype and virulence capacity of the STEC isolates.

Purpose: In this study, the efficacy and sensitivity of a custom design FDA-ECID microarray to accurately determine the serotype of STECs directly from produce enrichments was assessed. Currently used methods can take up to two weeks to first isolate a pure isolate from fresh produce and subsequently be analyzed by serology. The custom designed microarray is used to characterize all pathogenic *E. coli* including STECs through its molecular serotyping component and was used to detect STECs directly from enriched broths to minimize the time to a final result.

Methods: Produce was spiked with known CFU of *E. coli* O157:H7 following the BAM protocol. Total DNA was isolated from overnight enriched cultures and hybridized onto the FDA-ECID array. Molecular serotyping was performed using alleles from the *fliC*, *wzx*, and *wzy* genes that are represented on the array. For virulence determination different probe sets were used based on the *eae* alleles, *stx1* and *2* genes, and other virulence factors.

Results: *E. coli* O157:H7 was accurately typed using the FDA-ECID array when up to 50 - 100 CFU were present in the initial inoculum. Competition with the presence of *Salmonella* and *Listeria* when added with *E. coli* O157:H7 did not interfere with an accurate serotype determination.

Significance: Results show that the FDA-ECID array is an effective alternative to serology in serotyping environmental *E. coli* isolates. By minimizing the time to results from two weeks to two days from overnight enrichments can help to reduce the overall analysis time for food samples.

P3-13 Biomarker Identification from Next Generation Sequencing Data for Foodborne Pathogen Detection and Verification

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Introduction: Next-generation sequencing (NGS) technology has recently been widely applied in clinical and public health laboratory investigations for pathogen detection and surveillance. Major gaps currently exist in NGS data analysis and data interpretation. Topic modeling is an active research field in machine learning and has been mainly used as an analytical tool to structure large textual corpora for data mining.

Purpose: The purpose of this study is to implement topic modeling in NGS data analysis for biomarker identification in foodborne pathogen detection and verification.

Methods: A framework was developed to pursue data mining on NGS datasets by topic modeling. It consists of four major procedures: NGS data retrieval, preprocess, topic modeling, and data mining of the LDA topic outputs. The preprocessed NGS sequences were transformed into corpus, in which each document was reasonably viewed as "a bag of words assumption" which was essential for effectiveness of topic modeling approach.

Results: The NGS data set of 119 *Salmonella* isolates were retrieved from National Center for Biotechnology Information (NCBI) database and was used as an example in this work to show the working flow of this framework. The output topics by LDA algorithms could be treated as features of *Salmonella* strains to accurately describe the genetic diversity of *fliC* gene in various serotypes. The distinguished SNPs were identified by the following data mining methods as the potential biomarkers.

Significance: The implementation of topic modeling in NGS data analysis framework provides us a new way in NGS data analysis for elucidating genetic information and biomarker identification, therefore, enhance the NGS data analysis and its applications on pathogen identification, source tracking, and population genome evolution.

P3-14 Characteristics of Isolates of *Clostridium perfringens* from U.S. Foodborne Outbreaks

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Introduction: It is estimated that one in six Americans or 50 million people acquire a foodborne illness each year in the United States. Of these, *Clostridium perfringens* is estimated to be responsible for 1 million. This pathogen induces foodborne illness by expression of the *C. perfringens* enterotoxin gene, *cpe*.

Purpose: The purpose of this study was to evaluate the presence/absence of enterotoxin genes and genetic characteristics of *C. perfringens* recovered from foodborne outbreaks in the US.

Methods: We evaluated 90 isolates from 18 outbreaks representing 13 states and one cruise ship. Isolates were genotyped for the *cpe* gene by PCR and draft genome sequences from representative isolates were generated using the Ion Torrent PGM. Draft genomes were compared using reference-free SNP analysis.

Results: Of 90 isolates, 56 (62%) harbored the *cpe* gene indicating potential to cause diarrheal illness; however, 34 (38%) of the isolates did not carry the gene and were incorrectly identified as the causative agent of the outbreak. Based on SNP analysis, the genome sequences of 25 *cpe*-positive isolates shared more SNPs with each other than the genomes of 5 *cpe*-negative isolates; moreover, the sequences of *cpe*-positive and *cpe*-negative strains isolated in these outbreaks did not cluster together. Among the *cpe*-positive strains, those food and stool isolates from outbreak related strains clustered together.

Significance: Toxin-negative *C. perfringens* is commonly misattributed as the etiology of foodborne illness, and therefore we suggest that all isolates be screened for the *cpe* gene. Furthermore, genome sequencing of *C. perfringens* isolated from food and individuals with foodborne illness can be used to rapidly establish epidemiologic associations.

P3-15 Genotyping Human Norovirus and Hepatitis A Virus Recovered from Watershed Sites in a Major Leafy Vegetable Production Region in California

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Introduction: The foodborne viral pathogens, human noroviruses (NoV) and hepatitis A virus (HAV), are significant contributors of produce-associated outbreaks.

Purpose: To develop a rapid and simple genotyping tool for foodborne viruses, a focused, low-density DNA microarray was developed in conjunction with a rapid and high-throughput fluorescent method.

Methods: Probes (25-mer) were designed to target the NoV variable genomic region C at the 5'-end of ORF2, encoding the major capsid in both NoV genogroup I (GI) and genogroup II (GII). Additional capture probes were designed to target region 3, the VP1/P2A junction region at the end of the capsid region and beginning of the non-structural proteins in HAV genotypes IA and IB.

Results: Validation experiments indicated that this fluorescent array method accurately genotyped NoV GI and GII reference strains, resulting in high fluorescent signal values. To determine the assay sensitivity threshold, various amounts of in vitro cRNA transcript were tested on the array, and the results indicated that the use of microarrays has a potential detection limit of < 10 transcript copies for NoV GI and GII strains and < 100-1000 GII transcript copies of HAV strains. To determine the feasibility of using this array method for detecting foodborne viruses in environmental samples, water in lakes, streams, rivers and ponds that were part of watersheds adjacent to a leafy vegetable production region in the Salinas Valley along the Central California Coast were sampled. After conducting the genotyping analysis of over 100 water samples, NoV genotypes GI.2, GI.4, and HAV IB were the more commonly identified with a prevalence percentage above 50%. A lower prevalence below 40% was observed for GI.3a, G1.6, GII.1, GII.2, GII.4, GII.6, and GII.12.

Significance: Our findings led us to conclude that the use of low density microarrays in conjunction with a fluorescent signal amplification method enabled the accurate and rapid detection of NoV GI and GII strains as well as HAV from environmental samples.

P3-16 Microarray SNP Analysis as a Tool for *Escherichia coli* Subtyping

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Introduction: *Escherichia coli* is a highly diverse species with both pathogenic and non-pathogenic members, the former of which have been the focus of numerous food safety efforts. As a result, a wealth of genomic sequence data is now available for this organism. A rapid, cost-effective assay that makes use of this genomic data would be an invaluable tool in understanding the evolution and diversification of the species.

Purpose: We describe the use of a novel, high-density DNA microarray representing informative single nucleotide polymorphisms (SNPs) from *E. coli* mined from approximately 300 whole genome sequences. The custom FDA-ECID microarray has been designed and manufactured using next-generation Affymetrix PEG-GeneAtlas technology. This array is a rapid resequencing-based genomic tool for *E. coli* characterization and subtyping.

Methods: Three hundred whole genome sequences were used to identify ~125,000 conserved 25-mers each containing a central SNP. Of these, ~10,000 informative SNPs were selected for inclusion and are represented on our custom FDA-ECID microarray using a SNP-typing probe strategy.

Results: Using our optimized SNP-calling algorithms, we have analyzed data from a vast collection of temporally and geographically diverse *E. coli* isolates. The major phylogenetic lineages within the species were recapitulated using the array SNP data. In addition, the array data was compared to the same *in silico* SNPs from whole genome sequence (WGS) data as well as to a more comprehensive set of chromosomal backbone SNPs mined from the WGS archive. Comparisons of the microarray SNP data to the WGS data show greater than 95% similarity in classifying the isolates examined.

Significance: In summary, the FDA-ECID microarray is a powerful tool for molecular subtyping and phylogenetic analysis of *E. coli*.

P3-17 Early Recovery of *Salmonella enterica* from Cilantro Using Nonselective Preenrichment Broths

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Introduction: *Salmonella enterica* is a leading cause of foodborne illness in the United States. Current enrichment methods for *Salmonella* from produce require initial 24 hour incubation in a nonselective broth and additional 24 hour incubation in two selective media, Rappaport-Vassiliadis (RV) medium and Tetrathionate (TT) broth. Decreasing this time to detection would greatly impact traceback analysis efforts during outbreaks and routine surveillance.

Purpose: This study evaluates the recovery of two *S. enterica* serovars, Newport and Tennessee, after a reduced initial incubation period in nonselective broth with the goal of decreasing the total time of culture enrichment from 48 to 24 hours prior to selective plating.

Methods: Seven cilantro samples (50 g) were inoculated with *S. enterica* Newport or Tennessee (< 10 CFU/50 g) and processed according to the FDA Bacteriological Analytical Manual (BAM) with modifications. Five nonselective enrichment broths (modified buffered peptone water (mBPW), universal preenrichment broth (UP), lactose broth (LB), Davis minimal broth (DMB), and trypticase soy broth (TSB)) were evaluated. Five aliquots of each broth were inoculated into TT and RV after a static 37°C incubation ranging from three to seven hours, incubated at 42.5°C overnight, and streaked onto Xylose-Lysine-Tergitol 4 selective agar.

Results: Five-hour static initial nonselective enrichment was the earliest timepoint in which both serovars were recovered from TT samples. Detection of *Salmonella* from earlier timepoints was variable with no consistent association between nonselective preenrichment broth, selective broth, and *Salmonella* detection.

Significance: The enrichment of *Salmonella* from cilantro was reduced from 48 to 24 hours with a five-hour static incubation in nonselective preenrichment broth prior to inoculation into selective broths, significantly decreasing the time to detection.

P3-18 An Independent Evaluation of an Alternative Rapid Method for the Enumeration of *S. aureus* in Soy Ingredients

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Introduction: The presence of *S. aureus* in foods poses several problems to the food industry, as they may indicate post-process contamination of the commodity and certain strains of the organism are known to produce a potent enterotoxin that can be a public health hazard. Rapid quantitative microbiological methods offer a variety benefits including increased turnaround time, reduced labor cost, and a streamlined laboratory process. These methods are typically validated against the reference method through a recognized validation organization. However, these validations are carried out on limited number of food product and it is important for an end user to conducting their own independent evaluation.

Purpose: The purpose of this evaluation was to proactively evaluate the performance of the AOAC OMA 2003.07 method against the FDA BAM Chapter 12 on a variety of low moisture, soy ingredients: Isolated Soy Protein (ISP), Soy Fiber (SF), Soy Fluid Lecithin (SFL), Soy Deoiled Lecithin (DL), and Soy Nuggets (SN).

Methods: For each matrix, 5 replicates at three contamination levels (10-100 CFU/g, 100-1,000 CFU/g and 1,000-10,000 CFU/g) were evaluated. Three uninoculated control replicates were included for each matrix.

Results: Results for each contamination level were analyzed for the difference of means and reverse transformed difference of means (including 95% confidence intervals). The results of the study indicated that 7 out of 18 contamination levels evaluated produced statistically significant results between the alternative and reference method (indicated by a confidence interval that falls outside of -0.5 to 0.5).

Significance: The data generated in this evaluation indicates that the rapid alternative method may not be a suitable alternative method for the detection of *S. aureus* in these select commodities. The results of this study confirm the importance to the food industry to conduct their own internal verification of matrices not included in the scope of a method during the validation process.

P3-19 An Independent Evaluation of Two Alternative Rapid Methods for the Detection and Enumeration of Coliforms in Soy Ingredients

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Introduction: Coliform bacteria are a commonly used indicator of sanitary quality of foods and water. They are defined as rod-shaped, Gram-negative, non-spore forming, and motile bacteria. Coliforms can be found in the aquatic environment, in soil and on vegetation. They are universally present in large numbers in the feces of warm-blooded animals and thus historically have been used to indicate that other pathogenic organisms of fecal origin may be present.

Purpose: The purpose of the evaluation was to proactively evaluate the performance of the OMA 996.02 method (3M™ High Sensitivity Coliform Count plate) and the AOAC-RI PTM #100401 method (Neogen®for coliform count) against the FDA BAM Chapter 4 reference method on a variety of soy ingredients: Isolated Soy Protein (ISP), Soy Fiber (SF), Soy Fluid Lecithin (SFL), Soy Deoiled Lecithin (DL), and Soy Nuggets (SN).

Methods: For each matrix, 5 replicates at three contamination levels (10-100 CFU/g, 100-1,000 CFU/g and 1,000-10,000CFU/g) were evaluated by each method. Three control replicates were analyzed at 0 CFU/test portion.

Results: The results of the statistical analysis using the difference of means with calculated 95% confidence intervals indicated no statistical difference between the rapid methods and the reference methods in 31 of 36 contamination levels analyzed. The OMA 996.02 produced 2 statistically different levels while the AOAC-RI PTM 100402 method produced 3 statistically significant levels.

Significance: The applicability of OMA 996.02 method (3M™ High Sensitivity Coliform Count plate) covers low levels of coliform bacteria in dairy products, and the applicability of AOAC RI PTM 100401 includes several non-soy matrices, however, the results of these studies indicate both methods perform statistically equivalent to the standard reference method for the five soy ingredients evaluated. This study supports the fact that the scope of this validation could be expanded.

P3-20 An Independent Evaluation of the Two Alternative Methods for Aerobic Count for the Enumeration of Total Viable Count in Soy Ingredients

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Introduction: Aerobic Plate Count (APC) testing has been an industry standard for the examination of background flora in a variety of foods to gauge sanitary quality. Alternative examination methods for APC have been developed to save space and cost for food producers. However, there is limited validation information available for these rapid methods with soy ingredients. There is great need to evaluate soy ingredients for use with these alternative methods.

Purpose: The purpose of the evaluation was to proactively evaluate the performance of OMA 990.12 method (3M™ Aerobic Plate Count) and the AOAC-RI PTM 011001 method (NeoFilm®for Aerobic Count) against the FDA BAM Chapter 3 reference method on a variety of low moisture, soy ingredients: Isolated Soy Protein (ISP), Soy Fiber (SF), Soy Fluid Lecithin (SFL), Soy Deoiled Lecithin (DL), and Soy Nugget (SN).

Introduction: Aerobic Plate Count (APC) testing has been an industry standard for the examination of background flora in a variety of foods to gauge sanitary quality. Alternative examination methods for APC have been developed to save space and cost for food producers. However, there is limited validation information available for these rapid methods with soy ingredients. There is great need to evaluate soy ingredients for use with these alternative methods.

Purpose: The purpose of the evaluation was to proactively evaluate the performance of OMA 990.12 method (3M™ Aerobic Plate Count) and the AOAC-RI PTM 011001 method (NeoFilm®for Aerobic Count) against the FDA BAM Chapter 3 reference method on a variety of low moisture, soy ingredients: Isolated Soy Protein (ISP), Soy Fiber (SF), Soy Fluid Lecithin (SFL), Soy Deoiled Lecithin (DL), and Soy Nugget (SN).

Methods: For each method, fifteen 25-g replicates, were analyzed at three separate inoculation level(10 – 100 CFU/g, 100 – 1,000 CFU/g and 1,000 – 10,000 CFU/g) using a cocktail of common aerobic bacteria. Three control replicates were analyzed at 0 CFU/test portion. All candidate method plates were incubated at 35 ± 1°C for 48 ± 2 hours and enumerated.

Results: The results of the statistical analysis using the difference of means calculated with 95% confidence intervals indicated no statistical difference between the rapid methods and the reference method with one exception. The low level for the Soy Nugget using the AOAC-RI PTM 011001 method produced statistical differences when compared to the reference method.

Significance: These studies indicate that the alternative enumeration methods perform equally or better than the standard reference method for the five low moisture soy products evaluated. The alternative methods reduce bench space, incubator space and storage space when compared with the standard reference method saving both time and money.

Methods: For each method, fifteen 25g replicates, were analyzed at three separate inoculation level(10-100CFU/g, 100-1000CFU/g and 1000-10000 CFU/g) using a cocktail of common aerobic bacteria. Three control replicates were analyzed at 0 CFU/test portion. All candidate method plates were incubated at 35 ± 1 °C for 48±2 hours and enumerated.

Results: The results of the statistical analysis using the difference of means calculated with 95% confidence intervals indicated no statistical difference between the rapid methods and the reference method with one exception. The low level for the Soy Nugget using the AOAC-RI PTM 011001 method produced statistical differences when compared to the reference method.

Significance: These studies indicate that the alternative enumeration methods perform equally or better than the standard reference method for the five low moisture soy products evaluated. The alternative methods reduce bench space, incubator space and storage space when compared with the standard reference method saving both time and money.

P3-21 Evaluation of a New Rapid Method for the Enumeration of Yeast and Mold Food Samples: Collaborative Study

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Introduction: Due to their heterotrophic nature, yeasts and molds are able to adapt and survive in a wide range of environmental conditions. The presence of these microorganisms in food commodities can indicate contamination, inadequately cleaning or inadequate storage conditions. The 3M™ Petrifilm™ Rapid Yeast and Mold Count (RYM) Plate uses a cold-water-soluble gelling agent and an indicator system to facilitate the enumeration of yeast and molds after 48 h of incubation at 25°C or 28°C, compared to 5 -7 days at 25°C for the reference methods.

Purpose: The purpose of this AOAC®OMA℠ collaborative study was to compare the new method to the ISO 21527:2008 parts 1 and 2 and the FDA BAM Chapter 18 method in raw ground beef and raw almonds.

Methods: A total of 16 laboratories participated. Eight artificially contaminated samples, covering three levels of contamination (low level 10 -100 CFU/g, medium level 100-1,000 CFU/g, and a high level 1,000-10,000 CFU/g) and an uninoculated control level (0 CFU/g) were analyzed. For the new method, replicates were evaluated at both 48 and 60 h at both 25°C and 28°C. Unpaired replicates were analyzed following a harmonized FDA BAM and ISO reference method (enrichment in 0.1% peptone water).

Results: A paired *t*-test was conducted at $P = 0.95$ and no significant difference was observed between the new method and the harmonized reference methods. No statistically significant differences were observed between the new method when enumerated at 48 h vs. 60 h or when plates were incubated at 25°C or 28°C when compared to the reference methods. The repeatability values obtained were generally similar between the two methods.

Significance: The collaborative study demonstrated the robustness and high level of reproducibility of the new method when enumerating yeast and molds in raw ground beef and raw almonds.

P3-22 DETECT/L- Food and DETECT/S- Food: Finished Product Testing for Listeria and Salmonella with Revolutionary Time to Results

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Introduction: *Listeria* and Salmonella are growing concerns for producers of a variety of Ready-to-Eat and produce products. Recalls in these key categories due to *Listeria and Salmonella* contamination have been on the increase over the past decade. In many cases limited shelf life prevents testing for *these pathogens* before shipping due to the long time to result required for such tests.

Purpose: Following on the heels of the release of the first enrichment-free in-shift environmental assay for *Listeria* in 2014, Sample6 is proud to announce the availability of DETECT/L- Food and DETECT/S- Food, finished-product test for *Listeria* and *Salmonella* with a revolutionary time to result.

Methods: Our food testes leverage our proprietary Biollumination Platform, which enables single cell detection through the use of bacteriophages.

Results: In addition to time to result, these food tests uses up to 90% less enrichment media than existing assays, which preserves lab and incubator space and generates less waste. Our novel test makes all of these improvements while maintaining minimal sample prep and hands on time.

Significance: Our Listera and Salomnella food matrix tests will be available soon.

P3-23 Evaluation of Filter-plating Methods for Simplified Detection of *Campylobacter* Associated with Broiler Cecal Samples

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Introduction: *Campylobacter* is natural member of the gut microflora in many commercial broilers and as such can become a contaminant on edible surfaces during processing. Culturing gut contents or feces can be a means to determine flock status prior to live-haul. However, the wide variety of non-*Campylobacter* background bacteria in these complex samples contaminate growth media and can make it very difficult to isolate *Campylobacter*.

Purpose: The purpose of this study was to evaluate the detection of *Campylobacter* from broiler cecal samples by surface plating with and without the addition of a 0.45 µm filter on three different selective agars.

Methods: We cultured cecal contents from 100 commercial broiler flocks and carcass rinses from 50 of those flocks over the course of 17 months. *Campylobacter* was recovered on three different selective media: Campy-Cefex Agar, Campy-Line Agar and RF-*Campylobacter jejuni/coli* agar. For cecal contents, each medium was tested with and without the additional selection of a 0.45 µm filter. Filters were laid on the agar surface and the diluted sample was placed directly onto the filter. After the sample had dried, filters were removed and all plates (filtered and direct) were incubated at 42°C under microaerobic conditions for 48 h.

Results: We found about half (52%) of the tested flocks were positive for *Campylobacter;* positive flocks were detected during each month of the year. Overall, the *Campylobacter* status of cecal contents from one carcass was predictive of the status of a carcass rinse from the same flock. Placing a complex sample such as cecal contents onto a 0.45 µm filter improved the detection of *Campylobacter* by eliminating non-*Campylobacter* background colonies.

Significance: These data suggest that detection of *Campylobacter* from the complex environment of broiler ceca is improved by surface plating methods utilizing a 0.45 µm filter which eliminates non-*Campylobacter* background flora.

P3-24 Evaluation of 3M™ Petrifilm™ Rapid Yeast and Mold Count Plate for Enumeration of Yeasts and Molds in Beverages

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Introduction: Yeasts and molds are significant in the beverage industry; they can cause product spoilage and some species are pathogenic via the production of toxins. These organisms are important quality indicators, however the 5 - 7 days for traditional methods is a burden on the beverage industry. 3M™ Petrifilm™ Rapid Yeast and Mold (RYM) Count Plate were developed to address the need for rapid detection.

Purpose: The performance of Petrifilm RYM was evaluated by comparing yeast and mold counts in beverages with the USFDA BAM procedure.

Methods: A total of eight beverage samples were analyzed; five naturally contaminated and three spiked with heat-treated *Saccharomyces cerevisiae* and *Aspergillus niger* spores. Three contamination levels were prepared; low (1-100 CFU/ml), medium (100-1,000 CFU/ml) and high (>1,000 CFU/ml). Two methods were used: Plate Count Agar pour-plate with 0.01% chloramphenicol incubated at 25 + 1°C for 5 - 7 days (USFDA BAM 2001); Petrifilm RYM incubated at 25 + 1°C and 28 + 1°C for 48 + 2 hours and 72 + 3 hours.

Results: Petrifilm RYM incubated at 25°C and 28°C for 48 h and 72 h, gave Pearson correlation coefficient scores (r) of 0.9986, 0.9990, 0.9996 and 0.9995, respectively. The accuracy profile was accomplished by setting the β-expectation tolerance intervals (β-ETI) at 80% and the acceptability limits (λ) at ± 0.3 and + 0.4 log units/ml. At 28°C, β-ETI limits were within λ = + 0.3 log units/ml. At 25°C, β-ETI limits fell outside λ = + 0.3 log units/ml but were within λ = + 0.4 log units/ml.

Significance: The 3M method correlated to the standard method at the incubation conditions of 25°C (48 h and 72 h) and 28°C (48 h and 72 h). Petrifilm RYM can detect yeast and mold in beverages within 48 - 72 hours whereas the standard method takes 5 - 7 days.

P3-25 Comparative Study between 3M™ Petrifilm™ *Salmonella* Express System and Reference Method ISO 6579 for *Salmonella* Species Detection in Thai Fruit and Vegetable Products and Seasoning

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Introduction: *Salmonella* spp. are important pathogens implicated in the contamination of fruit, vegetable and seasoning products. Rapid and convenient detection of *Salmonella* helps food processors make fast, informed decisions.

Purpose: This study aimed to compare the 3M™ Petrifilm™ *Salmonella* Express System with a reference method (ISO 6579) for the detection of *Salmonella* spp. in fruit, vegetable and seasoning products from Thailand.

Methods: A total of 116 samples were tested with both methods. Samples were spiked with 5 serovars of *Salmonella* spp. (Anatum, Weltevreden, Stanley, Rissen and Typhimurium) at low (1–50 CFU/25 g), medium (>50 - 600 CFU/25 g) and high (>600 - 40,000 CFU/25 g) levels. These serovars represent the most frequently detected contaminants in food products analyzed by the Regional Medical Sciences Center 1, Chiang Mai. Twenty-five grams of each spiked sample was homogenized, divided and analyzed by 3M™ Petrifilm™ *Salmonella* Express System and ISO 6579. Six replicates of 2.0 CFU/25 g, 4.0 CFU/25 g and 8.0 CFU/25 g contamination levels in frozen green soybean were analyzed by 3M™ Petrifilm™ *Salmonella* Express System for LOD_{50} detection.

Results: 3M™ Petrifilm™ *Salmonella* Express System results revealed 100% sensitivity, 100% accuracy and 100% specificity. The six replicates analysis showed 1/6, 3/6 and 6/6 positive results for 2.0 CFU/25 g, 4.0 CFU/25 g and 8.0 CFU/25 g contamination levels, respectively. The limit of detection (LOD_{50}) using Spearman-Karber method (Excelpros 20) showed approximately 7 (or 6.55) CFU/25 g.

Significance: 3M™ Petrifilm™ *Salmonella* Express System is a rapid method with high efficacy, high accuracy, and is less time consuming than standard agar methods. The LOD_{50} in food samples gave acceptable results in this study.

P3-26 The Use of the RapidChek SELECT™ *Salmonella* Test System to Detect *Salmonella* Species in Seafood Samples

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Introduction: Human salmonellosis continues to be a significant worldwide public health problem in many different raw food products. RapidChek SELECT *Salmonella* test system can detect low levels of *Salmonella* spp. in different types of seafood.

Purpose: The aim of the study was to evaluate the performance of the *Salmonella* test system compared to the FDA BAM (Chapter 5 *Salmonella*) for the detection of *Salmonella* spp. in shrimp and catfish.

Methods: Shrimp and catfish samples (25 g) were inoculated with a low level, (1 cell per sample) of *Salmonella* Typhimurium ATCC 14028. Twenty-five samples, 20 low-level inoculated and 5 non-inoculated samples, of each matrix were analyzed by both methods. Samples for the test method were enriched in 225 ml of primary media, transferred to secondary media, and evaluated with test strips at a total time of just 22 h. For the FDA BAM method, samples were enriched and incubated in Lactose broth, transferred to Tetrathionate broth and Rappaport-Vassiliadis broth, and struck to HE and XLD agars for a total of 62-76 h.

Results: The test method detected 11 and 10 low-level inoculated shrimp and catfish samples, respectively, while the FDA BAM reference method detected 12 and 7 samples as positive. All samples were confirmed by the cultural method. There was no significant difference between the two test methods and equivalent performance was demonstrated. The overall Chi-square was 0.197 resulting in 111% accuracy, 100% average sensitivity and 100% average specificity.

Significance: The *Salmonella* test system detects low levels of *Salmonella* spp. in shrimp and catfish in as little as 22 hours. This should enable food producers to quickly and efficiently identify *Salmonella* spp. in a variety of seafood types, with reduced time and labor inputs while maintaining test method performance compared to the cultural reference method.

P3-27 Comparative Detection of *Salmonella* in Philippine Desiccated Coconut Using 3M™ Molecular Detection Assay *Salmonella,* 3M™ Petrifilm™ *Salmonella* Express System, and Bismuth Sulphite Agar

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Introduction: *Salmonella* spp. is an important foodborne pathogen worldwide, considered as a leading cause of food poisoning. Recently, there have been cases of Philippine desiccated coconut being confirmed positive for the presence of *Salmonella*, which were only detected when the products were already in the importing countries.

Purpose: The study aimed to compare sensitivity and selectivity of three *Salmonella* methods: 3M Molecular Detection Assay *Salmonella;* 3M Petrifilm *Salmonella* Express System; and spread plating on bismuth sulphite agar (BSA).

Methods: For the sensitivity assay, desiccated coconut samples were decontaminated at 121°C for 20 minutes and spray inoculated with decreasing levels of *Salmonella enterica* serovar Typhimurium (ATCC 14028) ranging from ca. 1 to 5 log CFU/g. Selectivity testing was conducted using decontaminated desiccated coconut samples inoculated with the following mixtures: *Salmonella* + *Escherichia coli* O157:H7, *Salmonella* + *Listeria monocytogenes, Salmonella* + methicillin-resistant *Staphylococcus aureus* (MRSA), and *Salmonella* + *E. coli* O157:H7 + *L. monocytogenes*+ MRSA. All samples were then subjected to three detection methods.

Results: The 3M Molecular Detection Assay *Salmonella* and 3M Petrifilm Salmonella Express System were able to detect *Salmonella* at levels as low as 1.6 log CFU/g (39 cells/g) while direct plating on bismuth sulphite agar detected as low as 2.6 log CFU/g (398 cells/g). The detection of *Salmonella* in all three methods was not hampered by the presence of any non-*Salmonella* microorganism tested in this study.

Significance: These data suggest that the 3M Molecular Detection Assay *Salmonella* and 3M Petrifilm Salmonella Express System may be used as rapid detection methods for *Salmonella* in desiccated coconut products.

P3-28 Improved Enrichment and Detection of *Listeria monocytogenes* in Raw Animal Proteins

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Introduction: *Listeria* are ubiquitous soil organisms that are present in wide variety of raw materials, including raw animal products that enter food facilities. *Listeria* have a slow growth rate and can be difficult to propagate in environments which are high in competing microbiota, such as some raw meat, poultry, fish and seafood.

Purpose: The aim of this study was to compare a newly developed molecular method to the traditional ISO cultural method for detection of *Listeria*, including *L. monocytogenes*, in raw animal proteins.

Methods: Raw refrigerated meat, poultry, fish and seafood samples (n = 66) were obtained from sources in France and the USA. Samples were naturally contaminated with *Listeria* or were artificially contaminated using a variety of *Listeria monocytogenes* isolates. Samples were analyzed using the cultural ISO 11290-1 method and using the new molecular method. For the new method, samples were diluted 1:20 in demi-Fraser broth, incubated for 28 h and subsequently tested using a modification of existing molecular detection methods. All presumptive results from the new methods were confirmed from enrichment broth onto selective and differential agars with further testing per ISO.

Results: Results for the two methods agreed for 45 of the 66 samples. The new molecular method detected 18 positive samples that were not identified by the ISO cultural method, and only missed three positives that were identified by the ISO method.

Significance: Disagreements between two test methods may occur due to contaminant distribution and other factors, but if methods are equivalent, a balanced ratio of positive to negative disagreements is expected. This study found enhanced detection by the new molecular method for *Listeria* in raw animal proteins, relative to the ISO cultural method, evidenced by an imbalance between positive and negative disagreements. This improved performance is available as a next-day result, compared to the ISO method which takes over one week.

P3-29 Development of Enrichment Broth for Detection of *Campylobacter jejuni/coli* in Fresh Produce and Poultry

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Introduction: *Campylobacter jejuni/coli* is one of the leading causes of bacterial foodborne illnesses across the world. As a standard method, Bolton broth for enrichment media and mCCDA for selective agars are most commonly used to detect *C. jejuni/coli* in food in many countries. However, the standard method has potential limitations, including false positives.

Purpose: The purposes of this study were to develop enrichment broth (Bolton broth with 12.5 mg/l rifampicin, R-Bolton broth) and to determine a newly designed R-Bolton broth-subsequent selective media combination for *C. jejuni/coli* detection in vegetables and poultry.

Methods: In order to evaluate survivability of *C. jejuni/coli* according to concentration of rifampicin, *C. jejuni/coli* were inoculated and incubated in Bolton broth with each concentration, 0 mg/l, 10.0 mg/l and 12.5 mg/l, of rifampicin at 42°C for 48 h microaerobically. For the determination of a selective agar, 125 of *Campylobacter* spp. and other bacteria were incubated in R-Bolton broth, followed by streaking onto several selective agars, respectively. Finally the combination of R-Bolton broth and CampyFood Agar (CFA) was applied to chicken and 3 kinds of vegetables for validating its performance.

Results: First of all, no significant differences in *C. jejuni/coli* growth were found among enrichment media with various levels of rifampicin. In addition, the combination of R-Bolton broth and CFA showed the highest specificity (99.2 %). When applied to food samples, such as, romaine lettuce, chicken, tomato and Korean leek, the combination decreased false-positive results by 58.3%, 55%, 16.7% and 8.3% for respectively, compared to a standard method.

Significance: It was demonstrated that combination of newly developed R-Bolton broth and CFA is useful to detect *C. jejuni/coli* in food with suppressing non-*Campylobacter* bacteria.

P3-30 RAPID'*Campylobacter*: A Reliable Medium for the Enumeration of *Campylobacter* in Poultry Meat Samples

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Introduction: Collection of reliable quantitative data on *Campylobacter* contamination on broiler carcasses is required in order to correctly assess the risk of human campylobacteriosis posed by handling and consumption of broiler meat.

Purpose: The aim of the present study was to evaluate RAPID'*Campylobacter* agar (RAPID; Bio-Rad, France) for quantification of *Campylobacter* in poultry samples and to compare results with those from the widely used modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) and Campy Food®Agar (CFA; bioMérieux, France).

Methods: Analysis of 12 artificially and 36 naturally contaminated samples was performed in parallel on RAPID, mCCDA and CFA plates. One ml from each homogenized sample was spread plated on 2 agar plates (0.5 ml on each plate), while 0.1 ml of further dilutions was plated using a spiral plater. After incubation, at least 4 presumptive positive colonies were confirmed by microscopic examination and PCR test. The agreement between *Campylobacter* counts obtained by the different agars was calculated using Lin's concordance correlation coefficient (*CCC*) and by Bland–Altman plot.

Results: Concordance correlation coefficient (*CCC*) between overall (artificially and naturally contaminated samples) *Campylobacter* counts on RAPID and on mCCDA agars was 0.973 (95% CI: 0.949 - 0.986). When plotting overall *Campylobacter* counts obtained by CFA against counts from RAPID plates, the *CCC* was 0.978 (95% CI: 0.961 - 0.988). Further, the Bland-Altman plot showed low variation in *Campylobacter* counts obtained between RAPID and the other two agars mCCDA and CFA.

Significance: RAPID agar is a highly sensitive and selective medium for the recovery of *Campylobacter*. The results obtained are in agreement with other commonly used agars, and therefore, RAPID media is a reliable tool for the *Campylobacter* enumeration in poultry meat samples.

P3-31 Evaluation of High Sensitivity Test Method for Fecal Indicators in Food, Water and from Surface Sponges

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Introduction: Food plants sometimes need to increase method sensitivity to sanitary/fecal indicators. Peel Plate EC HV is a ready to use *E.coli* and coliform method, in a conventional 100 mm Petri dish, utilizing gram negative selective medium with dual indicators to differentiate *E. coli* as blue/purple colonies, and coliform, as red colonies, at 35°C in 24 h.

Purpose: The purpose of this evaluation was to compare the new method in comparison to the reference methods, VRB agar with MUG overlay and alternatively AOAC 996.02, using solid dairy extracts, irrigation water, and environmental surface sponges.

Methods: Heavy cream (35 % fat) was co-inoculated at five concentrations with *Enterobacter* and *E. coli* and split into 5 replicates. Samples were sent to 5 laboratories and tested in duplicate by the new and reference methods, VRBA/MUG overlay and AOAC 996.02 at 32°C for 24 h. Ice cream, egg nog, condensed milk, and container rinses were inoculated similarly and tested in one laboratory. Irrigation water and environmental sponge samples (ISO 18593) were inoculated at 3 bacterial levels split into 5 samples and tested in duplicate at 35°C for 24 h.

Results: Results were evaluated for repeatability (S_r) and by paired-*t*-test for statistical difference, > 0.5 log. The Peel Plate EC HV results were not significantly different from reference methods in detecting total coliform in the dairy solids at 32°C and in distinguishing *E.coli* from coliform at 35°C in the water and environmental samples. Production of indicator colors is not consistent in all dairy products at 32°C, so while heavy cream and condensed milk detected *E.coli* and results were not significantly different from reference method, eggnog and ice cream did not produce the distinguishing colors.

Significance: Peel Plate EC HV demonstrates detection < 1 CFU/5ml with comparable reference method detection of total coliform at 32°C in dairy solids and *E. coli*/coliform detection to water and surface sponges at 35°C.

Poster

P3-32 Total Coliform Method for the Detection of Coliform Including *E. coli* in Pasteurized Dairy Products

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Introduction: United States dairy products are checked for coliform following the Pasteurized Milk Ordinance. Peel Plate EC is a simplified total coliform method that uses dual indicators to detect total coliform (including *E. coli*) at 32°C in 24 hours without the need for confirmatory analysis.

Purpose: The purpose of this evaluation was to perform inclusivity/exclusivity and an inter-laboratory comparison of the new method in comparison to the reference methods, VRB agar with BGLB confirmation and alternatively AOAC 989.1, in different dairy products.

Methods: Methods were evaluated at 32°C for inclusivity using 56 coliform strains and exclusivity study using 31 non-coliform strains. Heavy cream (40% fat) and whole (3.3% fat), chocolate (2% fat), and skim (1%) milks were inoculated with different heat stressed coliform and *E. coli* at 5 different contamination levels. Five laboratories received 5 replicates from each contamination level. All samples were tested in duplicate.

Results: Peel Plate EC detected 55 of 56 coliform strains, compared to 41 by AOAC 989.1, in inclusivity study. In exclusivity, the new method excluded 28 of 31 compared to 26 by AOAC 989.1. For the method comparison, results were evaluated for repeatability (S_r) and by paired *t*-test for statistical difference, > 0.5 log. In whole milk, chocolate milk, and cream the Peel Plate EC was not significantly different from the reference method; while, with whole milk and chocolate the AOAC 989.1 showed some statistically lower results. Peel Plate EC with skim at the 3 highest contamination levels were statistically lower from reference methods. Replicate pairs from new method had similar or better S_r than the reference. All *E.coli* tested were detected at 32°C but color differentiation was strain and dairy matrix dependent; thus *E. coli* in dairy matrices is only reliably scored as coliform.

Significance: Peel Plate EC demonstrates high selectivity with easy total coliform interpretation in dairy matrices at 24 h.

P3-33 Evaluation of a Simplified Total Aerobic Count Method for a Variety of Dairy, Meat and Environmental Sponge Samples

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Introduction: Food products and their production surfaces are checked for aerobic bacteria count to assess sanitation and predict shelf life. Peel Plate AC (Aerobic Count) is a pre-prepared self-wicking 47 mm diameter method that uses a color inducing substrate, TTC, to detect bacteria as red spots in 48 h.

Purpose: The purpose of this evaluation was to inter-laboratory evaluate the new method in comparison to the standard plate count (SPC) and AOAC 986.33 reference methods in pasteurized dairy products, ground beef and using environmental sponge samples.

Methods: Dairy products were evaluated at 32°C for 48 h. Whole milk (3.3% fat), chocolate milk (2% fat), heavy cream (20% fat), and skim milk (1% fat) were inoculated with a variety of stressed bacterial strains at 5 contamination levels. Five laboratories received 5 replicates from each level. All samples were decimal diluted and tested in duplicate. Ground beef and stainless steel surfaces were inoculated to low, medium and high concentrations. Ground beef extract (50 g/450 ml) and 25 ml sponge-surface-extracts were tested (n = 5 pairs) at 35°C for 48 h by two laboratories.

Results: Colony counts (CFU/ml or g) were evaluated for repeatability (S_r) and by paired *t*-test for statistical difference, > 0.5 log. The Peel Plate AC was not significantly different from the reference methods in the whole milk, chocolate, cream, ground beef and surface evaluations. Both Peel Plate AC and AOAC 986.33 methods showed > 0.5 log statistically lower results in the skim milk study indicating a better resuscitation of stressed bacteria by the SPC method. Replicate pairs from Peel Plate AC had similar S_r as both reference methods.

Significance: Peel Plate AC aerobic bacteria determination (32°C for 48 h) was equivalent to reference methods in most dairy preparations tested. At 35°C for 48 h, the new method performed similarly with ground beef and environmental sponge samples. Peel Plate AC offers a new testing alternative for aerobic plate count determination in dairy and food production.

P3-34 A Method Comparison of the 3M™ Petrifilm™ Rapid Aerobic Count Plate Method for the Enumeration of Total Viable Count

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Introduction: Colony count methods provide numerical estimations of the total viable microorganisms in food products. The accuracy of the colony count method is dependent upon many factors. The 3M™ Petrifilm™ Rapid Aerobic Count Plate (RAC) was developed with a new indicator technology that makes colonies easier to interpret and can produce results within 24 - 48 h compared to 48 - 72 h using the reference methods.

Purpose: The purpose of this independent evaluation was to compare the new method to the FDA/BAM Chapter 3 method and the SMEDP Chapter 6 method for a broad range of foods as part of the AOAC RI™ PTM validation process.

Methods: A total of 16 matrices were evaluated: raw ground beef, raw ground pork, raw ground turkey, chicken carcass rinsate, fresh swai, fresh tuna, fresh tiger shrimp, easy-peel shrimp, cherry tomato wash, frozen blueberries, Mediterranean apricots, creamy salad dressing, fresh pasta, vanilla ice cream, dry milk powder, and pasteurized skim milk. Naturally contaminated samples for each matrix that covered three target levels of contamination (low level 10 -100 CFU/g, medium level 100-1,000 CFU/g, and a high level 1,000-10,000 CFU/g) were analyzed. Pasteurized skim milk was evaluated after artificial contamination with a bacterium at 4 levels (uninoculated, low, medium, and high). Each contamination level was evaluated using 5 replicates.

Results: The Mean Log Difference and repeatability were calculated. No significant difference was observed between methods in 13 out of 16 matrices. For fresh tuna and fresh tiger shrimp, a statistically significant difference was observed for the low and high enumeration levels. For raw ground turkey and fresh tiger shrimp, a statistically significant difference was observed for the medium enumeration level.

Significance: This new method demonstrates reliability as a rapid alternative for the enumeration of total viable count in as little as 24 h for a variety of matrices.

P3-35 NeoFilm® for Yeast and Mold as an Alternative to Dilution Plating for the Enumeration of Yeast and Molds in Foods

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Introduction: NeoFilm Yeast and Mold is a simple, effective device used for the enumeration of yeast and molds. A 1 ml sample homogenate is applied to a thin membrane pad, which is then incubated for 48 - 72 h at 25°C. The membrane pad is comprised of a nonwoven fabric that contains a layer of microbial nutrients deposited in film.

Purpose: To compare recovery levels of yeast and molds in NeoFilm Yeast and Mold, using either PBS or peptone water as a diluent, against the FDA/BAM reference method plating. Method performance was assessed for 5 food types: breaded chicken nuggets, dry pet food, orange juice concentrate, yogurt, and cake mix.

Methods: For each food, five separate sample homogenates were prepared with both diluents at three target inoculation levels: 10^2, 10^3, and 10^4. Each homogenate was either plated by the reference method or on NeoFilm. Data were statistically analyzed using a difference of means, *P*-value, and correlation coefficients.

Results: When using PBS as diluent, there were statistically significant differences only for orange juice concentrate at the 10^3 level ($P = 0.01$), with reference yielding a higher count than NeoFilm, and cake mix at the 10^3 level ($P = 0.01$), with NeoFilm yielding a higher count than reference. When using peptone water as diluent, there were statistically significant differences only for dry pet food at the 10^2 level ($P = 10^{-4}$), with reference yielding a higher count than NeoFilm, and cake mix at the 10^2 and 10^3 levels ($P = 0.01$ and 0.001), with NeoFilm yielding higher counts than reference.

Significance: A high level of agreement was observed for the enumeration of yeast and molds as determined by NeoFilm Yeast and Mold and FDA/BAM reference methods. Incubation times of 48 h for peptone water and 72 h for PBS are recommended.

P3-36 Performance Evaluation of the ISO Reference Method, Italian Reference Method, and a Chromogenic Rapid Method for the Detection of *E. coli* and Coliforms in Natural Bottled Water

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Introduction: Bottled water can be contaminated by coliforms and/or *Escherichia coli*. These bacteria are considered as indicators of fecal pollution, and their detection in bottled water indicates the potential contamination by pathogenic enteric microorganisms.

Purpose: The aim of the study is to compare three different methods, the Italian reference method, the ISO 9308-1:2002 method and a chromogenic rapid method (RAPID'E.coli 2 Agar-Bio-Rad) for the detection of coliforms and *E. coli* in bottled mineral water.

Methods: The inclusivity and exclusivity of the three different methods were studied using 4 *E. coli* strains, 10 of other coliform bacteria strains and 35 strains of water-borne pathogens. The LOD of the three methods were defined analyzing 18 bottled of 1.5 liter natural mineral water, spiked with three different concentrations of coliforms or *E.coli* in presence of competitive bacteria. The ANOVA and Tukey's Multiple Comparison Test was used for evaluating the comparison among the quantitative results obtained from the three different methods.

Results: The 100% sensitivity and specificity was found comparing the results obtained by the three methods. Both Italian reference method and ISO reference 9308-1 have highlighted difficulties in discriminating colonies of *E. coli* and coliforms from other Enterobacteriaceae bacterial strains. Chromogenic media do not require any confirmation step of the colonies when it is used for enumerating coliforms and *E. coli*. Tukey's Multiple Comparison Test of the means of the counts of *E. coli*, using the three cultural methods, showed no statistical significant differences.

Significance: The results have demonstrated that the Italian reference method and the new rapid method (RAPID'E.coli 2 Agar-Bio-Rad) are as sensitive and specific as ISO method 9308-1:2002. Both methods could be used in alternative to ISO method to evaluate the contamination level of coliform and *E. coli* in drinking water, bottled water and mineral water.

P3-37 *Yersinia enterocolitica* in Fresh Vegetables: Evaluation of the Persistence during the Storage and the Effectiveness of the Washing Treatment

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Introduction: Over the past few decades, leafy fresh vegetables have been increasingly recognized as significant reservoirs of foodborne pathogens. Contaminated Vegetables with *Yersinia enterocolitica* and cases of yersiniosis, epidemiologically correlated with the consumption ready to eat (RTE) vegetables, are recently well documented in Europe.

Purpose: The aim of this work is to evaluate (1) the survival of *Y. enterocolitica* along the entire shelf life of fresh leafy vegetables and "Ready-to-Eat" (RTE) vegetables, and (2) the effectiveness of different washing treatments to reduce contamination of *Yersinia enterocolitica* on the surface of leafy vegetables.

Methods: Leafy vegetables were spiked with different concentration of pathogenic *Yersinia enterocolitica* (bio-serotypes O:3/4), stored at refrigerator temperature for six days and daily analyzed. Other spiked leafy vegetables were submitted to different domestic washing treatments (water, sodium hypochlorite at 60 and 220 ppm). All the samples were analyzed using both ISO 10273 standard cultural method and real-time PCR method.

Results: The results demonstrated that the concentration of *Yersinia enterocolitica,* detected on the surface of vegetables, does not decrease even when they were stored at refrigerator temperature for a week. Only the washing with 220 ppm of sodium hypochlorite can guarantee the 1-logarithm reduction of the original contamination of *Y. enterocolitica* in the vegetables.

Significance: *Yersinia enterocolitica* could persist in the vegetables for the entire shelf life and its presence could not be completely eliminated using domestic or industrial washing practices. The application of good agricultural practices and HACCP system, also in primary production, are the only measures to reduce or eliminate biological contaminants and achieve the objective of food safety in RTE vegetables.

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P3-38 Performances Assessment of the 3M™ Petrifilm™ Plates According to the ISO 16140 Standard for Total Viable Count, *Enterobacteriaceae* Count, Coliforms Count and *E. coli* Count in Pet Foods and Environmental Samples

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Introduction: The 3M™ Petrifilm™ Aerobic Count Plate, *Enterobacteriaceae* Count Plate, Coliforms Count Plate, *E. coli* Count Plate are ready culture medium systems which contain standard methods nutrients, a cold-water-soluble gelling agent, and a tetrazolium that facilitates colony enumerations.

Purpose: An independent study was conducted at ADRIA as part of the NF VALIDATION approval process, in order to extend the scope of the ISO 16140 validation studies to pet food and environmental samples. The ISO 4833, 21528-2, ISO 4832 and ISO 16659 methods were used as reference methods.

Methods: Each ISO 16140 method comparison study of these four Petrifilm plates gathered a linearity study done on 2 (matrix/strain) pairs, a relative accuracy study with 20 samples minimum tested in duplicate.

Results: The Petrifilm Aerobic Count Plate, *Enterobacteriaceae* Count Plate, Coliform Count Plate and *E. coli* Count Plate show all satisfying linearity performances, with linear correlation coefficients superior to 0.98. The intercepts close to 0 and the slopes close to 1 were validated for the tested categories in the accuracy study. Biases between the Petrifm plates and the related ISO methods are characterized by low values. The repeatability of the four Petrifilm plates are similar to the compared ISO standards.

Significance: The Petrifilm Total Viable Count Plate, *Enterobacteriaceae* Count Plate, Coliforms Count Plate and *E. coli* Count Plate are reliable alternative methods for Total Viable count, *Enterobacteriaceae* count, Coliform count and *E. coli* count in pet foods and environmental samples. These four Petrifilm plates offer important economic savings by reducing handling time.

P3-39 Simultaneous Detection of *Salmonella*, *E. coli* O157:H7 and STEC Top 6 in a Single Enrichment Step: A Validation Study for Raw Beef Products

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Introduction: STEC and *Salmonella* are among the most relevant foodborne pathogens to the meat industry. USDA-FSIS have recently homogenized their laboratory methods for *E. coli* O157:H7, STEC and *Salmonella* in order that surveillance programs can be conducted from the same samples and following the same sample preparation and enrichment protocols.

Purpose: The objective of this study was to evaluate the performances of a newly developed PCR based method for the simultaneous detection of STEC Top 6, *E. coli* O157:H7 and *Salmonella* in 375-g test portions of raw beef products from a single enrichment, in comparison to the USDA FSIS reference methods.

Methods: The evaluation consisted of method comparison between the alternate method and the USDA/FSIS reference methods for 2 different food matrices (ground beef-GB- and beef trim-BT). The alternate method was conducted at 2 different incubation times, 10 h and 20 h, one temperature 41.5°C, and presumptive positive results for *Salmonella* were confirmed by 3 different strategies.

Results: Low levels of contamination were between 0.60 & 0.72 CFU/test portion for *Salmonella,* and 0.43 and 0.49 CFU/test portion for STEC (*E. coli* O157:H7 and O45) in GB and BT, respectively. For each of the incubation times and for both levels of contamination, low and high (10x low), the PCR based method detected all the contaminated samples for both *Salmonella* and STEC. All the uncontaminated samples were showed negative by both methods. The statistical analysis with POD (Probability of Detection) showed the PCR-based method to be as good as the USDA reference methods for all the bacterial targets and at both incubation times. All the confirmation methods used for *Salmonella* isolation were showed equivalent in the confirmation of the PCR screening results.

Significance: This new molecular method was fast and accurate for the simultaneous detection of STEC Top 7 and *Salmonella* in beef samples.

P3-40 Comparison of Enrichment Media and Detection Methods for the Detection of *Salmonella* and *E. coli* O157:H7 from Natural Spent Sprout Irrigation Water after 8 h and 18 h Enrichment

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Introduction: A study was conducted to determine the efficacy of detecting low contamination levels of *Salmonella* and *E. coli* O157:H7 in samples of spent irrigation water (SIW).

Purpose: To evaluate the effectiveness of enrichment media and detection methods in detecting pathogens in SIW samples following short enrichments.

Methods: Eighteen test portions of 100 ml SIW were artificially inoculated with low levels of *Salmonella* and another 18 test portions were inoculated with *E. coli* O157:H7 to achieve contamination level of 3 to 45 CFU/100 ml. Samples were left at room temperature for 1 h, mixed with 300 ml of BPW or mEHEC media for 1:4 sample:media ratio and incubated at 41°C for 8 or 18 h. *Salmonella* and *E. coli* O157:H7 levels were quantified after 8 h on XLT4 and Sorbitol MacConkey agar, respectively. This served as the culture confirmation to ensure sufficient levels. After enrichment, *Salmonella* levels in BPW and mEHEC was 1.2 x 10^7 and 1.7 x 10^7 CFU/ml, while *E. coli* O157:H7 levels was 1.1 x 10^7 and 1.2 x 10^7 CFU/ml, respectively. Samples were tested for *Salmonella* using both methods, while method 1 was used for detection of *E. coli* O157:H7. All tests were repeated three times with three samples tested each time.

Results: Method 1 showed 100% sensitivity for both organisms regardless of enrichment media and times. Method 2 showed some inconsistency with the 8 h BPW *Salmonella* samples, but was able to detect consistently using the mEHEC after 8 and 18 h. As a result, Method 1 was chosen for short enrichment tests, whereas both systems were used for longer enrichment times.

Significance: Low levels of *Salmonella* and *E. coli* O157:H7 can be accurately detected in SIW samples after short incubation times using molecular methods. It is important to internally validate the methods to ensure fitness for the purpose.

P3-41 Improvement of Methods for the Enumeration of *Staphylococcus aureus* to Eliminate Competing Flora in Raw-milk Cheeses Produced in Local Non-federally Registered Canadian Establishments

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Introduction: Baird-Parker (BP) agar is a moderately selective and differential medium used for the enumeration of *S. aureus* in foods. Although food laboratories have been using this medium for years, and despite the presence of inhibiting reagents and its high glycine concentration, there are still challenges associated with the growth of non-staphylococci microorganisms that produce brown to black colored colonies on BP agar.

Purpose: This project compared various selective media with the goal of inhibiting the growth of competing microorganisms and reducing the time required to obtain a final result.

Methods: A selectivity study was conducted using 35 *S. aureus* and 22 non-*S. aureus* strains isolated from various dairy products. Linearity of BP, HiCrome™, HardyCHROM™ and BPm agars was evaluated on samples of Emmental cheese spiked at concentrations close to the regulatory limit for *S. aureus* in raw-milk cheeses. The performance of each agar was also assessed using a total of 116 contaminated cheeses. Each agar was incubated for 24 and 48 h in various conditions: temperature of 35 and 42°C in regular or modified atmosphere.

Results: BPm was the only agar to produce performance results comparable to BP ($P = 0.46$), while significantly inhibiting competing flora, when incubated at 35°C for 48 h. Although the incubation time of BPm is the same as BP, its use improved the limit of quantification of the method in presence of microorganisms showing morphological characteristics similar to *S. aureus* that were found to be overgrowing on BP. Use of BPm also reduces the turnaround time by avoiding the need to repeat analyses and to confirm non-*S. aureus* colonies unnecessarily.

Significance: The addition of BPm as a reference agar will allow regulatory organizations to accurately report the amount of *S. aureus* as an indicator of the potential presence of enterotoxin or a lack of adherence to good manufacturing practices.

P3-42 Validation of Compact Dry EC Medium for the Enumeration of *Escherichia coli* in Fresh Fruit and Vegetable, Raw Meat and Raw Milk Cheese Samples at an Incubation Temperature of 42°C

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Introduction: Canadian government regulations require the enumeration of *Escherichia coli* in food products such as fresh fruit and vegetables (FFV), raw ground meat, meat trim and cheese. The analytical methods used are MFHPB-27 (Direct Plating) for FFV and MFHPB-34 (*E. coli* Petrifilm™) for other food samples, both of which use a non-selective incubation temperature of 35°C. For these food products, in which considerable competitive bacterial flora may be present, the colony count is a challenge.

Purpose: The Compact Dry EC (CDEC) has been validated by the manufacturer for enumeration of *E. coli* in foods at 35°C. Because of its physical properties, the CDEC plate can be incubated at 42°C for inhibition of competitive microorganisms' growth, which facilitates the determination of accurate counts of *E. coli*.

Methods: Selectivity of the CDEC, determination of its linearity compared to a reference medium and determination of its limit of quantification (LOQ) for the targeted food category has been evaluated. The selectivity study was conducted using 69 strains of coliforms and 50 non-*E.coli* and non-coliform strains. The linearity of CDEC was done on lettuce, raw ground beef and camembert cheese spiked at concentrations close to the regulatory level specific for *E. coli*. The performance of the CDEC was further evaluated using a total of 179 contaminated foods.

Results: The established LOQ (10 CFU/g for FFV, 50 CFU/g for other food products) meets the Canadian regulatory standards related to *E. coli*. The visual and statistical evaluation (linear regression, comparison of means and standard deviations) of the data obtained using CDEC at 42°C for 24 h showed that this medium can be an alternative approach for *E. coli* enumeration in FFV, raw meat and raw milk cheese.

Significance: CDEC allows for a 42°C incubation temperature, providing food laboratories with an alternative method for evaluating foods with considerable competitive flora.

P3-43 Evaluation of a Reporter-labeled Strain of *Salmonella enterica* subsp. *enterica* serovar Typhimurium as a Control in Ground Beef and Media Assays

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Introduction: Salmonellosis is the leading cause of death and hospitalization attributed to foodborne illness in the United States and is a significant problem worldwide. In *Salmonella* food testing, to effectively discriminate between positive controls and actual food adulterant contamination, strains should contain unique and easily detectable reporters.

Purpose: To evaluate the use of a *Salmonella enterica* subsp. *enterica* serovar Typhimurium strain labeled with NanoLuc®Luciferase (Promega) as a positive control for use in media and ground beef testing.

Methods: A plasmid encoding the NanoLuc® reporter was transformed into *Salmonella* Typhimurium strain CDC 6516-60 (ATCC® 14028™). The stability of the reporter was determined through unselective serial passage and plated on tryptic soy agar. Following the FDA Bacteriological Analytical Manual for *Salmonella* (Chapter 5) protocol, the labeled *Salmonella* Typhimurium was spiked into ground beef samples and grown in lactose broth overnight and used as an inoculum for tetrathionate broth and Rappaport-Vassiliadis broth before being subsequently plated on xylose lysine deoxycholate agar and Hektoen enteric agar. The process was also performed in the absence of ground beef as a media-only control.

Results: Following unselective daily serial passage, the reporter was visible for ≥ 3 days in 100% of tested colonies grown on tryptic soy agar. The signal strength of the reporter was readily detectable by visual inspection of cells grown on agar plates from both the media-only and the media with ground beef assays.

Significance: This study demonstrates that the NanoLuc®-labeled *Salmonella* Typhimurium strain can be routinely used as a positive control for media testing and in the detection of microbial pathogens in food.

P3-44 ESIA ONE DAY: A One Broth One Plate ISO 16140 Validated Method for *Cronobacter* Species Detection in 48 Hours, Confirmation Included

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Introduction: *Cronobacter* spp. are opportunistic foodborne pathogens that can cause severe infections in preterm and full-term infants. bioMérieux propose a new validated protocol. The final result could be in 48 h with a proprietary confirmation test.

Purpose: The aim was to evaluate the performances of ESIA One Day™ versus the reference method for the detection of *Cronobacter* spp in infant food products and production environmental samples.

Methods: The comparative study was performed by comparison to the reference method ISO/TS 22964:2006 and following the protocol EN ISO 16140:2003 in infant formulas, milk powders and dehydrated complements of infant formulae, matrices used in infant formulae and soy milk, environmental samples from milk industries. The protocol of alternative method is an enrichment in buffered peptone water supplemented with vancomycin. Thirty gram samples were diluted to one-tenth and 300 g samples to quarter. After an incubation time of 18 ± 2 h at 37 ± 1°C, enriched broth was isolated on ESIA™ medium. Plates were incubated 21 h at 44 ± 1°C. Typical colonies (blue) were confirmed directly by conducting Fast Crono Confirmation test, by inoculating ID 32E gallery or by Rapid ID 32E.

Results: The study proved that there were no significant difference between ESIA One Day™ method and ISO/TS:22924 standard. Sensitivity and specificity of the alternative method is equivalent to the reference method. Moreover, the shorter confirmation protocol could give a result in 5 min.

Significance: ESIA One Day™ method, validated following the protocol EN ISO 16140:2003, is easy to implement in a routine laboratory workflow and will help the end-user by improving the time to results.

P3-45 Non-Pathogenic Microorganisms Complicate Recovery of *Listeria monocytogenes* from Buffered *Listeria* Enrichment Broth

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Introduction: The recovery of *Listeria monocytogenes* from buffered *Listeria* enrichment broth (BLEB) can be complicated by non-pathogenic *Listeria* species in the test sample which typically reach higher populations. Different species of *Listeria* demonstrate variation in their tolerance to background microorganisms during selective enrichment further increasing the inter-species post-enrichment population differentials and further hindering *L. monocytogenes* recovery.

Purpose: This study evaluates the effects of *Citrobacter braakii* on the recovery of *L. monocytogenes* when additional species of *Listeria* are present in the test sample.

Methods: The effects of *C. braakii* on the final populations of *L. monocytogenes* (n = 100), *Listeria innocua* (n = 55), *L. seeligeri* (n = 14), and *L. welshimeri* (n = 31) were determined. Select pathogenic and non-pathogenic *Listeria* strains were paired based on sensitivity to *C. braakii* growth and the pairs were used to inoculate BLEB along with *C. braakii*. The population differentials between the *Listeria* pairings were determined. These same *Listeria* pairs were co-inoculated along with *C. braakii* into cheese, guacamole, and crab meat and *L. monocytogenes* recovery using Oxford and Rapid'*L. mono*™ chromogenic agars was evaluated.

Results: The enrichment populations of individual strains of *Listeria* when grown in the presence of *C. braakii* ranged from 1.8 to 8.0 (mean = 7.0 ± 1.1) log CFU/ml for *L. monocytogenes*, 3.7 to 8.2 (mean = 7.5 ± 0.9) log CFU/ml for *L. innocua*, 1.3 to 7.8 (mean = 6.7 ± 1.6) log CFU/ml for *L. seeligeri*, and 1.3 to 8.1 (mean = 7.6 ± 1.2) log CFU/ml for *L. welshimeri*. The population differentials between paired species of *Listeria* were too great for recovery of *L. monocytogenes* from spiked food samples when using either recovery medium.

Significance: The presence of background microorganisms can further increase the population differentials between competing strains further increasing the difficulty of recovering *L. monocytogenes* even when using a species differentiating medium.

P3-46 Semi-Quantitative Method to Estimate Levels of *Campylobacter*

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Introduction: Research projects utilizing live animals and/or animal systems often require reliable, accurate quantification of *Campylobacter* following treatments. Even with marker strains, conventional methods designed to quantify are labor and material intensive requiring either serial dilutions or MPN procedures to estimate the numbers of *Campylobacter* in ceca.

Purpose: The objective of this study was to devise an economical method which could accurately estimate the colony forming units per gram (CFU/g) of *Campylobacter* in the ceca of chicks.

Methods: Day old chicks, housed in isolation units, were gavaged with a marker *Campylobacter coli* (Cc^{GR}), euthanized at 7 or 14 days and the ceca aseptically removed. All cecal samples were diluted 1:3 (w:v) in Tecra Broth (TB) and stomached for 60 s. Cc^{GR} recovery was conducted on Campy Cefex agar plates w/ 200 ppm gentamicin using a semi-quantitative method (SQM) previously used for *Salmonella* and by enumeration. Ceca were obtained from a local processing plant, divided into three aliquots and inoculated with a known CFU of the Cc^{GR} at three levels. The ceca and inoculum were weighed, 3 times the volume of TB added and then enumerated as described for the above cecal samples.

Results: The SQM was comparable to the conventional enumeration method in 20/20 7-day and 19/20 14-day old broiler chicks. In addition to the bird study, a controlled experiment verified the reliability of the SQM. With inoculated ceca using three levels of Cc^{GR} (10^1, 10^3, and 10^5 CFU/g), the SQM showed no statistical difference ($P > 0.05$ by paired *t*-test) from the CFU/g of inoculated cecal material and was more accurate than the standard method when predicting the inoculated number of cells.

Significance: This SQM provides researchers with an accurate, inexpensive and useful method to enumerate *Campylobacter* marker strains utilized in research studies.

P3-47 Innovative Solution for the Detection and Confirmation of the TOP7 Shiga Toxin-producing *E. coli* (STEC) Serogroups in 375g Raw Beef

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Introduction: Shiga Toxin-producing *Escherichia coli* (STEC) are an important cause of foodborne illness. This pathogen can be responsible for gastrointestinal diseases ranging from diarrhea to hemorrhagic colitis and hemolytic uremic syndrome. As a result, the food industry needs fast, sensitive and complete methods for STEC detection to ensure a safe food supply.

Purpose: VIDAS UP *E. coli* Serogroups (ESPT) was developed as a global method using phage recombinant proteins for the immuno-concentration (IC) of *E. coli* serogroups O157, O26, O103, O111, O145, O45 and O121 from food enrichments. IC will be used both for screening and confirmation steps. The aim of this study was to compare the new alternative method with the ISO 13136 standard.

Methods: For the alternative method, 375 g artificially contaminated raw beef products were ¼ diluted in a selective pre-warmed BPW and enriched for 10 h at 41.5 ± 1°C. For the reference method, 25 g samples were 1/10 diluted in BPW and enriched for 20 h at 37 ± 1°C. DNA was extracted using VIDAS ESP1 protocol or commercial lysis and analyzed by PCR (*eae*, *stx*, O-groups). Any positive samples were confirmed by immuno-concentrating the STEC strains with VIDAS ESP2 protocol or commercial IMS from the enrichment broth and streaking the immuno-concentrated samples onto selective agar plates. After incubation, typical colonies were further confirmed by SLIDEX *E. coli* tests and/or PCR tests.

Results: Among the 56 tested samples, 41 were obtained positive by both the methods. The results obtained for the two methods, in the tested experimental conditions described above, are comparable.

Significance: This study has demonstrated that the VIDAS ESPT assay is a promising tool to screen and isolate STEC strains from food enrichment. This complete alternative solution will provide technical and economic advantages to the food industry for routine testing.

P3-48 AFNOR Validation of RAPID' *P. aeruginosa*: A Shorter Method for Detection and Enumeration of *Pseudomonas aeruginosa* in Bottled Water

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Introduction: The standardized ISO 16266 reference method for the detection and enumeration of *P. aeruginosa* involves the use of CN agar. The lack of specificity of this medium requires subsequent laborious confirmatory tests. The reference method is consequently costly and time-consuming. The use of selective chromogenic agar increases the ease of use. Results are based on color change enzymatic reaction without confirmation, reducing the time to results.

Purpose: RAPID'*P.aeruginosa* was developed to allow the direct enumeration (without confirmation) of *Pseudomonas aeruginosa* using the membrane filtration method for the testing of water for human consumption. The principle is based on the detection of an enzymatic activity typical of *P. aeruginosa*. The selective mixture makes it possible to inhibit the majority of interfering flora in particular *Pseudomonas* non-*aeruginosa*. An evaluation based on NF VALIDATION rules and ISO 16140 standard was conducted using a short (24 h) incubation time.

Methods: The chromogenic medium was evaluated by a comparison study performed by ADRIA Développement according the ISO 16140 requirements in comparison to the ISO 16266 reference method.

Results: Inclusivity study carried out with 25 *P. aeruginosa* strains showed that 24 strains were detected within 22 h on the chromogenic medium while the incubation time for CN Agar was 48 h. Among the 24 strains belonging to non-*P. aeruginosa*, none was able to grow on the chromogenic medium while 11 strains grew on CN agar, with one confirmed false positive. Relative accuracy was performed with 21 bottled water samples spiked with different levels of *P. aeruginosa*. Results showed that numerations obtained with chromogenic medium were equivalent to those obtained with CN agar.

Significance: RAPID'*P. aeruginosa* proved to be an effective alternative method for the detection and enumeration of *P. aeruginosa* in bottled water. The high specificity of the medium decreases time-to-results and costs associated with confirmation steps.

P3-49 Development of a Modified USDA FSIS MLG 5B.03 Screen-based Workflow to Detect and Isolate Shiga Toxin-producing *Escherichia coli* (STEC) Big Six Serogroups and *E. coli* O157:H7 in Ready-to-Eat Food and Produce

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Introduction: Shiga Toxin-producing *Escherichia coli* (STEC) is an enteric pathogen that causes hemorrhagic diarrhea, which can progress to hemolytic-uremic syndrome (HUS). Foodborne outbreaks causing HUS were initially associated with *E. coli* O157:H7 (EHEC), but a growing number of incidences were linked to the STEC "Big Six" serogroups (O26, O45, O103, O111, O121, and O145). After observing outbreak and policy trends, BFL-FDACS initiated a study to expand testing into screening and confirmation of STEC Big Six serogroups in Ready-to-Eat (RTE) food and produce.

Purpose: The purpose of this study was to construct a workflow for screening RTE food and produce by optimizing enrichment, DNA extraction, detection, and isolation parameters and apply those to appropriate matrix extensions. This study built upon the USDA MLG 5B.03 method, which only validated meat products.

Methods: The STEC Big Six *E. coli* serogroups and O157:H7 were spiked into a variety of RTE food matrices and enriched. Extracted DNA was screened for *stx1*, *stx2*, and *wzy* genes, and a secondary screen for Big Six serogroups, *eae*, and *stx* was performed using USDA FSIS MLG 5B.03. Screening results steered the cultural isolation and confirmation of the appropriate *E. coli*.

Results: The enrichment (mBPW) and extraction (MagNA Pure Compact) were optimized in the initial matrix with 100% detection and confirmation (n = 6). Matrix extensions on RTE sprouts, hot dogs, and cilantro demonstrated the method can detect and confirm STEC in 100% of spiked samples (n = 12 for each commodity). ChromSTEC and EMB agars most effectively isolated STEC.

Significance: BFL-FDACS has successfully developed a two-step STEC screening and isolation method for RTE foods and produce that can be expanded to test *E. coli* isolated from other laboratory food samples. Improved detection and classification of STEC found in food would result in a more effective surveillance program that will enhance public safety.

P3-50 Evaluation of the FDA BAM Method for Detection of Low Levels of *E. coli* O157:H7 in Artificially Inoculated Alfalfa Seeds

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Introduction: Contaminated sprouts have been linked to numerous illness outbreaks. Seeds are often identified as the contamination source via epidemiological means. Analytical methods capable of identifying causative pathogens in contaminated seeds are needed to support regulatory actions.

Purpose: Evaluate the BAM method for detection of *E. coli* O157:H7 in inoculated alfalfa seeds. The sensitivity of culture method vs. qPCR for screening contaminated samples was compared.

Methods: Test seeds spiked with different levels (0.1, 1, or 10 % w/w) of inoculated seeds (containing ~1 log CFU/g of *E. coli* O157:H7) were divided into 25-g aliquots. Each aliquot was mixed with 225 ml of pre-enrichment media (mBPWp) and incubated according to the BAM. Presence of the pathogen was determined either by culture method (streaking or spread plating on TC-SMAC or R&F *E. coli* O157:H7 agar) or by qPCR using SmartCycler or ABI-7500.

Results: Culture method and qPCR showed similar sensitivity. Both detected *E. coli* O157:H7 in all samples spiked at a 1% level (*E. coli* O157:H7 concentration of -1 log MPN/g). At a 0.1% spiking level (*E. coli* O157:H7 level of -2 log MPN/g), presence of the pathogen was indicated in 9/20 and 8/20 samples using culture method and ABI-7500 test, respectively. In seeds spiked at a 0.01% level (*E. coli* O157:H7 level < -2.52 log MPN/g), the pathogen was detected in 1/20 samples using culture method but was not detected by ABI-7500. The sensitivity of the culture method can be significantly improved by incorporating immunomagnetic separation (IMS). With IMS, the number of positive samples increased from 9/20 to 18/20 and from 1/20 to 8/20 in seeds spiked at 0.1% and 0.01% level, respectively.

Significance: The BAM method performed well for detection of low levels of *E. coli* O157:H7 in alfalfa seeds and will be useful in supporting outbreak investigations and other surveillance activities.

P3-51 Screening and Isolation Methods for *Salmonella* Dublin from Bovine Sources

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Introduction: *Salmonella* Dublin is the host-adapted serovar for cattle and poses a significant economic loss to the dairy industry as well as a zoonotic risk. Intermittent shedding by carrier animals has been linked to several foodborne disease outbreaks due to consumption of raw milk. An improved method to screen and selectively isolate *Salmonella* Dublin from bovine samples is needed for effective herd management and to reduce risk of foodborne illness.

Purpose: Develop and evaluate molecular and cultural methods to differentiate *Salmonella* Dublin in various bovine samples.

Methods: *Salmonella* Dublin strains (n = 25) and non-Dublin strains (n = 5) were inoculated (100 CFU/g) into bovine samples (colostrum, blood, feces) and their growth was evaluated in five selective enrichments [(Rappaport-Vassiliadis (RV; 2 formulations), tetrathionate broth (TT; 2 formulations), selenite cysteine broth (SC)] by a real-time PCR targeting *inv*A and *vag*C genes. Differentiation between Dublin and non-Dublin strains was evaluated using Xylose Lysine Desoxycholate agar with arabinose (XLD+A), Modified Semisolid RV (MSRV), Simmon's Citrate Agar (SCA), Congo Red Agar (CRA). All media were incubated at 37°C for 24 h prior to evaluation with the exception of CRA (25°C, 7 days).

Results: The highest cell densities (as determined by Ct values from PCR assay) and easiest isolation of *Salmonella* Dublin were achieved with TT (USDA formulation) and SC enrichments. Dublin strains displayed significantly different phenotypes than non-Dublin strains on MSRV and SCA; however, this was mostly due to Dublin's inability to grow or demonstrate motility. Extended incubation on CRA produced the most distinct differentiation between the Dublin (smooth/white) and non-Dublin isolates (rough/red).

Significance: A simplified cultural method coupled with molecular screening was deemed suitable for isolating and differentiating *Salmonella* Dublin from background *Salmonella* spp. This method can be used to analyze a wide range of microbiologically complex bovine samples for shedding status and herd health decisions.

P3-52 Accuracy and Precision of Coliforms Testing in National Shellfish Sanitation Program

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Introduction: Laboratories and analysts participating in the National Shellfish Sanitation Program (NSSP) are certified by inspection as well as successful proficiency test (PT) participation. FDA PTs are analyst based and permit laboratories to use preferred methods to test shellfish samples.

Purpose: Quantitatively analyze variability of analysts, laboratories and methods enumerating coliforms in shellfish waters based on 2013 and 2014 FDA PTs.

Methods: Eight growing area and eight process water simulated shellfish samples were inoculated with coliforms at 10-80 CFU/100 ml. The inoculation procedures, mixing methods and homogeneity/stability were verified according to ISO 17043 and 13528. Participants utilized NSSP-approved methods (most probable number (MPN) or membrane filtration). A total of 119 analysts (48 laboratories) in 2014 and 121 analysts (48 laboratories) in 2013 tested simulated shellfish samples for total and/or fecal coliforms.

Results: Accuracy and precision were calculated according to ISO 13528. Analyst z-scores were based on robust consensus means. Fecal coliform reproducibility SD was 0.279 and 0.177 in 2013/14 for MPN, and 0.263 and 0.165 in 2013/14 for membrane filtration. Similar patterns were seen in process water samples where total coliform reproducibility SD was 0.305 and 0.197 in 2013/14 for MPN, and 0.275 and 0.158 for membrane filtration. Test on equivalency according the Q-Hampel method showed MPN and membrane filtration did not have equivalent performance across 8 growing and 8 process water samples in 2013. Means for MPN and membrane filtration were not equal, but the mean deviation fell within tolerated limits across 8 growing water and 8 process water samples in 2014.

Significance: Analysis of data from two consecutive annual PTs demonstrates method variability plays a role in coliforms testing and could affect enforcement of shellfish regulations. This study shows how large-scale PTs in relevant matrices can be used to generate method performance data and evaluate overall performance of laboratories.

P3-53 Evaluation of Modified Semisolid Rappaport Vassiliadis Motility Agar (MSRV) as a Selective Enrichment Medium for the Isolation of *Salmonella* from Mung Bean, Alfalfa, and Clover Sprouts with the Bacteriological Analytical Manual (BAM)

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Introduction: A variety of sprout types have been identified as sources of *Salmonella*. Development of a culture method for use with sprouts will benefit sprout surveillance efforts and assist in identifying origins of contamination in sprout related outbreaks.

Purpose: Modified Semisolid Rappaport Vassiliadis (MSRV) motility agar is evaluated as a selective enrichment medium in isolating *Salmonella* from artificially contaminated test portions of alfalfa, mung bean, and clover sprouts.

Methods: Comparison of Fractional Positive Values (FPV) for MSRV, RV, and TT selective enrichment media were performed with Fisher's Exact 2-tailed F-test ($P < 0.05$). The FPV is that number of test portions identified as positive among 20 analyzed. Preenrichments were performed with

Lactose broth (LB; BAM method) and Universal Preenrichment Broth (UPB). FPVs of the preenrichment media/selective enrichment media were also compared to evaluate effectiveness of LB and UPB ($P < 0.05$)

Results: RV media was more effective than TT and MSRV (FPVs of 6, 1, and 0) in isolating *Salmonella* Newport from mung beans, as was UPB over LB (all FPVs = 0) ($P < 0.05$). For isolating *Salmonella* Stanley and *Salmonella* Oranienburg from clover sprouts with LB preenrichment media, respective FPVs for TT of 8 and 13 were higher than FPVs for RV and MSRV of 0 ($P < 0.05$). The FPVs for MSRV were lower than FPVs for RV and TT with UPB preenrichment media ($P < 0.05$). No differences in FPVs occurred in trials with alfalfa sprouts except between UPB and LB with UPB being more effective as preenrichment media ($P < 0.05$).

Significance: The use of MSRV for the isolation of *Salmonella* from alfalfa, mung bean, and clover sprouts is not recommended, however UPB is more effective as preenrichment media than LB for these sprout types ($P < 0.05$).

P3-54 A Rinse/Membrane Filtration Method for Detection of Bacterial Pathogens in Cantaloupe and Jalapeño Pepper

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Introduction: Shiga Toxin-producing *Escherichia coli*, *Salmonella enterica*, and *Listeria monocytogenes*, are among the major pathogens that can be found as contaminants of fresh produce. To reduce the risk of disease, more efficient methods for detection of these pathogens in produce are needed.

Purpose: To compare a novel rinse/membrane filtration method (RMFM) to more traditional sponge rubbing or stomaching in preparing fresh produce samples for detection of pathogenic bacteria.

Methods: Decontaminated jalapeño peppers and cantaloupes were inoculated with three levels of inoculum of each organism separately (*S. enterica, L. monocytogenes* and *E. coli* O157:H7; 10^2, 10^4 and 10^6 CFU). Bacteria were recovered using the RMFM in parallel with homogenization by stomacher and a sponge surface rubbing method, followed by standard culture methods (BAM). To determine relative accuracy, relative specificity and relative sensitivity, the MFM was compared to the other methods following the ISO16140:2003 protocol. The detection limit was determined by the MPN technique and results analyzed (Kruskal Wallis and Fisher tests).

Results: When 10^4 and 10^6 CFU were inoculated, all bacteria were detected in 100% of samples by the three methods. However, with an inoculum of 10^2 CFU, *S. enterica* was detected in 20%, 60% and 20% of samples using RMFM, stomacher and sponge respectively. *L. monocytogenes* was detected in 40%, 60% and 20% of samples using RMFM, stomacher and sponge respectively; whereas *E. coli* was detected in 80%, 60% and 60% of samples by RMFM, stomacher and sponge, respectively. The limit of detection of RMFM (1.0 - 2.3 MPN/g) was similar to the other methods (0.9 - 2.8 MPN/g). In all cases, specificity and relative accuracy was more than 95%.

Significance: Overall, the RMFM performed similar or better than the homogenization and sponge surface sampling methods. The RMFM method might be a good alternative for processing large numbers of samples for pathogen detection.

P3-55 Evaluation of a Simplified *E. coli*/Coliform Method for a Variety of Water and Produce Rinse Samples

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Introduction: Water and produce rinse samples are tested for the presence of generic *E. coli* and coliform as sanitary and process control measurements. Peel Plate EC (*E. coli* and Coliform) is a pre-prepared self-wicking method with β-galactosidase (GAL) and β-glucuronidase (GLU) substrates to detect *E. coli* as blue/purple colonies and coliform as red colonies. The method has been validated in various food matrices. The 47 mm test area of the test and the absence of lid contact with the test medium allows the use of membrane-filters to concentrate liquid/water samples and apply the filter as test samples.

Purpose: The purpose of the study was to evaluate the new method in comparison to reference methods using mixed cellulose acetate filters of water and produce rinse samples.

Methods: Bottled water, irrigation water, produce flume, and diluted-cooling-water (5% propylene glycol) samples were co-inoculated with different coliform and *E. coli* strains at low, medium and high concentrations. One hundred ml samples of bottled water, produce rinse (mixed greens) chlorine neutralized with thiosulfate, and the diluted cooling water were filtered through 0.45 µm mixed cellulose filters and plated in comparison m-Endo LES agar method and confirmed by BGLB/EC-MUG broth. Irrigation water was filtered, 100 ml, and tested in comparison to EPA method 1604.

Results: In the method comparisons, results were evaluated for repeatability (S_r) and by paired *t*-test for statistical difference, > 0.5 log. The Peel Plate EC was not significantly different from the reference methods in the detection of coliform or *E. coli*.

Significance: Peel Plate EC detected *E. coli* and coliform at 35°C in 24 h similarly to reference methods in water and rinse samples and is a testing alternative for detecting fecal indicators in food production environments.

P3-56 Cold Plasma Inactivation of *Salmonella enterica*, Shiga Toxin-producing *Escherichia coli*, and *Listeria monocytogenes* by a Novel Surface Dielectric Barrier Discharge Device

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Introduction: Atmospheric cold plasma offers a dry, non-thermal, and rapid process for decontamination of food products and a novel cold plasma device based on Surface Dielectric Barrier Discharge (SDBD) has been developed in our laboratory.

Purpose: The objective of this study was to evaluate the inactivation efficacy of the SDBD-based cold plasma device against the major foodborne pathogens *Salmonella enterica* (*Se*), Shiga Toxin-producing *Escherichia coli* (STEC), and *Listeria monocytogenes* (*Lm*).

Methods: Five-strain cocktails of *Se*, STEC, or *Lm* cultures (10^7 CFU/sample) were spot inoculated onto sterile glass cover slips, air dried, and treated at various distances (1, 3, 5, and 7 cm) from the cold plasma actuators for different treatment times (2 and 4 min). Log reductions, calculated by comparing with inoculated untreated samples as controls, obtained from at least two replicating trials, were used to determine decimal reduction times (D-values) at each distance for each pathogen mixture.

Results: Inactivation of bacterial cells was observed at all distances and at both treatment times but with decreasing efficiency at increasing distance. Average log reductions for 4 min treatments at 1 cm were 4.1 for *Se*, 3.4 for STEC, and 2.3 for *Lm.* D-values (min) at 1 cm for *Se*, STEC, and

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Lm were 1.3, 0.9, and 1.8, respectively. These results confirm that the SDBD design induces a localized airflow that contains reactive species that decreases with distance from the actuator and that treatment time has an additive effect rather than a compounding effect.

Significance: This study confirms the ability to inactivate bacterial pathogens with cold plasma by SDBD and that induced airflow is the means of reactive species delivery to contaminated surfaces.

P3-57 Streamlined Food Pathogen DNA Extraction Using a Small Benchtop Automated Instrument

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Introduction: Every year, pathogenic microbe contamination in foods causes serious and sometimes fatal infections worldwide. It is, therefore, important to monitor food sources carefully. Application of molecular techniques is allowing fast and selective foodborne pathogen testing.

Purpose: Here we examine the utility of a small benchtop automated instrument, the Maxwell® 16, and novel cellulose-based magnetic particles for purification of amplifiable bacterial DNA from enriched food cultures.

Methods: Using enumerated aliquots of viable bacteria, we created 25 g food samples (lettuce, milk or ground beef) of specific pathogen contamination (1, 10, or 100 CFU). Bacteria tested were *Salmonella enterica*, *Listeria monocytogenes*, *Staphylococcus aureus* (milk and ground beef only) and Shiga Toxin-producing *Escherichia coli* O157:H7 (lettuce and ground beef only). After a standard overnight incubation to enrich for live microbes, we evaluated both a direct extraction (fast) protocol and a lysozyme treatment (maximum sensitivity) protocol for DNA extraction using a custom Maxwell® 16 kit. Following extraction, probe-based qPCR was used to detect purified microbial DNA. Extractions were performed in triplicate for each microbe, food type, and microbe concentration.

Results: We found that following both the fast and maximum sensitivity protocols, we were able to detect down to at least 10 viable microbes in each food tested, and in some cases the single microbe cultures were also positive. The difference in positive microbe detection between the fast and maximum sensitivity protocols was ≥ 5 Ct. The method was compared to an AFNOR and AOAC-certified manual purification protocol and found to give comparable results.

Significance: This study supports the use of Maxwell® 16 for automated purification of microbial DNA from enriched cultures for food safety testing.

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P3-58 Comparison of Four DNA Extraction Methods for Detection of *Salmonella* in Chia Powder Associated with a Foodborne Illness Outbreak in 2014

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Introduction: A multistate outbreak of multiple serotypes of *Salmonella* infections, linked to sprouted chia seed powder, were reported by CDC from January to June 2014. A total of 25 cases from 14 states were infected with the outbreak *Salmonella* strains: *Salmonella* Harford, and *Salmonella* Oranienburg.

Purpose: To compare the performance of four DNA extraction methods in their efficiency to extract *Salmonella* DNA from pre-enriched broth and selective enriched broths of naturally contaminated chia powder.

Methods: Around 200 g chia powder samples were received from a state lab. Twenty test portions (10 g each) were pre-enriched in 90 ml modified buffer peptone water for 24 h at 35°C. Aliquots of 1.0 and 0.1 ml from the incubated pre-enrichments were subcultured to 10 ml tetrathionate (TT) broth and to 10 ml Rappaport-Vassiliadis (RV) broth, respectively. From pre-enriched and selective enriched broths, DNA was extracted by using Bio-rad InstaGene matrix, Fortius LyseNow DNA extraction kit, Life Technology automated PrepSEQ nucleic acid extraction kit, and automated Qiagen Biosprint 96 one-for-all vet kit. *Salmonella* qPCR assay developed by FDA was performed using DNA extracted from 24 h pre-enriched broth and 48 h selective enriched broths (RV/TT).

Results: BAM culture result shows all 20 test portions positive. It indicates a high *Salmonella* contamination level greater than 9.34 MPN/g in this naturally contaminated chia powder sample. qPCR result (1 positive/20 test portions) from PrepSEQ extracted DNA in 24 h pre-enriched broth showed a significantly less detection rate than culture result. The average Ct values from LyseNow method are significantly higher than those from InstaGene and BioSprint one-for-all.

Significance: To compare efficiency of different DNA extraction methods in pre-enriched and selective enriched culture will help improve sensitivity and reliability of the detection of *Salmonella* by qPCR assay from food products with different *Salmonella* contamination levels.

P3-59 Optimization of Cold Plasma Actuator Design for Decontamination of *L. monocytogenes*

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Introduction: As a relatively new microbial inactivation technology, non-thermal or cold plasma has been gaining a lot of interest in food safety applications. Various modes of plasma generation have been explored but most of these designs require high power input and an artificial gas flow, therefore complicating their usages.

Purpose: The objective of this study was to optimize cold plasma actuator design for efficient inactivation of foodborne pathogens.

Methods: Surface Dielectric Barrier Discharge (SDBD) cold plasma devices were constructed with electrodes arrangement either asymmetrically or symmetrically. The airflow dynamic of the devices were evaluated by Particle Image Velocimetry (PIV) and their efficacy in microbial inactivation was examined by using a five-strain cocktail of *Listeria monocytogenes* that were spot-inoculated onto sterile glass coverslips, placed at various distance (1, 3, 5, and 7 cm) from the plasma source, with inoculated untreated samples as controls.

Results: It was observed that the asymmetric arrangement of electrodes resulted in higher velocities and more turbulent flow than that of symmetric arrangement. The PIV data was supported by microbial inactivation data, in which significant ($P < 0.05$) higher log reduction of inoculated *L. monocytogenes* was achieved by the devices with asymmetric arrangement of electrodes than those of symmetric ones, with log reductions of 4.8 ± 0.5 vs 3.5 ± 0.5 at 1 cm and 2.3 ± 0.3 vs 1.1± 0.2 at 3 cm for asymmetric and symmetric devices, respectively.

Significance: These data show that the arrangement of electrodes in cold plasma device had significant effect on its efficacy in inactivation of *L. monocytogenes* and such optimization could be explored in the design of simpler cold plasma device.

P3-60 Efficacy of Gaseous Chlorine Dioxide in Killing *Salmonella enterica* on the Surface of Eggshells as Affected by Relative Humidity

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❖ ***Developing Scientist Competitor***

Introduction: Chlorine dioxide (ClO_2) in aqueous and gaseous forms has been widely used to inactivate foodborne pathogens from foods and food-contact surfaces. Compared to aqueous ClO_2, gaseous ClO_2 has been known to be more effective in killing foodborne pathogens and to leave less residue on food and food-contact surfaces after treatment. Despite these advantages, the effectiveness of gaseous ClO_2 against *Salmonella enterica* on eggshells has rarely been reported.

Purpose: This study was done to evaluate the lethal activity of gaseous ClO_2 against *S. enterica* on the surface of eggshells and to determine the optimum relative humidity (RH) conditions for the treatment.

Methods: Eggshells were inoculated with *S. enterica* contaminated chicken feces, were placed in airtight containers (1.8 liters) in which RH had been adjusted to 23, 43, 68, 85, or 100%, and were exposed to gaseous ClO_2 (peak concentration: 141.7 ppm) for 0, 0.5, 1, 2, 4, and 6 h at 25°C. After treating, the viable cell numbers of *S. enterica* on the eggshells were determined.

Results: The initial populations of *S. enterica* on eggshells were 5.9 log CFU/egg. After treatments with gaseous ClO_2 at 23, 43, 68, 85, and 100% RH for 6 h, the populations of *S. enterica* on eggshells decreased by 0.8, 0.9, 3.1, 5.9, and 5.9 log CFU/egg, respectively. At 85 and 100% RH, *S. enterica* on the surface of eggshells was completely inactivated within 4 h.

Significance: It was observed that gaseous ClO_2 was effective in inactivating *S. enterica* on the surface of eggshells and the lethality of gaseous ClO_2 against *S. enterica* on eggshells was maximized as the RHs were increased > 85%.

P3-61 Biofilm Formation of *Salmonella* Enteritidis and Its Tolerance to Chlorine Treatment under Different Environmental Stress Conditions

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❖ ***Developing Scientist Competitor***

Introduction: Biofilm formation of *Salmonella* spp. on food contact surface may increase the risk of foodborne illness. In food processing environment, *Salmonella* may be exposed to different stresses, such as low temperature, acidic or alkaline antimicrobials, and low nutrient conditions. However, little is known about how these stress conditions affect *Salmonella* Enteritidis biofilm formation and its resistance to sanitizer treatment.

Purpose: This study aimed to determine the effects of temperature, pH, and nutrient availability on *Salmonella* Enteritidis biofilm formation on stainless steel coupons (SSCs) and its resistance to chlorine treatment.

Methods: Biofilm formation was investigated on SSCs using *Salmonella* Enteritidis ATCC 13076 (bdar morphotype) and F124 (rdar morphotype). SSCs were immersed in tryptic soy broth (TSB) or diluted TSB (1/20 TSB) at various pH conditions (pH 5.3, 7.3, and 8.3) and incubated at 4 or 25°C. Biofilm density was evaluated after 2, 4, and 7 days of incubation and the formed biofilms were subsequently exposed to 50 ppm chlorine (pH 6.8) for 1 min. Mean values were compared using ANOVA.

Results: The density of *Salmonella* Enteritidis biofilm and its resistance to chlorine treatment were not greatly influenced by incubation time or pH. However, biofilm formed in 1/20 TSB had a significantly ($P < 0.05$) higher density compared to that formed in TSB. In addition, 1/20 TSB enhanced biofilm resistance to chlorine at 25°C, especially for *Salmonella* Enteritidis F124 that had 0.84-log reduction in 1/20 TSB but 3.79-log reduction in TSB at pH 7.3. Due to the greater sensitivity of biofilm formed at 4°C, no significant difference ($P > 0.05$) in resistance to chlorine between TSB and 1/20 TSB was observed.

Significance: This study may help to design better strategies to control *Salmonella* biofilm formation under different stress conditions.

P3-62 Efficacy of Detergent and Sanitizers on Shiga Toxin-producing *Escherichia coli* (STECs) Attached to Stainless Steel

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✦ ***Undergraduate Student Award Competitor***

Introduction: Pathogens attached to product contact surfaces have the potential to contaminate food products at varying stages of processing. Shiga Toxin-producing *Escherichia coli* (STEC) serogroups (O157:H7, O26, O45, O103, O111, O121, O145) that are adulterants in non-intact beef products can attach to commonly used surfaces based on our previous research.

Purpose: To evaluate the effectiveness of detergent and sanitizers and combinations of both in their ability to remove attached STECS from stainless steel (SS) and polyurethane coupons.

Methods: Using a STEC cocktail (one strain from each serogroup), cells were allowed to attach and grow on SS and polyurethane coupons for 24 h at 25°C. Coupons were rinsed to remove loosely attached cells and treated with detergent (detergent/water), sanitizer (water/sanitizer), bleach (water/bleach), detergent/sanitizer, detergent/bleach, control (water only), or no treatment. Coupons were immersed into the solutions for 5 min for each step and a concentration of 200 ppm for sanitizer and bleach. STEC cells were removed from the coupons, serial dilutions were made in BPW and plated onto Tryptic Soy Agar, incubated at 37°C for 24 h, and counted. Counts were converted to log CFU/cm^2 before statistical analysis.

Results: A significant ($P < 0.0001$) treatment effect occurred, with quaternary ammonium, bleach, and combinations of both with detergents being the most effective treatments. All treatments significantly reduced STECs when compared to the untreated coupons (14 log CFU/cm^2). Log reductions ranged from a 5 log CFU/cm^2 difference observed on coupons treated with detergent, to a 12 log CFU/cm^2 reduction with bleach only.

Significance: These results show that a complete cleaning and sanitation program is important to processing plants and food service establishments to reduce the amount of STECs that could attach and grown on surfaces such as SS and polyurethane. Proper sanitary methods are important to processing plants, food establishments, and food markets.

P3-63 Pulsed Light: Inhibitory Activity against Norovirus and Mechanism of Action

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Introduction: Pulsed light is a non-thermal processing technology which has been proved to be effective for the inactivation of bacteria in liquid media such as clear beverages. Drinking water is commonly associated with norovirus outbreaks, the leading cause of non-bacterial gastroenteritis. Pulsed light could therefore be a promising technology for inactivation of norovirus, yet little is known about viruses.

Purpose: This study assessed pulsed light efficiency for the disinfection of liquid media contaminated with human norovirus surrogate (MNV-1) according to their composition. The mechanisms involved in the inactivation of MNV-1 were also investigated.

Methods: Viruses were exposed to pulsed light in several suspensions (PBS buffer, hard water, mineral water, turbid water and sewage treatment effluent) to different fluences (from 0.7 to 9.0 $J.cm^{-2}$) in a Xenon SINTERON 500 device. The log reductions of infectious MNV-1 were determined by performing plaque assay. The mechanisms involved in the antiviral activity of pulsed light were elucidated by analyzing the morphology (electron microscopy), the viral proteins (SDS-PAGE) and the RNA integrity (Bioanalyzer, UPLC-MS/MS) of the treated MNV-1.

Results: The pulsed light device emitted a broadband spectrum (200–1000 nm) at a fluence of 0.7 $J.cm^{-2}$ per pulse with 2 % UV at 8 cm beneath the lamp. Reductions in viral infectivity exceeded 3 log in less than 3 seconds (5 pulses, 3.5 $J.cm^{-2}$) in clear suspensions. The presence of bentonite (turbidity) interfered significantly with viral inactivation ($P < 0.05$), leading to a reduction of 2.0 ± 0.4 log at the maximum turbidity (1000 NTU). Furthermore, pulsed light appeared to disrupt MNV-1 structure and degrade viral protein and RNA.

Significance: The results suggest that pulsed light technology could provide effective alternative means of inactivating noroviruses in wastewaters, in clear beverages or in drinking water, and so contribute to reduce the burden of norovirus illness.

P3-64 Efficacy of a Portable Aerosolized Hydrogen Peroxide Delivery Method for Inactivating Human Norovirus and Its Surrogates

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Poster

Introduction: Human norovirus (HuNoV) can persist on surfaces for weeks, and improved methods for environmental control of HuNoV are needed.

Purpose: To test the efficacy of a novel portable device for delivery of aerosolized hydrogen peroxide against GI.6 and GII.4 HuNoV, as well as bacteriophage MS2, Feline Calicivirus (FCV), and Tulane Virus (TV).

Methods: Sterile stainless steel strips (1 x 5 cm) were inoculated with 25 µl of either fecal stocks (HuNoV) or viral stocks (surrogates). Inoculated strips were allowed to dry and placed throughout a 1,040-ft^3 BSL3 containment lab. The air handler unit was turned off, and an 8% aerosolized hydrogen peroxide (aHP) solution was delivered into the room using the RDS3110 portable unit (Aeroclave LLC, Winter Park, FL) until the target concentration of aHP was met (ranging from 0.2 to 0.35 ml/ft^3). The target concentration was held for 5 min, and the air handler unit was then turned back on to reduce aHP levels to below 2 ppm. Exposed virus was eluted in PBS and stored at -80°C prior to analysis. Impact of aHP on viral fitness was determined using RT-qPCR for HuNoV samples, and plaque assays for surrogate viruses. All experiments were performed in triplicate.

Results: The amount of aHP injected was significantly associated with the degree of virus inactivation ($P < 0.05$). Exposure to 0.35 ml-aHP/ft^3 achieved a 2.5-log reduction in RT-qPCR units for both GI.6 and GII.4 HuNoV, and a 5.5, 4.3, and 3.4-log reduction in PFU for MS2, FCV, and TV, respectively.

Significance: FCV and MS2 were more sensitive than HuNoV or TV to aHP. TV may be a better surrogate than FCV for future disinfection studies. The RDS3110, using an 8% H_2O_2 solution, is a portable and effective means for HuNoV disinfection for users concerned with hazards associated with higher concentrations of H_2O_2.

P3-65 Efficient Sanitation Method of Jars Used for Fermenting Step of Korean Traditional Sauce Production

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Introduction: Traditionally, the Korean soybean sauce/paste were placed and fermented in a jar for a certain period. It is very limited to clean and sanitize the jar because the use of detergent or sanitizer will cause the quality deterioration or off-odors. Other sanitation methods are also known hard to eliminate unfavorable microbes in jar.

Purpose: This study was to evaluate the efficiencies of sanitation methods applied for jar and to propose an effective treatment using the hot air by burning solid-fuels.

Methods: The inside and bottom surfaces of jars were swabbed using 3M pipette-swab before and after the treatments. The sanitation methods used commonly including straw fire, 30% alcohol, 20% liquor (*soju*), boiling water, baking soda solution and 2% solution of glacial acetic acid were applied to clean and sanitize the jars while the thermal treatment with solid-fuels was also evaluated. The number of total aerobic bacteria, lactic acid bacteria, and mold and yeast were analyzed by following method of Korea Food Code.

Results: The various treatment methods were shown to reduce the total aerobic bacteria only by maximum of one log CFU/100 cm^2; boiling water treatment was 4.95 to 3.67 log CFU/100 cm^2 and 2% solution of glacial acetic acid for one hour immersion was 3.96 to 2.36 log CFU/100 cm^2, while a jar treated with heat of solid fuel was shown no growth of microorganisms from initial 4.59 log CFU/100 cm^2. Excessive use of solid-fuel could break the jar and thus, the proper ratio between solid-fuels and jar volume should be calculated to prevent the too high temperature to break a jar, such as about 70 g of solid-fuel for 20 liter and 280 g for 130 liter jars.

Significance: These results suggest an effective thermal treatment of jars, and therefore, it contributes to improve the hygienic management of jars used in traditional sauces fermentation process.

P3-66 The Effects of *Ginkgo biloba* Extract on the Growth, Motility and Biofilm Formation of *Salmonella* and *Listeria* Isolates from Poultry

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Introduction: *Salmonella* and *Listeria* are common foodborne pathogens in poultry and have caused a large number of outbreaks worldwide. Biofilm formation of *Listeria* and *Salmonella* is common in food industry and is also a mechanism of antimicrobial resistance.

Purpose: The purpose of this study was to investigate the effect and mechanism of *G. biloba* extract against *Listeria* and *Salmonella* biofilm formation.

Methods: Antibiofilm assay was performed by incubating *Listeria* and *Salmonella* isolates from poultry on cell culture slide with or without *G. biloba* extract. Biofilm was also stained with the LIVE/DEAD BackLight bacterial viability kits and observed by fluorescence microscope. *Listeria* and *Salmonella* isolates were inoculated on motility agar with or without *G. biloba* extract to investigate the influence of *G. biloba* extract on motility. Effect of *G. biloba* extract on the growth of *Listeria* and *Salmonella* were also evaluated by measuring the optical density of bacterial culture incubated with or without *G. biloba* extract.

Results: *G. biloba* extract at 100 µg/ml significantly inhibited initial cell attachment and biofilm formation of *Listeria* and *Salmonella*. *G. biloba* extract reduced the swimming motility of *L. innocua* and *L. welshimeri* and the swarming motility of *Salmonella* Enteritidis and *Salmonella* Stanley. Swarming and swimming motility inhibition may be one of the mechanisms of the antibiofilm effect of *G. biloba* extract against some serotypes of *Salmonella* and *Listeria*. *G. biloba* extract also inhibited the growth of *Listeria* but have no influence on *Salmonella*.

Significance: The findings of this study provide the basis for the application of *G. biloba* extract as food additives to promote the quality and safety of poultry products.

P3-67 Contact Time and its Effect on Cross-contamination of *Enterobacter aerogenes* from Stainless Steel, Ceramic Unglazed Tile, Carpet, and Wood to Food

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❖ ***Developing Scientist Competitor***

Introduction: The popular culture notion of the "five second rule" states food dropped on the floor for less than five seconds is "safe," because bacteria need time to transfer. The rule has been explored to a very limited degree in the published literature.

Purpose: This study quantifies cross-contamination rates of *Enterobacter aerogenes* from four common household surfaces to two representative foods considering contact time and the matrix containing the organism.

Methods: Two inoculum matrices were used: tryptic soy broth (TSB) containing an overnight culture and peptone buffer containing cells from overnight culture separated by centrifugation. Household surfaces (ceramic unglazed tile, indoor/outdoor carpet, wood, stainless steel) were inoculated with a nalidixic acid resistant strain of *E. aerogenes* and dried for 5 h. Surfaces contained ~7.0 log CFU/surface after drying. Squares (4x4 inches) of white bread and gummy squares were dropped on the respective surfaces from 5 inches and left to rest for four different times (0 to 300 s), and the transferred cells enumerated. Each unique condition was replicated 20 times for 1,280 total measurements.

Results: *E. aerogenes* showed increased transfer from tile, wood or stainless steel to white bread with increased contact time. Transfer ranged from below detection limit (0 sec, buffer inoculum on tile to white bread) to a high of 19.0% (TSB inoculum on tile to white bread at 300 s). The transfer from carpet to white bread was always below the detection limit (2 log CFU/sample) for each experiment. The transfer from all surfaces, inoculum suspension types, and contact times to gummy squares was below the detection limit except for tile and wood at 300 s in TSB inoculum, where ~0.20% transfer occurred.

Significance: Longer contact times promote greater transfer. Surface type (both contact surface and food) often has a more significant effect than contact time.

P3-68 Evaluation of Repeatability of Surface ATP Test Devices at Different Environmental Temperatures

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Introduction: The hygiene of food contact surfaces is critical to ensure the safety and quality of foods. The use of surface ATP hygiene monitoring systems is widespread and provides food business operators with rapid, measurable and cost effective tests. This enables them to take corrective actions in a timely manner and improve surface hygiene. Therefore, it is essential that the ATP hygiene monitoring system provides accurate and reliable results.

Purpose: To evaluate the repeatability of seven brands of surface ATP test devices and luminometers at different environmental temperatures, in order to assess performance differences, and whether repeatability varies at different temperatures.

Methods: The performance of the luminometers and ATP test devices was evaluated at 5°C, 10°C, 20°C and 35°C using an environmental chamber. All devices were tested by pipetting 10µl of a 4 x 10^{-9} M ATP solution onto the mid-section of the swab/sponge bud of each device. Testing was completed following the instructions provided by the device manufacturer. The repeatability was assessed by calculating the Coefficient of Variance (%CV).

Results: Ten repeat samples at each temperature for all manufacturers were obtained to evaluate the repeatability. The results suggest that only one of the seven devices had consistently repeatable results (%CV < 20) at all temperatures studied.

Significance: There are considerable differences between the repeatability performances of the brands; it is essential that hygiene monitoring systems provide a repeatable result to ensure consistency and accuracy of results. Effective and accurate surface assessment can be used for optimization of sanitation procedures and continual improvement.

P3-69 Quantitative Assessment of Cabin Crew Hand Hygiene Knowledge, Attitudes and Self-reported Practices

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❖ ***Developing Scientist Competitor***

Introduction: The importance of food safety of airline catering is recognized, with meals produced on the ground and consumed later in the sky. As professional food handlers, cabin crew are responsible for receiving, storing, reheating and serving food on-board as well as handling chemicals, sick people, infants, special meals, cleaning toilets. Thus, hand hygiene is a critical issue. Many studies have been conducted on handwashing in different sectors of catering industry but not for airline catering, particularly cabin crew.

Purpose: This study assessed cabin crew handwashing/hygiene knowledge, attitudes, and self-reported practices. Findings will help identify malpractices and also inform development of future food safety training of cabin crew.

Methods: An online quantitative questionnaire was developed using qualitative in-depth interview data from cabin crew training managers/supervisors and previous cognitive research literature. The questionnaire was administered to a purposeful sample of 2500 cabin crew from 20 airlines.

Results: Overall, 307 questionnaire responses were obtained (response rate 12.28%). Results revealed that 60.3% cabin crew reported previous training/instruction in food safety/hygiene (70% female; 30% male). The majority (95%) of those trained knew the main reason for washing hands on-board was to remove 'dirt and bacteria'; however, in contrast, 90% of untrained cabin crew were unaware. Of concern, 11.1% of respondents reported that they did not 'always' wash their hands after visiting the toilet (M = 4.28 untrained, M = 4.17 trained). A positive attitude towards use of antibacterial sanitizers was determined by trained and untrained staff. The majority (61.6%) of trained staff reported awareness not to handle foods with bare hands, however, 64.7% of untrained cabin crew acknowledged to not washing bare hands when touching any of body parts, e.g., hair.

Significance: These findings could be used by airlines with no/limited food safety training to cons cabin crew hand hygiene malpractices and thus introduce appropriate training to reduce the potential risk of foodborne disease from on-board aircraft.

P3-70 The Performance Level of Food Safety Management Practices for School Food Service Providing More Than Two Meals a Day in Korea

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Introduction: School food service has been estimated to have the highest cases of foodborne illnesses by location in Korea. Especially, the number of outbreaks for school food services that provided food 2 or 3 times daily was 5.7 times higher than for once-a-day servings in 2013.

Purpose: The purpose of this study was to identify the elements that require improvement in order to ensure the safety of school food service by assessing sanitary management practices in school food services providing meals 2 or 3 times a day.

Methods: Three sequential inspections were performed to assess the level of hygiene practices of 23 school food service providers in three cities within the southern Gyeongbuk province between November and December 2014. HACCP-based hygiene and safety inspection checklists consisted of the three sections: mandatory hygiene and sanitation management, recommended hygiene and sanitation management, and compliance of food hygiene law.

Results: Surveyed schools scored 72.17 ± 8.80 points out of 100 on average. The lowest scores (% of compliance) were 5.08/9 (56.4%) for facilities and equipment management and 3.13/6 (52.2%) for personal hygiene in all three inspection sections. Proper hand-washing as well as adequate washing and sanitization of rubber gloves were not in compliance in any of the surveyed schools.

Significance: As the lack of prerequisite programs and personal hygiene management were identified as the main elements to improve, training programs to support the implementation of prerequisite programs by school food service providers should be emphasized.

P3-71 Efficacy of Food Hygiene Chemicals against *Pseudomonas aeruginosa* Biofilms Grown Using ASTM E-2871-12

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Introduction: Biofilms are microbial communities that can contaminate food environments and are difficult to eradicate with many cleaners and sanitizers at the levels at which they are typically used. A test method (ASTM E-2871-12) has been developed as a way to grow biofilms that can then be used to evaluate the ability of cleaners and sanitizers to inactivate them.

Purpose: The purpose of this study was to determine the reduction of *Pseudomonas aeruginosa* biofilms when exposed to standard and elevated levels of cleaners and sanitizers.

Methods: *P. aeruginosa* (ATCC 15442) biofilm was exposed to 21 cleaners and sanitizers using ASTM E-2871-12. Most products were tested at three concentrations. Concentrations tested included the standard use concentrations and levels that were significantly higher than is normally used.

Results: The results were grouped into 3 different categories, products with log reductions below 3, significantly above 3, and significantly above 4. The chemicals that demonstrated a log reduction significantly greater than 3 at 95% confidence level were: A mixed halogen sanitizer diluted 1:100 and an undiluted alcohol and quaternary ammonium blend. The chemical concentrations that demonstrated a log reduction significantly greater than 4 at 95% confidence level were: A mixed halogen sanitizer diluted 1:10, a chlorinated alkali cleaner diluted 1:10, chlorine dioxide at 25 ppm, and chlorine dioxide at 100 ppm. All other cleaners and sanitizers tested inactivated less than 3 log of the biofilm.

Significance: The results show that many chemicals that are currently used as cleaners and sanitizers do not kill a *P. aeruginosa* biofilm at the concentrations that they are typically used, indicating that if biofilm elimination is the goal then the chemicals may need to be used at elevated levels or process changes may need to be used to make them effective against biofilms.

P3-72 Ultraviolet-C Efficacy against a Norovirus Surrogate and Hepatitis A Virus on a Stainless Steel Surface

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Introduction: Norovirus (NoV) and hepatitis A virus (HAV) infections are considered to be the most common causes of non-bacterial gastroenteritis around the world. The transmission of pathogenic microorganisms to food via contaminated surfaces is a significant problem in food processing. Stainless steel is widely used in food manufacturing and processing industries. The U.S. Food and Drug Administration has approved the use of UV-C on food products for controlling surface microorganisms.

Purpose: We investigated the effectiveness of UV-C radiation (10–300 mWs/cm^2) in controlling the infectivity of NoV, using murine norovirus-1 (MNV-1) and an HAV on a stainless steel surface that could be a major source of cross-contamination and foodborne enteric viruses and was to compare reduction responses of the viruses on the UV-C treated surface.

Methods: Stainless steel surface was used to examine the effects of low doses of UV-C radiation on MNV-1 and HAV titers (initial, approx. 5 log PFU of MNV-1 or HAV). The Weibull model was used to determine decimal reduction value (d_R-values, 90% reduction) of UV-C dose against MNV-1 or HAV.

Results: The Weibull model was used to generate non-linear survival curves and calculate d_R-values for MNV-1 (R^2 = 0.91) and HAV (R^2 = 0.95). Total MNV-1 and HAV titers significantly decreased (P < 0.05) with higher doses of UV-C (10–300 mWs/cm^2). MNV-1 and HAV were reduced to 0.03–4.43 and 0.03–2.62 log PFU/ml, respectively, on the stainless steel surfaces by low-dose UV-C treatment. The d_R-value for MNV-1 (35.85 mWs/cm^2) was significantly (P < 0.05) lower than that of HAV (53.94 mWs/cm^2) after UV-C treatment. Therefore, this study shows that HAV is more resistant to UV-C radiation than MNV-1.

Significance: This study suggests that low doses of UV-C light on food contact surfaces could be effective to inactivate human NoV and HAV in restaurant, institutional, and industrial kitchens and facilities.

P3-73 Evaluation of the Effects of the Selected Physical and Chemical Effects to Reduce Human Norovirus Surrogate in Cell Culture Lysate

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Introduction: Human norovirus (NoV) is a main cause of viral gastroenteritis worldwide. The incidence rate by NoV is steadily increasing in the foodborne diseases. Chlorine, ethanol, quaternary ammonium and ultrasound have been used to inactivate NoV in foods or tool surface. Many studies about virus inactivation methods are in progress, but more research needs to be developed.

Purpose: This study was conducted to investigate the effects of selected electrolyzed water, hydrogen peroxide, sodium chloride on the inactivation of MNV-1 as a NoV surrogate in the cell culture lysate.

Methods: The physical and chemical methods such as slightly acidic electrolyzed water (SAEW, chlorine 30 ppm for 0 to 60 min), hydrogen peroxide (H_2O_2, 0 to 50,000 ppm), sodium chloride (NaCl, 0 to 30%) were selected to inactivate MNV-1. The reduction effects of three disinfectants were evaluated by plaque assay.

Results: The total MNV-1 titers were significantly ($P < 0.05$) decreased with stepwise increase of exposure time of SAEW and concentration of H_2O_2 and NaCl. After MNV-1 cell culture lysate was exposed to SAEW for 5, 10, 20, 30, 60 min, the MNV-1 was reduced up to 2.10 log PFU/ml. After MNV-1 cell culture lysate was treated with 10,000 to 50,000 ppm H_2O_2 within 5 min and the MNV-1 was reduced up to 2.51 log PFU/ml. Compared to reduction of MNV-1 treated with SAEW and H_2O_2, the MNV-1 treated with 5-30 % NaCl was only reduced to less than 1.0 log PFU/ml. According to these results, MNV-1 was most reduced by 50,000 ppm H_2O_2 and the treatment of NaCl was shown the least reduction.

Significance: SAEW and H_2O_2 could be applied as a good viral sanitizer whereas NaCl was not shown great efficacy against MNV-1. Especially, SAEW can use as an effective treatment against NoV surrogate such as MNV.

P3-74 Inactivation of *Cronobacter sakazakii* in Head Lettuce by Using a Combination of Ultrasound and Sodium Hypochlorite

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Introduction: *Cronobacter sakazakii* has attracted increasing attention owing to its ability to grow as low as 5.5°C. NaOCl is commercially used in the food industry. Ultrasound detach bacteria from the surfaces of fresh-cut produce. There is a need to further synergistic effects of combination treatments using ultrasound on food decontamination.

Purpose: We determined the synergistic effects of an ultrasound (US) and NaOCl (50–200 ppm) combination on *C. sakazakii* reduction in fresh head lettuce, in comparison with US or NaOCl alone, and its effects on the quality of the lettuce.

Methods: Fresh head lettuce was used for investigating the synergistic effects of US (37 kHz, 380 W for 5–100 min) and NaOCl (50–200 ppm) against *C. sakazakii*. The synergistic effect of combined US and NaOCl were calculated using the following equation: synergistic reduction value = A – (B + C), where A is log reduction by combined US and NaOCl, B is log reduction after single US, and C is log reduction after single NaOCl.

Results: US was not enough to inactivate *C. sakazakii* (0.01–0.58 log reduction), whereas NaOCl significantly ($P < 0.05$) reduced *C. sakazakii* (0.58–2.77 log reduction). The combination (4.44 log reduction) of 100 min US and 200 ppm NaOCl resulted in an additional 1.7 log reduction of *C. sakazakii*. Synergistic effects were observed in most combined treatments, although the most synergistic values were all < 1.0 log CFU/g. The highest synergistic values were 1.08 log CFU/g when treated with a combination of 100 min US and 200 ppm NaOCl. Moreover, the pH and °Brix after 5–100 min US and 50–200 ppm NaOCl did not differ significantly from that in head lettuce treated with US or NaOCl alone.

Significance: Combination of 100 min US and 200 ppm NaOCl could be a potential approach for the post-harvest leaf vegetable processing to enhance the microbial safety of leafy vegetables without any changes in food qualities.

P3-75 Comparison of Antiviral Activity between Monomeric, Dimeric and Oligomeric Proanthocyanidins against Human Norovirus Surrogates

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❖ ***Developing Scientist Competitor***

Introduction: Proanthocyanidins (PACs) are dimers, oligomers, and polymers of catechins linked between carbons, C4 and C8 (or C6). Polymerized PACs in blueberries contain B-type linkages, with different profiles and amounts than cranberries that contain A-type linkages. B-type PAC can be converted to A-type PAC by radical oxidation that influences their bioactivity. Both B-type and A-type PAC reportedly have antiviral activity against human noroviral surrogates, that needs further understanding to prevent or treat human norovirus infections.

Purpose: The objectives of this study were to compare the antiviral activity between monomeric catechin monohydrate, procyanidin B2, B-type PAC from blueberries (B-PAC) and A-type PAC from cranberries (C-PAC) on the infectivity of human norovirus surrogates, feline calicivirus (FCV-F9) and murine norovirus (MNV-1), at 37°C.

Methods: FCV-F9 and MNV-1 at 5 log plaque forming units (PFU)/ml were treated with equal amounts of 0.5 or 1 mg/ml catechin monohydrate, procyanidin B2, blueberry PAC, cranberry PAC or phosphate buffered saline (pH 7.2, control) at 37°C over 6 h. Infectivity was determined using plaque assays and data from triplicate experiments were statistically analyzed.

Results: Monomeric catechins at 1 mg/ml reduced both viruses to undetectable levels only after 24 h. Procyanidin B2 at 1 mg/ml reduced FCV-F9 to undetectable levels within 6 h and MNV-1 by only 1.29 log PFU/ml after 24 h. Polymeric B-PAC at 1 mg/ml reduced both FCV-F9 and MNV-1 to undetectable levels within 3 h, while polymeric C-PAC at 0.6 mg/ml reportedly caused 5.02 and 2.95-log reduction of FCV-F9 and MNV-1, respectively, within 1 h.

Significance: Overall, polymeric catechins (PACs) showed a higher and faster antiviral effect compared to monomeric catechins. This study provides insights between the antiviral activity of different structural polyphenols that should help in the design of appropriate preventive and anti-noroviral therapies from natural sources.

P3-76 Clove Bud Oil, Its Active Component and Combination Treatments with Plant Extracts Inactivate Multi-Drug Resistant *Salmonella* Newport on Organic Leafy Greens

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❖ ***Developing Scientist Competitor***

Introduction: Nearly 40% of foodborne outbreaks involving *Salmonella* are linked to fresh produce. The USDA-NOP mandates for chemical-free production practices have left the organic industry with few treatment options. Plant essential oils and extracts have shown notable antimicrobial activity against foodborne pathogens *in vitro,* are generally recognized as safe (GRAS) by the FDA, and may provide an effective treatment alternative for the organic produce industry.

Purpose: Developing natural alternative treatments is necessary for reducing contamination by foodborne pathogens. We investigated the antimicrobial activity of clove bud oil, its active component eugenol, and combination treatments consisting of a plant extract and the essential oil (combinations A and B) against multi-drug resistant *Salmonella enterica* serovar Newport on organic leafy greens.

Methods: Iceberg lettuce, romaine lettuce, bunched mature spinach and baby spinach were dip inoculated with *Salmonella* Newport, treated with either PBS, 3% H_2O_2, 0.1 - 0.5% essential oil or its active component and combination A or B (5% plant extract and 0.1% essential oil) for 2 min and stored at 4°C. Samples were taken for enumeration on day 0, 1 and 3.

Results: Reductions in *Salmonella* populations for all treatments ranged from 0.36 - 3.7 log CFU/g on all four leafy greens, with the essential oil (0.5%), its active component (0.5%) and combination B being most effective on bunched mature spinach with reductions of 3.7, 3.7 and 3.6 log CFU/g, respectively. Reductions from individual treatments of essential oil (0.1%) and plant extract (5%) ranged from 0.40 - 2.5 log CFU/g and were less effective than the combination treatments (0.87 - 3.6 log CFU/g). Hydrogen peroxide yielded a maximum reduction of 1.7 log CFU/g showing no residual activity during storage, whereas plant antimicrobials showed residual activity that was concentration- and storage time-dependent.

Significance: This data supports the potential use of plant antimicrobials as alternative sanitizers to reduce the microbial burden of organic leafy greens.

Poster

P3-77 Microemulsion Loaded with *Laurus nobilis* Oil Inhibited the Growth of *Penicillium expansum* and the Production of Patulin

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✦ ***Undergraduate Student Award Competitor***

Introduction: *Penicillium expansum* is one of the most common and harmful pathogens in apples and pears. Patulin produced by *P. expansum* is a harmful mycotoxin which threatens human and animals health. Essential oils are widely reported as an environmental friendly agent to control postharvest diseases, however, the insolubility in water and the instability limited the application of essential oil.

Purpose: To prepare a stable and soluble essential oil microemulsion, which has great antifungal activity against *P. expansum* and has the ability to control the production of patulin.

Methods: Microemulsion was prepared with Tween-20 as surfactant, ethanol as co-surfactant, *Laurus nobilis* oil as oil phase and water as aqueous phase. *In vitro* antifungal activity was assessed with agar diffusion method. Spore germination rate was counted with microscope. *In vivo* antifungal activity was determined in wounded pear. Patulin was extracted from both *in vitro* and *in vivo* culture and analyzed with HPLC followed with the AOAC Official Method.

Results: The 1000 μg/ml microemulsion completely inhibited the growth of *P. expansum in vitro*. The 400 μg/ml microemulsion inhibited 70.6 ± 0.02% spores germination. The 1600 μg/ml microemulsion decreased 43.2 ± 0.3% decayed incidence of pears. The content of patulin, respectively, decreased 86.4 ± 1.2 % and 97.9 ± 2.1% *in vitro* and *in vivo* when the presence of 400 μg/ml microemulsion.

Significance: This study structured a new food-grade microemulsion which has great antifungal activity against *P. expansum* and has the ability to inhibit the produce of patulin.

P3-78 Impact of Different Washing Methods on Epiphytic Bacterial Communities on Organic Lettuce

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Introduction: Most small-medium scale farmers minimally handle leafy greens post-harvest. In Maryland, leafy greens are typically washed with or without the addition of sanitizer to wash water and stored, refrigerated, for a few hours to a few days prior to sale. The influence of post-harvest handling on the microbiota of organic produce has not been fully investigated.

Purpose: The goal of this study was to investigate whether different washing methods and storage times changed the indicator bacterial counts as well as the epiphytic bacterial communities on lettuce.

Methods: Four treatments were evaluated: unwashed, washed in tap water, in 100 ppm bleach solution or 30 ppm peroxyacetic acid solution (Tsunami® 100), immediately after washing or following 5 days of low temperature storage at 4°C. Indicator microorganisms (*E.coli,* coliforms, aerobic bacteria, yeast and mold) were quantified by culture methods, and bacterial community profiles were compared using Automated Ribosomal Intergenic Spacer Analysis (ARISA), to identify differences among treatments. ANOVA and ANOSIM were used to assess significant differences in the data.

Results: Culture methods showed that washing significantly reduced microbial indicators initially (APC, yeast, and molds; $P < 0.05$), but the difference between unwashed and washed samples diminished after storage. Bacterial community analysis revealed a significant difference among treatments using operational taxonomic unit relative abundance data in three experiments: global R = 0.70; global R = 0.30, global R = 0.49 ($P < 0.001$ in all cases). Significant differences among treatments persisted after 5 days of storage in all experiments; global R = 0.56, $P < 0.001$; global R = 0.22, $P < 0.05$; global R = 0.97, $P = 0.001$). Therefore, although reductions in indicator bacterial counts obtained after washing diminished after storage, differences in bacterial community profiles persisted. Different washing treatments had long-lasting effects on the epiphytic bacterial communities.

Significance: Impacts of washing on lettuce microbiome could result in different food safety risks through interactions between foodborne pathogens and other species of epiphytic bacteria.

P3-79 Antimicrobial Effect of Wuweizi (*Schisandra chinensis*) Extracts on Foodborne Pathogenic Bacteria in Synthetic Media and Orange Juice

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Introduction: The herbal tea Wuweizi (*Schisandra chinensis*) is considered to be a natural health drink in China. Previous research suggests that Wuweizi possesses antimicrobial activity.

Purpose: The purpose of this study was to evaluate the effect of Wuweizi extracts on the survival of major foodborne pathogenic bacteria in synthetic and food models.

Methods: Ten grams of Wuweizi were boiled in 200 ml of distilled water for 45 min. Various dilutions of the extract were tested against *Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Salmonella* Typhimurium via the agar well diffusion assay as well as in nutrient broth. In addition, pasteurized orange juice supplemented with 5% Wuweizi extract was separately inoculated at 6 log CFU/ml with the three pathogens and stored at 7°C or 21°C. Over time the pathogens in the juice were enumerated on plate count agar. Finally, chemical analysis was conducted on the extract to determine its acidity, and total phenolic, flavonoid and anthocyanin contents.

Results: Wuweizi extract generated inhibition zones of 1.58, 2.78, and 1.63 cm against *E. coli* O157:H7, *L. monocytogenes,* and *Salmonella* Typhimurium, respectively. *L. monocytogenes* and *Salmonella* Typhimurium in nutrient broth containing 2.5% Wuweizi extract were killed within 6 h. At 21°C, the extract at 5% inactivated all three pathogens in orange juice within 24 h. At 7°C, *L. monocytogenes* in orange juice was killed within 2 d; whereas, *E. coli* O157:H7 or *Salmonella* Typhimurium did not survive longer than 4 d. The pH of Wuweizi extract was 2.51. The total phenolic, flavonoid, and anthocyanin contents were measured at 851 mg GAE/l, 64.3 mg quercetin/l, and 22.9 mg cyd 3-glu/l, respectively.

Significance: The antimicrobial property of Wuweizi extract may be attributed to its high contents of organic acids and phenolic compounds. The extract provides an alternative and green solution to enhance the microbiological safety of food.

P3-80 Reducing Non-O157 Shiga Toxin-producing *E. coli* on Fuji Apple by Cinnamon Oil

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❖ ***Developing Scientist Competitor***

Introduction: Non-O157 Shiga Toxin-producing *E. coli* (STEC) has gained increasing awareness due to several recent outbreaks in which fresh produce is the main vehicle contributing to the transmission of non-O157 STEC to humans. Compared to *E. coli* O157:H7, currently far less attention has been paid to intervention of non-O157 STEC on produce; on the other hand, due to rising health concern about chemical sanitizers, intervention using natural compounds is gaining public interest. Cinnamon is a commonly used spice reported to have antimicrobial activity.

Purpose: To evaluate the efficacy of cinnamon oil washing in reducing non-O157 STEC on Fuji apples.

Methods: Unwaxed Fuji apples were cleaned, air dried and dip inoculated in 10^7 CFU/ml non-O157 STEC three strain cocktail (O26:H11, O121:H19, and O145:NT) solution. Surface-inoculated apples were air dried for 1 h, then washed with phosphate buffered saline (PBS), 0.5%, and 1.0% cinnamon oil solution for 30 s and 60 s. Residual bacteria on apples as well as in washing solutions were enumerated onto LB plate.

Results: Dip inoculation resulted in ~6.8 log CFU non-O157 STEC per apple, and PBS wash (control) resulted in ~1-log reduction. Inhibitory effect of cinnamon oil against non-O157 STEC on apple is time and dose dependent: 60 s washing in 1% cinnamon oil solution achieved about 3-log CFU reduction per apple, which is 2 log more reduction than that of PBS wash; 60 s wash in 0.5% cinnamon oil or 30 s wash in 0.5% and 1.0% cinnamon oil solution resulted in ~2.0 or 1.5-log reduction per apple, respectively. In addition, ~6 log CFU/ml of non-O157 STEC were detected in spent PBS wash solution, while it was under detection level in wash solution with cinnamon oil.

Significance: Cinnamon oil has the potential to be used as an alternative sanitizer in fresh apple industry.

P3-81 Effect of Sub-inhibitory Concentrations of Cinnamon Oil on Shiga Toxin Production by *E. coli* O157:H7

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Introduction: Cinnamon is a widely used spice with broad-spectrum antimicrobial activity. *E. coli* O157:H7, which produces Shiga Toxin (Stx) as its major virulence factor, is an important foodborne pathogen associated with bloody diarrhea and Hemolytic Uremic Syndrome.

Purpose: This study tested the inhibitory effect of cinnamon oil against *E. coli* O157:H7, and for the first time, evaluated its influence on Stx2 production, Vero cell cytotoxicity, and Stx2 phage induction.

Methods: Antimicrobial activity was measured by disc diffusion assay, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and death curve. Western blotting, real-time quantitative RT-PCR and *in vitro* Vero cell culture were used to analyze Stx2 production, *stx2* mRNA expression and cytotoxicity, respectively. Stx phage induction was measured by plaque forming units coupled with real time quantitative PCR.

Results: MIC and MBC were 0.025% and 0.05% (v/v), respectively; two times of MBC showed bactericidal effect within 15 min, while three fold of MBC resulted in a 6-log reduction during 30 min incubation. Sub-inhibitory concentrations of cinnamon oil decreased Stx2 production, *stx2* mRNA expression, and cytotoxicity in a dose dependent manner ($P < 0.05$); ¾ or 1 MIC reduced Stx2 production and *stx2* mRNA expression to undetectable level. Consistently, the phage titer and S*tx2* phage DNA decreased proportionally to cinnamon oil concentrations, and beyond the detection level when cinnamon oil concentration increased to ¾ and 1 MIC.

Significance: Cinnamon oil has the potential to be used as a promising natural compound to control *E. coli* O157:H7 and Stx production.

P3-82 The Influence of Essential Oils on Developmental Structures and Morphology on the Yeast Pathogen *Eremothecium ashybii*

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Introduction: Essential oils possess antimicrobial properties as a result they can be used as bio-preservatives and to control crop contamination thereby ensuring food safety. Contamination of food products by fungi affects shelf life, influences food safety negatively and frequently results

in qualitative and quantitative losses of food commodities. The search for alternative antifungals to reduce commodity losses due to fungal contamination post-harvest and infections of plants on farms is currently in demand.

Purpose: The current study investigated the influence of essential oils on growth and morphology of the yeast pathogen *Eremothecium ashbyii*.

Methods: Essential oils (Bergamot, citronella, eucalyptus, rosemary, rose geranium, sage, tea tree and thyme) were characterized using Gas Chromatography Mass Spectrometry. The agar diffusion assay together with light and scanning electron microscopy were used to study the influence of essential oils on growth and morphology of *E. ashbyii*.

Results: Bio-assay results showed that oils characterized by a high content of monoterpenes showed higher antifungal activity while oils characterized mainly by oxygenated monoterpenes displayed weak activity. Furthermore, tested oils affected yeast growth by first targeting developmental structures (asci and ascospores) with increased mitochondrial activity, indicating their possible anti-mitochondrial activity. Microscopy revealed that oils mainly characterized by a high content of monoterpenes completely inhibited developmental structures or affected their development whereas hyphae appeared granular and wrinkled.

Significance: The current study successfully exposed that tested oils can be used to develop alternative novel biological anti-germination agents to combat the spread *E. ashbyii* and possibly other fungal plant pathogens.

P3-83 Antimicrobial Activities of Gaseous Essential Oils against Foodborne Pathogens and Spoilage Microorganisms

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❖ ***Developing Scientist Competitor***

Introduction: Essential oils (EOs) are volatile liquids obtained from plants and can have antibacterial activities. Many researchers have reported the antimicrobial activities of EOs in liquid phase, however, meager research attention has been given to the antimicrobial activities of gaseous EOs.

Purpose: This study was done to screen gaseous EOs with inhibitory activities against foodborne pathogens (*Salmonella enterica* and *Listeria monocytogenes*) and spoilage microorganisms (*Erwinia* spp. and *Pseudomonas aeruginosa*) and to determine the minimal inhibitory concentrations (MICs) and minimal lethal concentrations (MLCs) of gaseous EOs against those microorganisms.

Methods: In total, 100 types of commercial EOs were obtained. Experimental apparatus to screen and measure the antimicrobial activities of gaseous EOs were constructed using airtight container and medium with pH indicator. Using the apparatus, EOs with inhibitory activities against *S. enterica, L. monocytogenes, Erwinia* spp., and *P. aeruginosa* were screened, and their MIC and MLC values were measured.

Results: Thirty-five types of gaseous EOs showed inhibitory activities against *S. enterica*, and gaseous cinnamon leaf oil showed the lowest MIC (0.0391 µl/ml) and MLC (0.0391 µl/ml). Against *L. monocytogenes*, 43 types of gaseous EOs showed antimicrobial activities, and gaseous cinnamon bark, cinnamon leaf, clove bud, oregano, oregano spanish, and thyme thymol oils showed the lowest MIC (0.0781 µl/ml) and MLC (0.0781 µl/ml). For *Erwinia* spp. and *P. aeruginosa*, 17 and 13 types of gaseous EOs, respectively, showed antimicrobial activities. Gaseous thyme thymol, oregano, and cinnamon leaf oils showed the lowest MIC (0.625 µl/ml) and MLC (0.625 µl/ml) against *Erwinia* spp. Against *P. aeruginosa*, gaseous garlic oil had the highest antimicrobial activity and its MIC and MLC values were 0.625 µl/ml.

Significance: Gaseous EOs showing antimicrobial activities against *S. enterica, L. monocytogenes*, *Erwinia* spp., and *P. aeruginosa* were screened, and their MIC and MLC values were determined.

P3-84 Synergistic Lethal Effects between Plant Extracts against *Listeria monocytogenes* and *Staphylococcus aureus*

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Introduction: There has been a growing interest on plant extracts to replace synthetic preservatives in foods. However, the addition of plant extracts in foods may adversely change the sensorial properties of foods. The combinations of plant extracts with synergistic lethal effects may minimize the sensorial changes of foods by reducing the amounts of individual extracts added.

Purpose: This study was done to screen plant extracts for inhibitory activities against *Listeria monocytogenes* or *Staphylococcus aureus*, and to determine the combinations of plant extracts with synergistic lethal effects against those pathogens.

Methods: Plant extracts (625 types) were tested for their antimicrobial activities against *L. monocytogenes* or *S. aureus* using an agar well diffusion assay. For selected plant extracts, their minimal inhibitory concentrations (MICs) against *L. monocytogenes* or *S. aureus* were determined. Finally, synergistic lethal effects between plant extracts against those pathogens were evaluated using a checkerboard test.

Results: *Morus alba*, *Rhus chinensis*, *Carex pumila*, *Sappan lignum*, *Coptis chinensis*, *Siegesbeckia glabrescens*, and *Dryopteris erythrosora* extracts showed antimicrobial activities against *L. monocytogenes* or *S. aureus*. *D. erythrosora* extract showed the lowest MIC (0.0039 mg/ml) against *L. monocytogenes* followed by *M. alba* (0.0313 mg/ml) and *C. chinensis* (0.50 mg/ml). A combination of *D. erythrosora* and *M. alba* and a combination of *D. erythrosora* and *C. chinensis* showed synergistic lethal effects against *L. monocytogenes*. *D. erythrosora* extract showed the lowest MIC (0.0039 mg/ml) against *S. aureus* followed by *M. alba* (0.0156 mg/ml) and *C. chinensis* (0.25 mg/ml). A combination of *C. chinensis* and *M. alba* and a combination of *C. chinensis* and *D. erythrosora* showed the synergistic lethal effects against *S. aureus*.

Significance: Plant extracts with inhibitory activities against *L. monocytogenes* or *S. aureus* were screened, and their MIC values were evaluated. Combinations of plant extracts with synergistic lethal effects against *L. monocytogenes* or *S. aureus* were determined.

P3-85 Phenolic Composition and Antimicrobial Activity of Seaweed Extracts

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Introduction: Seaweeds are sources of compounds with biological activity, which can be used as functional ingredients. They produce secondary metabolites such as phenolic compounds, which give stronger antioxidant activity. However, little is known about its antimicrobial activity.

Purpose: The main purpose of this study was to evaluate the phenolic compounds content and antimicrobial activity of Nori, Kombu, Wakame and Hijiki seaweed extracts.

Methods: The extracts were prepared with ethanol (60, 80 and 100%) at different times (2 and 7 days of extraction, at room temperature). Phenolics were quantified by the Folin - Ciocalteu method, and the antimicrobial activity was evaluated by the Minimum Inhibitory Concentration (MIC) against *Escherichia coli* (ATCC 25922), *Salmonella* Enteritidis (ATCC 13076), *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 7644) and *Klebsiella pneumonia* (ATCC 13883).

Results: For Wakame, the higher content of phenolics, 1.66 mg GA/g, were obtained by 2 days of extraction with 80% ethanol, while for Nori, Kombu and Hijiki (7.95, 0.54 and 6.04 mg GA/g, respectively) showed higher extraction with ethanol 60% as solvent, with two days of extraction. The ethanolic extracts of seaweed did not show antimicrobial activity against *Escherichia coli*, *Salmonella* Enteritidis and *Staphylococcus aureus*. With respect to *Listeria monocytogenes* and *Klebsiella pneumoniae*, it was observed that all ethanolic extracts to 100% showed inhibitory activity, regardless of the extraction time. May be evidenced the ethanolic extracts to 100% of Nori (7 days), Hijiki (2 days), Wakame (2 days) and Kombu (7 and 2 days), had the best performance, with MICs between 19.53 and 39.06 µg/ml for *Klebsiella pneumoniae*.

Significance: Based on the data, it can be said that several crude extracts of seaweed can be considered bioactives. There was no correlation between the concentration of phenolic compounds and the antimicrobial activity of the extracts.

P3-86 Chemical Composition and Biological Activities of Strawberry Pulp Stored under Refrigeration

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Introduction: Fruits have a very important role in our diet, as they are natural sources of nutrients, vitamins and other important compounds for preventing various diseases. Strawberries are rich in Vitamin C and phenolic compounds, bioactive compounds known for their ability to scavenge free radicals.

Purpose: Evaluate the chemical composition and biological activities of ethanolic extracts of raw strawberry pulp, during the times 0, 7, 14 and 21 d of storage under refrigeration.

Methods: After obtaining the pulp, at each time, this was subjected to extraction with 100% ethanolic at 70°C for 30 min. The quantification of phenolic compounds was made by the method of Folin Ciocalteu. The antioxidant activity was evaluated by DPPH method (IC_{50}), and the antimicrobial activity, by the Minimum Inhibitory Concentration (MIC_{50}) against yeasts and bacteria.

Results: The phenolic content showed the highest value at the time 21 d, of 38.84 ± 2.2 mg GAE/g. Time 0 showed the highest antioxidant activity, with IC_{50} = 138.38 µg/ml, in the time 21 the IC_{50} was 287.15 µg/ml. For antimicrobial activity at time 0, there was activity, with MIC_{50} of 15.63 µg/ml for *C. krusei* yeast and for the bacteria *B. cereus*. Both *S. aureus* and *E. coli*, at 0 and 7 days, showed MIC_{50} of 500 µg/ml. In the 7 and 14 days, the best results were to MIC_{50} of 15.63 and 31.25 µg/ml, respectively, against *B. cereus*. At time 21 days, the MIC_{50} decreased to 125 µg/ml against *B. cereus*.

Significance: Storage time interferes with the chemical composition and biological activities of strawberry pulp, increasing the concentration of phenolic compounds, and the antioxidant and antimicrobial activities were reduced. It is important to the rapid consumption of strawberry pulp stored under refrigeration so you can take advantage of its functional properties.

P3-87 Combined Efficacy of Essential Oil Compounds and Bacteriophage to Control *Staphylococcus aureus* with Potential Application

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❖ ***Developing Scientist Competitor***

Introduction: Multidrug-resistant (MDR) *Staphylococcus aureus* are increasingly prevalent and control via standard antibiotic treatment has become challenging. Combinations of different antimicrobials represent an approach for combating MDR bacteria. Antimicrobials with potential food industry application include essential oils (EO) and bacteriophage (phage).

Purpose: Here, we aimed to investigate the combination of EOs and phages as alternative antimicrobials to control *S. aureus*.

Methods: Four EO compounds were evaluated by disc diffusion assay to determine inhibitory effects against five strains of *S. aureus*. Next, a 48-hour growth inhibition assay was performed using a 96-well bioassay. Phage adsorption assays were performed up to 120 h at 6, 13, and 37°C to determine lytic activity, and phage concentration over time was determined by plaque assay. Combinations of phage and EO against *S. aureus* were also evaluated at 37°C using a 96-well bioassay.

Results: Disc diffusion assays indicate that the zones of inhibition (IZ) of alpha-pinene (IZ=10 – 23 mm) and alpha-pinene + limonene (IZ=11 – 15 mm) have a greater inhibitory effect against *S. aureus* strains when compared to other EOs tested. Growth inhibition assay at 37°C confirmed these results with 1.5% alpha-pinene inhibiting *S. aureus* growth up to 30 to 40 h depending on the strain. Phage adsorption assays indicate that phage K has high lytic activity at 37°C when compared to 6 and 13°C possibly impacting applicability in food industry. Results from the combined effect of EO and phage indicate that this combination inhibits *S. aureus in vitro* at 37°C more effectively than phage or EO alone although there is variability between strains. For application in the food industry, these antimicrobials were evaluated for their efficacy against *S. aureus* on raw chicken pieces at 6, 13 and 25°C. Results indicate that at 25°C 1.5% alpha-pinene inhibits *S. aureus* growth better as compared to other antimicrobial combination. At 6 and 13°C, there was no significant effect of EO and phage alone or in combination against *S. aureus* when applied on the raw chicken pieces.

Significance: These results indicate that EOs and phage can be used as potential antimicrobials against *S. aureus in vitro*. For these antimicrobials to work *in vivo* such as raw meat products, better delivery method of the antimicrobials should be employed for a uniform application on meat.

P3-88 Antiviral Activity of Medicinal Plant Extracts against Murine Norovirus and Feline Calicivirus

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Introduction: Human noroviruses (hNoVs) were considered as significant causes of nonbacterial gastroenteritis around the world. However, no antiviral drugs were developed because culture system of noroviruses has been absent. Recently, there are several studies about medicinal plant extracts that have potential as novel antiviral agents.

Purpose: The purpose of this study was to investigate the antiviral activities of herbal extracts against human norovirus surrogates including feline calicivirus (FCV) and murine norovirus (MNV).

Methods: Twenty-nine extracts of *Z. latifolia, P. oleracea, S. chinensis, G. uralensis, C. longa, C. versicolor, I. obliquus, L. edodes, F. carica, C. aurantium, G. lucidum, C. militaris, C. sinensis, C. pinnatifida, A. annua, G. biloba, A.thumbergii, A. pilosa, C. sativum, V. vinifera, P. multiflorus, E. sessiliflorus, A. sativum,*

S. flavescens, A. fistulosum, C. officinalis, P. lactiflora, A. japonica, and *E. ulmoides* were used in this study. The effect of pre-, co-, and post-treatment of 29 medicinal plant extracts was investigated on virus-infected cells. Antiviral activity, cytotoxicity, and antioxidant activity was measured on Crandell-Reese Feline Kidney for FCV and RAW264.7 cells for MNV at 10, 20, 50, and 100 µg/ml concentration of medicinal plant extracts.

Results: Compared with antiviral drug and 29 herbal extracts, *C. sinenesis*, *A. japonica*, and *C. pinnatifida* showed the significant reduction of norovirus surrogates without cytotoxicity. The titer of FCV significantly reduced to 81.35 ± 9.48 and 65.00 ± 7.07% on CRFK cells pretreated with 100 µg/ml of *C. sinensis* and 20 µg/ml of *A. japonica* for 24 h, respectively. The titer of MNV was reduced to 53.71 ± 9.18 % on RAW264.7 cells pre-treated with 50 µg/ml of *C. pinnatifida* for 24 h.

Significance: Three herbal extracts of *C. sinenesis*, *A. japonica*, and *C. pinnatifida* could have potential to control norovirus without cytotoxicity.

P3-89 Antimicrobial Activity of *Thymus vulgaris* L. and *Origanum vulgare* L. Essential Oils against Pathogenic and Starter Bacteria in Brazilian Semi-Hard Cheese Model

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Introduction: Coalho cheese is a semi-hard Brazilian cheese with medium to high moisture obtained by a simple manufacturing process usually using mesophilic lactic starter cultures. Essential oils from *Origanun vulgare* L. (OVEO) and *Thymus vulgaris* L. (TVEO) are recognized as safe antimicrobial compounds, however there is a lack of information regarding the effects of these oils against starter and pathogenic bacteria in cheese.

Purpose: The aim of this study was to evaluate the activity of OVEO and TVEO against starter mesophilic cultures *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis, and strains of pathogens S. aureus* and *L. monocytogenes* in a coalho cheese-based model system.

Methods: Samples of coalho cheese (10 g) containing OVEO or TVEO at Minimal Inhibitory Concentration were inoculated with 1 ml of bacterial suspension (10^7 CFU/ml), blended for 5 min and incubated at 8 - 10°C. Systems without oils were assayed similarly. The number of viable cells in the systems was counted at various time points (0, 24, 48 and 72 h) by serial dilution and plating on M17 Agar, Brain Heart Agar added of sodium chloride 8.5%, or *Listeria* Agar for starter cultures, *S. aureus* and *L. monocytogenes,* respectively

Results: Starter and pathogenic bacteria in systems containing essential oils always exhibited lower viable cells counts ($P \leq 0.05$) compared to control systems. After 72 h, reduction of 2.85 log CFU/g, 1.85 log CFU/g and 0.53 log CFU/g was observed for *L. monocytogenes, S. aureus* and starter bacteria in cheese containing OEOV, respectively. In systems with added TVEO, a reduction of 2.15 CFU/g, 1.22 CFU/g and 0.64 CFU/g LAB was observed for *L. monocytogenes, S. aureus* and starter, respectively.

Significance: The results suggest OVEO or TVEO may inhibit pathogens frequently associated to coalho cheese. Both compounds have some inhibitory effects against starter lactic culture needed to produce this product.

P3-90 Activity of *Origanum vulgare* L. Essential Oil and Carvacrol against Planktonic and Biofilm Cells of *Staphylococcus aureus* Strains Isolated from Hospital Food Service Environments

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Introduction: The ability of *Staphylococcus aureus* to form biofilms enhances it survival in food processing and food service environments. The increased pressure for safe and natural foods has motivated research of the antimicrobial efficacy of natural compounds, such as *Origanum vulgare* L. essential oil (OVEO) and its major constituent carvacrol (CAR). There is a lack of information regarding the effects of these substances against *S. aureus* cells grown in biofilms.

Purpose: The aim of this study was to determine the minimum inhibitory concentration (MIC) of OVEO and CAR against planktonic and biofilm *S. aureus* cells.

Methods: Ten strong biofilm producing strains of *S. aureus* were previously isolated from hospital food service environments. MIC values of OVEO and CAR were determined in triplicate by microdilution in Tryptone Soya Broth with glucose 1g/100ml, using a microplate incubator/reader (OD 490_{nm}). OVEO *and CAR were used in concentrations from* 0.3 to 40 µl/ml. Biofilms were produced by culturing cells for 24 h before addition of antimicrobial substances. *S. epidermidis* ATTCC 12228 and *S. aureus* ATTCC 25923 were used as negative and positive control (strong producer) for biofilm formation, respectively

Results: MIC value of OVEO was 5 µl/ml against planktonic cells for eleven strains (n = 9; and control strains) and 10 µl/ml for one *S. aureus* strain. MIC value of CAR ranged from 2.5 µl/ml (n = 8; control negative strain) to 5 µl/ml (n = 2; control positive strain) against planktonic *S. aureus* cells. The MIC values of OVEO (10 µl/ml or 20 µl/ml) and CAR (5 µl/ml or 10 µl/ml) were always doubled for *S. aureus* cells in the biofilm state.

Significance: OVEO and CAR are effective in inhibiting growth of cells of *S. aureus* in planktonic and biofilms states, and cells in biofilms were more resistant to tested antimicrobials.

P3-91 Combined Application of Essential Oils from *Origanum vulgare* L. and *Rosmarinus officinalis* L. or Their Constituents to Inhibit *Staphylococcus aureus* in Food Based-model Systems

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Introduction: Essential oils and their constituents are 'natural' antimicrobials with potential application in food matrices. Little is known about the combined activity of these substances against multiple *Staphylococcus aureus* strains in food matrices.

Purpose: This study evaluates the effects of the essential oils *Origanum vulgare* L. (OVEO) and *Rosmarinus officinalis* L. (ROEO) and their major constituents carvacrol (CAR) and 1,8-cineole (CIN) applied in combination, against a cocktail of five *S. aureus* strains in cheese and meat-based models.

Methods: Samples (10 g) of semi-hard cheese or ground beef were mixed with OVEO+ROEO or CAR+CIN at the Fractional Inhibitory Concentration (FIC), where FIC is typically the Minimum Inhibitory Concentration (MIC)/4. These mixtures were inoculated with 1 ml of bacterial suspension (10^7 CFU/ml), blended for 5 min and incubated at 8 - 10°C. Systems without antimicrobials were assayed similarly. The viable cells were enumerated at various time points (0, 24, 48 and 72 h) by serial dilution and plating on Brain Heart Infusion agar with sodium chloride 8.5%

Results: The cheese and meat systems with OVEO+ROEO showed reductions of 1.8 log CFU/g and 1.6 log CFU/g, respectively, after 48 h; when CAR+CIN were used, reduction of 1.9 CFU/g and 1.7 CFU/g were observed for cheese and meat systems, respectively. Cheese and meat model systems containing essential oils or constituents at showed greater than a 2.8-log reduction in *S. aureus* counts after 72 h. Control experiments for both matrices without added antimicrobials showed no change in *S. aureus* concentration over 72 h.

Significance: The essential oils OVEO and ROEO and their major constituents CAR and CIN, are effective in combination in controlling multiple *S. aureus* strains in cheese and meat-based models. These results support the idea that the combination of these substances could be used to control the growth and survival of *S. aureus* in food.

P3-92 Effects of Compounds in *Olea europaea* on Growth of Major Enteric Bacterial Pathogens and *Lactobacillus* Strains

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❖ ***Developing Scientist Competitor***

Introduction: The olive *Olea europaea* is rich in phenolic compounds and tri-terpenoids, both of which are associated with multiple promising pharmacological activities including anti-oxidant, anti-inflammatory, antimicrobial, and antiviral effects. Due to the growing multi-drug resistant foodborne bacterial pathogens, alternative antimicrobials are essential. Bio-active compounds extracted from *Olea europaea* might be potential in reducing colonization of enteric bacterial pathogens as well as maintaining the growth of probiotics.

Purpose: The purpose of this study was to evaluate the antimicrobial property of 4 olive compounds, maslinic acid (MA), oleanolic acid (OA), hydroxytyrosol (HT), and oleuropein (OP), against major foodborne pathogens as well as their role on growth of *Lactobacillus* strains.

Methods: The minimum inhibitory concentration (MIC) of these 4 compounds on enterohemorrhagic *E. coli* EDL933 (EHEC), *Salmonella* Typhimurium LT2 (ST), and *Listeria monocytogenes* LM2 (LM) were determined by broth micro-dilution method. Growth conditions of *Lactobacillus casei* (LC), *L. rhamnosus* (LR), and *L. plantarum* (LP) were determined in MRS broth with and without 1% MA, OA, HT, or OP. Data were analyzed using ANOVA.

Results: The MIC of HT on EHEC, ST, and LM was found to be less than 0.125% (w/v), and the MIC of OP on EHEC, ST, and LM was showed to be 2%, 1%, and 0.5%, respectively. However, the MIC of MA or OA on three foodborne pathogens was found to be higher than 4%. Growth of LC, LR, and LP were stimulated by ≈0.5, ≈1.0, and ≈1.0 logs with 2% MA and by ≈1.0, ≈1.0, and ≈0.5 logs with 2% OA at 24 h. Two percent OP maintained the growth of *Lactobacillus*, whereas 2% HT exhibited inhibitory effects on them.

Significance: HT in olive could be a potential alternative antibiotic; OP, both showed antimicrobial effect and maintained in probiotic growth, and may aid in prevention of foodborne illness.

P3-93 In Vitro Antimicrobial Activity of Thyme, Achillea, Salvia and Rosemary Essential Oils against the Top Seven Verotoxigenic *Escherichia coli*

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Introduction: The reduction of chemically synthesized additives from foods in the organic market is a current demand in the food industry. Essential oils could potentially fill this need. They seem to have a high potential in terms of their antimicrobial activity against foodborne pathogens.

Purpose: To determine *in vitro* antimicrobial activities (minimum inhibitory concentration [MIC] and minimum bactericidal concentrations [MBC]) of 4 pure essential oils (EO; thyme, achillea, salvia and rosemary) against seven *E. coli*serotypes (O26, O45, O103, O111, O121, O145 and O157).

Methods: Antimicrobial activities were determined by micro-dilution techniques using 96-well microplates, Muller-Hinton broth and p-Iodonitrotetrazolium chloride to indicate bacterial growth. After initial determination of MIC and MBC, the obtained concentrations were tested at different pH (4 and 7) and temperatures (4 and 25°C); in the following treatments: T1 = pH 4 at 4°C; T2 = pH 4 at 25°C; T3 = pH 7 at 4°C; and T4 = pH 7 at 25°C by quadruplicates. Each experiment was conducted twice (n = 224).

Results: Thyme, achillea, and rosemary showed MIC activity at lower concentrations (2 mg/ml, 5 - 10 mg/ml, and 2 - 5 mg/ml; respectively); while for salvia, the MIC was determined at higher concentrations (12 - 25 mg/ml). The MBC was as follows: thyme (2 mg/ml), achillea (5 to 10 mg/ml), rosemary (2.5 to 5 mg/ml) and salvia (12 to 25 mg/ml). When EO's MIC and MBC were tested in T1, T2, T3 and T4, no effects were observed. When lower concentrations of EOs (1 - 5 mg/ml) were tested in T1 and T2, thyme, achillea and rosemary showed bactericidal effects, evidencing a synergistic effect with pH and temperature.

Significance: Data shows that EOs, especially thyme and rosemary, can be effective against the 7 VTEC serotypes tested and suggests that these EOs might be effective against VTEC in acidic foods and at refrigerated temperatures.

P3-94 Antimicrobial Activity and Total Soluble Phenolic Content in *Momordica balsamina* and *Momordica foetida* Extracts

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❖ ***Developing Scientist Competitor***

Introduction: *Momordica balsamina* (*inkakha*) and *Momordica foetida* (*inshubaba*) are two plants used in several countries, including Swaziland, both for food and as a traditional medicine to treat several diseases. Increasing resistance and allergic reactions to synthetic antibiotics and preservatives validates the search for potential antimicrobial agents from these indigenous vegetables.

Purpose: In this study, antibacterial activity of *M. balsamina* and *M. foetida* extracts against different pathogenic bacteria was evaluated and its relationship with soluble phenolic content determined.

Methods: Different oven dried plant parts of the two *Momordica* subspecies were extracted using methanol-water (7:3 v/v). Antimicrobial activity was determined using a disc diffusion assay against *Staphylococcus aureus* NCTC 10652, *Staphylococcus aureus* NCTC 10655, *Listeria monocytogenes* 11944, *Salmonella* Typhimurium LT2 and *E. coli* O157:H7 Shiga toxin minus (stx) Sakai. Total soluble phenolic content was determined using a Folin-Ciocalteu reagent and calculated as Gallic acid equivalents (GAE).

Results: *Momordica* extracts showed antimicrobial activity against Gram positive bacteria only. *M. balsamina* leaf and stem extracts displayed the highest inhibitory activity with zones up to 17 mm while the *M. balsamina* fruit extracts did not show any activity. *M. foetida* leaf and fruit extracts gave inhibition zones up to 14 mm, yet *M. foetida* stem extracts did not show any inhibition zone. Total soluble phenolic content in *M. balsamina* extracts ranged from 0.043 to 0.192 mg while for *M. foetida* extracts the range was 0.059 to 0.272 mg. Leaf and stem extracts for both *Momordica* plants showed higher phenolic contents than fruits extracts. There was a weak linear relationship between antimicrobial activity and phenolic content of *Momordica* extracts with R^2 values of 0.29, 0.39 and 0.44.

Significance: These results could indicate the potential use of *Momordica* extracts as antimicrobial agents and also provide the basis for isolation and identification of the biologically active substances in these extracts.

P3-95 Antimicrobial Activity of the Ethanolic Extract from Exotic Brazilian Herbs on Gram Positive Bacterial

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Introduction: The studies of natural products have been intensifying with consumer concern in to ingest healthier foods.

Purpose: The antimicrobial effectiveness of ethanolic extract of *Quassia amara, Solanum sciadostylis (Sendtn.) Bohs* and *Artemisia annua* on *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 11778, *Micrococcus luteus* ATCC 9341, *Enterococcus faecalis* ATCC 51299 and *Staphylococcus aureus* ATCC 6538 were ascertained using the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

Methods: Broth microdilution method in 96-well microplates was used and the inoculum was prepared at the concentration of 10^{-2} CFU/ml, starting from a 0.5 McFarland. Extract concentrations of 19 - 5,000 µg/ml were evaluated.

Results: The plant extracts showed varied activity on the test organisms with *Quassia amara* and Artemisia showing a stronger antimicrobial activity of 39 µg/ml and 312 µg/ml, respectively, on *Micrococcus luteus* compared to MIC of 2,500 µg/ml of *Solanum sciadostylis* and Artemisia extracts on *Staphylococcus aureus* and 1,500 µg/ml on both *Bacillus subtilis* and *Bacillus cereus*. *Staphylococcus aureus* exhibited the same MIC (2,500 µg/ml) for all extract analyzed. *Quassia amara*, *Solanum sciadostylis* and *Artemisia annua* extracts did not show any bactericidal activity between the concentrations of 19 – 5,000 µg/ml used for experiment on *Bacillus cereus* and *Enterococcus faecalis.* MBCs of *Artemisia annua* extracts of 1,250 µg/ml was generally lower than that of *Quassia amara* extracts 2,500 mg/ml on *Bacillus subtilis*. The MBCs of *Quassia amara*, *Solanum sciadostylis* and *Artemisia annua* extracts on *Micrococcus luteus* were the same (5,000 µg/ml).

Significance: This study confirms the antimicrobial potential of these plants extracts and supports the use in traditional medicine and would be an interesting source for discovery of novel antibiotics agents from plant sources. This study confirms the antimicrobial potential of these plants extracts and supports the use in traditional medicine and as interesting source for discovery of novel preservatives in food technology.

P3-96 Morphological Changes Induced by *Thymus vulgaris* Essential Oil on *Staphylococcus aureus* Cells

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Introduction: Microbial resistance to antibiotics, especially among staphylococcal strains, is a major threat to public health. Since resistance by certain strains of *Staphylococcus* to multiple antibiotics like methicillin emerged in the past decades, many strategies to control antibiotic resistance have been proposed. However, some staphylococcal strains have become resistant, at least to some extent, even to vancomycin – indicating a dire need for new alternative therapeutic approaches.

Purpose: To investigate the action of *Thymus vulgaris* essential oil on *Staphylococcus aureus* cell morphology.

Methods: The effect of thyme essential oil on *S. aureus* was evaluated by bio-assay preparation and minimal inhibitory concentrations (MICs). Morphological changes were assessed using gram staining, scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Results: Exposure to thyme oil induced alterations in the bacterial membrane of *S. aureus*, which led to loss of cell wall integrity, as demonstrated by gram staining, SEM and TEM. In addition, loss of cellular contents, irregular cytoplasmic membrane, swollen cells; shrinkage of the cell, incomplete cell division and the presence coagulated material, as indicated by SEM and TEM were observed. Therefore, thyme oil when compared to antibiotics, damages the cellular membrane of *S. aureus*, which eventually leads to cell death.

Significance: Thyme essential oil shows effective antimicrobial activity and is therefore considered a potential antimicrobial agent. Moreover, evidence provided in this study indicates that thyme essential oil might enhance the chances of developing new conventional and natural antimicrobial agents (drugs as well as food preservatives) and be good alternatives to synthetic chemicals.

P3-97 Bio-control of Shiga Toxin-producing *Escherichia coli* (STEC) O157:H7 Contaminated in Raw Beef by Using a Lytic Bacteriophage

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Introduction: Shiga Toxin-producing *Escherichia coli* (STEC) are dangerous foodborne pathogens usually transmitted to humans through raw or undercooked meats. Lytic bacteriophages or phages have recently emerged as the natural agents for controlling of STEC contamination in meats.

Purpose: The objectives of this study are to isolate lytic bacteriophages for STEC O157:H7 and to use them as the means for the decontamination of these bacteria in raw beef.

Methods: The lytic phages were isolated from bovine intestine samples by using the double layer technique with *E. coli* O157 as the hosts for propagation. The phage with widest lytic range was selected and used for the inactivation of STEC O157:H7 bacteria artificially inoculated in raw beef at 5 x 10^5 CFU/piece at 8°C and 25°C. The survival viable cells were then enumerated after 2, 4, 6 and 24 h incubation for comparing the differences between control and phage-treated samples. The significant differences were inferred by using *t*-test.

Results: The phage PE37 belonging to the non-*stx* gene member of the Myoviridae family was successfully isolated. The lytic range tests revealed that the PE37 lysed all 37/37 (100%) tested STEC O157:H7 strains isolated from human illness. At 8°C, after 24 h incubation, the treatment of raw beef pieces (n = 10) with the PE37 at Multiplicity of Infection (MOI) = 100 reduced the concentration of STEC O157:H7 by 0.82 log CFU/piece compared to phage-free control ($P < 0.05$). Likewise, the reduction of STEC O157:H7 viable count was approximately 3.1 log CFU/piece in the samples treated with the phage compared to non-treatment at 25°C after 24 h ($P < 0.05$).

Significance: The results from present study implicate that phage PE37 has potential characteristics applicable to controlling of STEC O157:H7 contamination in raw meats.

P3-98 Isolation and Characterization of Bacteriophages against Shiga Toxin-producing *E. coli* and Extended Spectrum Beta Lactamase (ESBL)-producing *E. coli*

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Introduction: *Escherichia coli* is the most common foodborne pathogen worldwide. Among *E.coli* strains, Shiga Toxin and ESBL-producing *E. coli* have been receiving more attention due to its virulence. The use of bacteriophages as bio-preservatives to control ESBL-producing *E.coli* and STEC is a promising measure.

Purpose: The aim of this study is to isolate phages, which infect STEC or ESBL-producing *E. coli*, and evaluate their ability to lyse bacterial hosts in vitro assay.

Methods: Fifteen samples of bovine intestine and chicken products were employed to isolate phages against either STEC or ESBL-producing *E. coli* by double agar overlay plaque assay. Lytic spectrum was performed to determine lytic capability of obtained phages on double layer agar by the spot on the bacterial lawn technique. Based on lytic range and growth, phage S194-CS5 was chosen to assess its potential to kill STEC O157:H7 in LB broth at 37°C for 24 h. Fresh LB broth (5 ml) was inoculated with 100 µl of *E. coli* O157:H7 (5.10^7 CFU/ml) and 100 µl of phage S194-CS5 at multiplicity of infection (MOI) of 10. Viable cells of *E. coli* O157:H7 were enumerated on Tryptic Soy Agar (TSA) at 2, 4, 6 and 24 h of incubation.

Results: A total of 53 phages were isolated, 36 phages using ESBL-producing *E. coli* as hosts and 17 phages employing STEC as bacterial targets. The phage with the widest host range infected 9 (41%) of the 22 ESBL-producing *E. coli* and 7 (70%) of 10 STEC strains. The phage S194-CS5 reduced viable cell count to about 2 log, 3 log, 3.2 log and 3.2 log at 2 h, 4 h, 6 h and 24 h of incubation, respectively.

Significance: These isolated phages can be exploited as bio-control agents to reduce ESBL-producing *E. coli* and STEC contamination in meats.

P3-99 Inactivation of *Listeria monocytogenes* by a Lytic Bacteriophage Cocktail on Roast Beef and Pastrami to Improve the Safety of RTE Beef Products

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Introduction: *Listeria monocytogenes* causes one of the major lethal foodborne infection, Listeriosis. This is an important food safety concern for RTE industries as evidenced by several major Listeriosis outbreaks linked to RTE meats and recalls.

Purpose: The goal of this study is to evaluate antimicrobial strategies using bacteriophages as a biological antibacterial treatment to eliminate potential *L. monocytogenes* contamination on RTE sliced beef products (roast beef and pastrami).

Methods: For phage evaluation experiments, meat slices were cut and spot-inoculated with *L. monocytogenes* cultures (N = 5) and with (1000 MOI Phage) or without (PBS) phages, vacuum packaged, and incubated at 4°C for 30 days. Samples were collected at 0 h, 30 min, 2 h, 1 d, 2 d, 5 d, 7 d, 14 d, 21 d, and 30 days; diluted, plated on PALCAM plates, incubated at 37°C for 24 - 48 h, viable bacterial colonies were counted. Potassium lactate (4.8%) was also evaluated as an antimicrobial. Data analysis was done by plotting CFU/g as a function of time for all treatment points.

Results: Phage treatment results showed a sharp decline of *Listeria* counts (CFU/g) after 30 min in the vacuum packaged roast beef at 4°C. A ~4-log difference from the control was observed in 2 h and no viable cells were detected after 24 h. In contrast, *L. monocytogenes* cells without phage treatments had a steady survival rate with ± 0.5 log cycles variability; regression analysis data showed a slight death rate over 30 d at 4°C. Interestingly, with potassium lactate treatment (4.8%) in vacuum packaged roast beef stored at 4°C, no reduction of bacterial cells was observed.

Significance: Results showed bacteriophage treatment was highly effective to control *L. monocytogenes* in roast beef which can be used as an alternative to prevent any undesired contamination at 4°C.

P3-100 Antimicrobial Packaging against *Escherichia coli* O157:H7 on Beef, Pork and Chicken Using Bacteriophage

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Introduction: *Escherichia coli* O157:H7 is the most common foodborne bacteria that causes bloody diarrhea and, occasionally, hemolytic-uremic syndrome.

Purpose: The purpose of this study is to examine the inhibition of *E. coli* O157:H7 in beef, pork and chicken using antimicrobial packaging containing bacteriophage.

Methods: *E. coli* O157:H7 bacteriophage BPECO 19 was obtained from the bacteriophage bank in Korea. For antimicrobial packaging, the commercial film and absorbance pad were treated on the surface of the each with 10,000, and 100,000 multiplicity of infection (MOI) of bacteriophage BEPCO 19. The meat samples with 3 cm x 3 cm were prepared in vacuum package and sterilized with gamma irradiation. Each meat spiked with *E. coli* O157:H7 10^5 CFU/sample was contacted with bacteriophage-containing film or pad and stored at 4°C. The populations of *E. coli* O157:H7 were counted on SMAC in 1, 2, 4, 8, 12, 24, 28, 72, 120, 168 h.

Results: Antimicrobial film was more effective than antibacterial pad for inhibiting *E. coli* O157:H7 on all types of meat sample. In beef, *E. coli* O157:H7 was reduced completely using antimicrobial film at 100,000 MOI in 1 h and at 10,000 MOI in 12 h, respectively. With antimicrobial pad, *E. coli* O157:H7 was controlled at 100,000 MOI in 12 h and reduced to 1.00 log/sample in 8 h. In pork and chicken, *E. coli* O157:H7 was not cultured at 100,000 MOI by antimicrobial film in 8 h and 2 h, respectively. Unlike beef, antimicrobial pad was effective to inhibit *E. coli* O157:H7 at 10,000 MOI in pork and chicken in 12 h and 8 h, respectively.

Significance: Antimicrobial packaging containing bacteriophage BPECO19 was successfully applied to inhibit *E. coli* O157:H7 on pork, beef and chicken kept in refrigeration.

P3-101 Application of Lytic Bacteriophages to Control *Salmonella* Newport Growth on Cherry Tomato "*Solanum lycopersicum* var. *cerasiforme*"

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Introduction: Bacteriophages have been envisioned as novel and safe tool to control different types of foodborne pathogenic bacteria. *Salmonella* are one of the most important foodborne pathogens that are associated with various outbreaks around the world. Contaminated tomatoes were implicated in several *Salmonella* associated outbreaks reported by CDC.

Purpose: The objective of this work is to identify and characterize different lytic bacteriophages against *Salmonella* Newport and applying these phages to enhance the safety of cherry tomato.

Methods: Different sewage samples were enriched and screened for the isolation of lytic phages specific against *Salmonella* Newport. The isolated phages were identified by TEM and restriction pattern using different restriction enzymes (*TaqI, SwaI, SspI and NdeI*). Their stability, host range patterns and ability to control the growth of *Salmonella* in broth and tomato were determined.

Results: Four lytic phages belong to family *Myoviridae* (CGG 4-1, CGG 4-2) and *Siphoviridae* (CGG3-1, CGG3-2) were selected from 15 isolated phages against *Salmonella* Newport based on their broad host range patterns against 26 tested *Salmonella* serovars. The four phages behaved differently when stored at various environmental conditions. One phage, CGG 4-2, was sequenced and showed no lysogenic or virulent genes in its genome. The isolated phages have a latent period of around 50 min and burst size of around 100. When cocktail of the isolated phages was used to control the growth of *Salmonella* Newport in broth medium, complete inhibition of bacterial growth was observed at 25 and 12°C for 24 h. On the other hand, 4.5-log units reduction in the bacterial count was obtained when applying the phage cocktail on contaminated tomato fruits (MOI = 10^5) stored at 25°C for 3 days.

Significance: These findings suggest that the isolated phages can be considered as a useful biocontrol option for *Salmonella* spp. to enhance the food safety.

P3-102 Isolation of Bacteriophage(s) for the Biocontrol of Pathogenic and Cephalosporins-resistant *Escherichia coli* in Foods of Poultry Origin

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Introduction: *Escherichia coli* has been identified as one of the major causes of foodborne disease worldwide. It causes outbreaks with a huge impact on public health. Bacteriophages are viruses infecting bacteria; they are host-specific, capable of only infecting susceptible species or even strains. Thus, phages as biocontrol agents could be used to lower the incidences of these outbreaks.

Purpose: The focus of the study was to isolate and characterize bacteriophage(s) for the biocontrol of pathogenic and antimicrobial resistant *Escherichia coli* in foods of poultry origin.

Methods: Bacteriophage lytic to pathogenic and antimicrobial resistant *Escherichia coli* was isolated by sample (food, water waste, poultry feces) enrichment method followed by an overnight incubation at 37°C. Presence of lysis on the lawn of the host indicator bacteria showed the presence of the phage which was then purified by gradient Cesium chloride purification and stored in 4°C and -80°C until required.

Results: The bacteriophage was identified morphologically by transmission electron microscopy; which classified the phage to family Myoviridae. This phage has specifically infected and lysed pathogenic and cephalosporins-resistant *Escherichia coli* isolated from poultry.

Significance: By virtue of its lytic ability on pathogenic and cephalosporins-resistant *Escherichia coli* isolated from poultry, this bacteriophage could be a natural alternative to antimicrobials for the biocontrol of pathogenic and susceptible cephalosporins-resistant *Escherichia coli* in chicken-derived foods. Thus could improve food safety and safeguarding public health.

P3-103 Characterization of *Campylobacter* Bacteriophages Isolated from Chicken Samples

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Introduction: *Campylobacter* is the major cause of human bacterial gastroenteritis in developed countries, and chicken meat products are considered to be a major source of campylobacteriosis. Controlling *Campylobacter* in chicken meat products reduces the risk of campylobacteriosis. Bacteriophages are one of the promising biocontrol agents, since they are specific to the host bacteria and not harmful to human.

Purpose: The purpose of this study was to isolate and characterize bacteriophage specific to *Campylobacter*.

Methods: *Campylobacter* strains were isolated from chicken meat and bovine liver samples. A total of 53 isolates (46 *C. jejuni* and 7 *C. coli*) were characterized by using RAPD and automated riboprinting. Of these, 10 strains were used as hosts for the isolation of lytic phages from chicken meat samples. Lytic spectra of the isolated phages were determined against *C. jejuni* and *C. coli* isolates. *C. jejuni* isolate L26 was inoculated in BHI broth at 10^5 CFU/ml in the presence of phage PHC10. Viable counts were determined after 2, 4, 6, 8, 10, 12 and 24 h incubation at 42°C. Significant difference was analyzed by Student's *t*-test.

Results: The characterization of 46 *C. jejuni* isolates showed that they belonged to 25 RiboGroups and 24 RAPD types. On the other hand, 7 *C. coli* isolates were classified into 3 RiboGroups and 3 RAPD types. Among 26 lytic phages for *C. jejuni*, the phage PHC10 had the broadest lytic spectra. The phage PHC10 lysed 28/46 (61%) of the *C. jejuni* isolates. All lytic phages did not lyse *C. coli* isolates. The phage PHC10 inhibited the growth of *C. jejuni* L26 for 12 h. The viable count was significantly lower than that of control even after 24-h incubation ($P < 0.05$).

Significance: The phage PHC10 seems to be an effective biocontrol agent against *C. jejuni*.

P3-104 Safety Aspects of Bacteriocinogenic *Lactobacillus curvatus* Strains Isolated from Smoked Salmon

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Introduction: *Lactobacillus curvatus* is a wide spread bacterium in the natural environment and its potential in producing bacteriocins represents an important opportunity for exploration it as tools for food biopreservation. Safety assessment for presence of virulence and antibiotic resistance genes in lactic acid bacteria (LAB) is an important task to be evaluated in order to be selecting these strains as commercial beneficial cultures.

Purpose: The aim of this study was to explore safety aspects of bacteriocinogenic *Lb. curvatus* strains based on presence and expression of genes related to the virulence factors, production of biogenic amines and antibiotic resistance.

Methods: *Lb. curvatus* ET06, ET30 and ET31 were isolated from smoked salmon, identified based on their biochemical and genetic characteristics including PCR with species-specific primers, and characterized as bacteriocin producers against some food spoilage microorganisms and foodborne pathogens. The strains were subjected to molecular and phenotypical tests to assess the presence of more than 50 genes related to virulence factors, production of biogenic amines and antibiotic resistance.

Results: *Lb. curvatus* ET06, ET30 and ET31 produce class IIa bacteriocins with 2.8, 3.1 and 4.5 kDa, respectively, with bactericidal activity against *Staphylococcus* spp., *Enterococcus* spp. and *Listeria* spp., including *L. monocytogenes* from various serological groups. *Lb. curvatus* ET06, ET30 and ET31 presented also low virulence profile, indicated by the presence of few genes related to antibiotic resistance and surface proteins, based on genetic and physiological tests.

Significance: Besides all beneficial properties studied for various LAB, most considered as GRAS, special attention needs to be paid to the possible presence of virulence factors, production of biogenic amines and antibiotic resistance. Horizontal gene transfer of virulence factors between pathogenic and LAB, including probiotics is a highly possible scenario in case of uncontrolled application of probiotics or starter cultures.

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Poster

P3-105 Diversity of Lactic Acid Bacteria Isolated from Food Spoilage Microflora in India

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Introduction: Lactic Acid Bacteria (LAB) have a unique ability to outcompete, survive and grow in various niches. LAB play a significant role in food spoilage. Diversity of LAB in spoilage is dependent on the intrinsic and extrinsic factors of food. Studies on the diversity of LAB in food spoilage microflora are lacking.

Purpose: To isolate, characterize and identify LAB from various food sources during spoilage.

Methods: Various foods (meat, poultry, seafood and produce) were stored either under refrigeration or room temperature till deemed spoiled. At specified intervals, samples were either swabbed (10 sq. cm) using a sterile swab or diluted in Buffered Peptone Water (BPW) (1:10), serially diluted (1:10 in BPW), spread plated on MRS agar and incubated anaerobically (30°C; 48 h). After incubation, typical LAB colonies were isolated and biochemically characterized (cell-wall type, catalase, oxidase, spore forming ability, acid-fast staining). Isolates exhibiting typical LAB biochemical characteristics were used to determine carbohydrate as well as protein substrate utilization patterns and identified using VITEK-2. Isolated were also grouped based on the substrate utilization patterns using cluster analysis.

Results: A total of 90 isolates were identified either as *Leuconostoc* spp*., Lactococcus* spp., *Streptococcus* spp., *Pediococcus* spp., or *Enterococcus* spp. Isolates exhibited varying levels of carbohydrate and protein utilization indicating diversity in the LAB.

Significance: Diversity in LAB can be further explored to determine their antimicrobial efficacies to improve food safety and increase shelf life.

Acknowledgements: BIG and C-CAMP, India

P3-106 Comparative Proteomic Analysis of *Lactobacillus pentosus* MP-10 for the Identification of Key Proteins Involved in Triclosan Tolerance

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Introduction: Lactobacillus pentosus MP-10 isolated from brines of naturally fermented Aloreña green table olives has potential probiotic traits including inhibition of human pathogenic bacteria, survival at low pH (1.5) and bile salt tolerance (3%). Lactobacillus pentosus MP-10 was intrinsically resistant to some antibiotics but sensitive to different biocides including triclosan.

Purpose: In order to elucidate cellular mechanisms that facilitate triclosan tolerance, the proteome response of Lb. pentosus MP-10 induced or not by triclosan has been analyzed in this study.

Methods: Qualitative and quantitative differences in proteomes were analyzed using two-dimensional electrophoresis (2-DE), tryptic digestion, liquid chromatography-mass spectrometry analysis and database search for protein identification.

Results: The results obtained showed that sub-lethal concentrations of triclosan induced discernible changes in the proteome of exposed *Lb. pentosus* MP-10 and provide insights into mechanisms of response and tolerance. In this way, the proteins differentially expressed in *Lb. pentosus* MP-10 in response to triclosan exposure were those involved in carbohydrate and protein metabolism to sustain energy supply for cell survival under stress conditions.

Significance: Our study suggests that reinforcing carbohydrate and protein metabolism are the major ways of leading to triclosan tolerance in Lb. pentosus MP-10.

P3-107 Effects of Natural Antimicrobials in Combination with Surfactants on the Reduction of Hepatitis A Virus and Murine Norovirus

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❖ ***Developing Scientist Competitor***

Introduction: Natural plant extracts and juices that have antimicrobial properties are useful alternatives to harsh chemical food-contact surface disinfectants. Recently, natural antimicrobials have shown promising antiviral properties, including efficacy against hepatitis A and human norovirus surrogates.

Purpose: The purpose of this study was to determine the efficacy of several natural antimicrobials alone and in combination with surfactants, against hepatitis A virus (HAV) and murine norovirus (MNV-1), a surrogate for human norovirus.

Methods: Grape seed extract, white vinegar, pycnogenol, pomegranate juice, cranberry juice, and sodium bicarbonate were evaluated for efficacy against MNV-1 or HAV at a 1 to 10 (virus to sanitizer) ratio in suspension. Antimicrobials with a pH below 4 were tested with sodium dodecyl sulfate (SDS) and basic compounds were combined with benzalkonium chloride. Following 1 minute contact time at room temperature, antimicrobials were neutralized using 10% FBS in PBS and an acid/base neutralizer. Antimicrobial activity was quantified following plaque assay (n = 3).

Results: MNV-1 (average inoculum titer was 6.17 log PFU/ml) was reduced by 2.33 and 2.65 log PFU/ml when treated with 0.01% or 0.1% grape seed extract, respectively, and by 1.59 log PFU/ml in 0.1% pycnogenol. Undiluted cranberry juice, pomegranate juice, and white vinegar were not effective (< 1 log PFU/ml reduction) alone. Combined with 0.1% SDS, reductions of 1.48 and 1.38 log PFU/ml were observed for cranberry juice and white vinegar, respectively. Reductions of HAV (average inoculum titer was 5.98 log PFU/ml) in 0.01% grape seed extract, 0.1% grape seed extract and 1% pycnogenol were 0.75, 2.00 and 1.33 log PFU/ml, respectively. Sodium bicarbonate (5%) did not inactivate HAV alone, however combined with benzalkonium chloride, a 1.22 log PFU/ml reduction was observed.

Significance: Results suggest the antiviral properties of some natural antimicrobials against foodborne viruses can be enhanced when paired with surfactants. Such combinations could be used as produce washes or food-contact surface disinfectants to help prevent transmission of foodborne viruses.

P3-108 Effect of Zero-valent Iron on Tulane Virus and Murine Norovirus

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Introduction: Zero-valent iron (ZVI) has been used to remediate groundwater of chemical contaminants and has been demonstrated to remove various biological contaminants from water.

Purpose: Here we study the effect of ZVI incorporation in sand filtration columns on the removal of Tulane virus (TV) and murine norovirus (MNV) from an aqueous system.

Methods: TV and MNV suspensions (100 ml at 10^3 to 10^4 RT-qPCR units) were loaded separately onto each of two columns (~130 cm^3 volume; 45-ml pore volume) of sand alone and a 1:1 ratio of sand:ZVI (particle size 0.005 to 0.125 in). Viral suspensions and a subsequent flush of sterile deionized water (300 ml per column) were pumped through the columns at a rate of 1 ml/min. Eighty 5-ml fractions of eluate were collected beginning five minutes after input. Every fifth fraction was tested for viral RNA by RT-qPCR. Two trials were conducted for each virus.

Results: MNV was detected throughout collection of the eluate from the sand column with a peak of over 10^3 RT-qPCR units at the beginning of the flush period. Fewer than 10 RT-qPCR units of MNV were recovered at any point during eluate collection from the ZVI:sand column. TV (average 20 RT-qPCR units at peak) was detected from eluate of the sand column. However, TV was not recovered from the ZVI:sand column eluate for the first trial, but was recovered in the eluate in the second trial comparable to the sand column.

Significance: Incorporation of ZVI in sand filtration systems may aid the removal of virus from water systems with potential application for enhanced safety of drinking and irrigation water. Comparison of the significant removal ($P < 0.05$) of both surrogates provides evidence for interaction of the viral capsid with ZVI.

P3-109 Peracetic Acid Induced Viable but Non-Culturable *Salmonella* in Poultry Meat

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Introduction: Some bacteria tolerate harmful environmental insults by entering the viable but non-culturable (VBNC) state. The VBNC cells lose the ability to grow in routine culture media yet maintain their metabolic activity as well as the ability to cause diseases. This phenomenon, described in over 60 different pathogenic species, poses a significant risk to public health as it renders the bacteria not only resistant to chemical interventions but impairs their growth on diagnostic culture media.

Purpose: The goal of this study was to examine the ability of commercial food disinfectants to induce the VBNC state in foodborne pathogens such as *Salmonella*.

Methods: A laboratory-based spray model was developed and was used to test *Salmonella enterica* bearing chicken parts sprayed with either peracetic acid (PAA) or 1, 3-Dibromo-5, 5-dimethylhydantoin (DBDMH). *Salmonella* recovered from the test samples were enumerated based on traditional culturing methods coupled with a direct viability assessment via LIVE/DEAD fluorescent dyes.

Results: Our results demonstrate the presence of over 1 log of *Salmonella* population that failed to grow on the agar media in response to PAA treatment ($P < 0.00$), while no VBNC population was detected in either the DBDMH-treated or un-treated control samples ($P > 0.05$). A detailed description of our spray protocol, experimental reproducibility and statistical analysis of the results are also provided.

Significance: Both PAA and DBDMH are widely used meat processing aids with the former being considered as one of the most effective disinfectants in the industry. The formation of the VBNC state *in Salmonella* after treatment with PAA poses a significant concern to public food safety as it may lead to underestimation of *Salmonella* occurrences in food samples.

P3-110 Antimicrobial Activity of Lactic Acid Bacteria Isolated from Fermented Taro Skins against *Salmonella* Typhimurium and *Listeria monocytogenes*

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❖ ***Developing Scientist Competitor***

Introduction: Taro is the staple of native Hawaiian diet and an important part of Polynesian culture. Pigs recover from gastrointestinal illness when fed cooked taro skins. It was hypothesized that lactic acid bacteria (LAB) may be present in the taro skins and responsible for the recovery.

Purpose: This study aimed to identify dominant bacterial species present in fermented taro skins and determine their antimicrobial potential against diarrhea-causing bacteria.

Methods: Three samples of cooked taro skins were obtained from a taro processing facility and incubated at 21°C. Aerobic plate count and LAB count of the sample were determined at 0, 24, and 34 h. Selected bacterial isolates were identified by sequencing the 16S rRNA gene. Culture supernatants of representative LAB isolates were evaluated on their bactericidal properties against *Salmonella* Typhimurium and *Listeria monocytogenes*. Moreover, the supernatants were neutralized with NaOH or treated with proteinase K before being further assessed on their antimicrobial activity.

Results: During fermentation, the LAB count of cooked taro skins increased from 3.0 log CFU/g to 8.7 log CFU/g in 34 h. All samples showed a dominance of LAB in the microbial populations. LAB constituted 91% of 37 sequenced isolates. *Leuconostoc mesenteroides* was the dominant LAB species (75%), with *Lactococcus lactis* and *Weissella soli* also present. These LAB species showed varying antimicrobial activity against tested diarrhea-causing bacteria. *Salmonella* Typhimurium died in culture supernatants of *L. mesenteroides* and *W. soli* within 2 h; *L. monocytogenes* died in a culture supernatant of *L. mesenteroides* within 8 h. Compared with control, the supernatants still showed antimicrobial effects after being neutralized. The proteinase treatment reduced the inhibition zones of the supernatants against the tested pathogens.

Significance: This study indicates the presence of beneficial LAB in fermented taro skins. The LAB species could potentially be utilized to enhance food safety.

P3-111 Antimicrobial Effect of Carvacrol and Cinnamaldehyde against *Salmonella* Tennessee in Peanut Paste at Different Water Activities and Fat Levels

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❖ ***Developing Scientist Competitor***

Introduction: Essential oil components carvacrol and cinnamaldehyde have shown effectiveness against *Salmonella* in microbiological media and various high water activity (a_w) foods. However, their effectiveness at low a_w has not been well established.

Purpose: To evaluate the antimicrobial activities of carvacrol and cinnamaldehyde against *Salmonella* Tennessee K4643 in peanut paste with different a_w and fat levels over 5 days at 25°C.

Methods: Low fat (< 5%) peanut pastes were made with defatted peanut flour, glycerol and DI water to achieve a_w of 1.0, 0.7, 0.5 and 0.3. High fat (50%) peanut paste was prepared with defatted peanut flour and peanut oil (a_w = 0.29). Carvacrol (0, 1250 and 2500 ppm) or cinnamaldehyde (2500

and 5000 ppm) were added to each sample. All samples were then inoculated with 8 log *Salmonella* Tennessee K4643 and stored at 25°C. Samples were taken at day 0, 1, 3 and 5 and *Salmonella* were enumerated on tryptic soy agar. Each experiment was performed in triplicate.

Results: In low fat peanut pastes, *Salmonella* at a_w 1.0 increased by 1 log over 5 days in the control. Neither compound reduced viable *Salmonella* at the maximum concentration at a_w 1.0. Lowered a_w (0.7 - 0.3) alone reduced *Salmonella* by 2.58 - 3.45 logs on day 5. Cinnamaldehyde was more effective than carvacrol. On day 5, the population reached an undetectable level with 2500 ppm cinnamaldehyde while 2.69 and 3.08 log remained with 5000 ppm carvacrol at a_w 0.5 and 0.3, respectively. Increased fat concentration greatly reduced the effectiveness of both compounds as *Salmonella* was not affected by either compound after 5 days ($P > 0.05$).

Significance: Both essential oil components effectively inactivated *Salmonella* Tennessee in low fat peanut paste at $a_w \leq 0.7$ with cinnamaldehyde demonstrating better inactivation than carvacrol. The effectiveness of both compounds was dramatically reduced by increased fat level.

P3-112 Antibacterial Effect of Wasabi against *Listeria monocytogenes* and *Salmonella* Typhimurium

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Introduction: *Listeria monocytogenes* and *Salmonella* Typhimurium are two of the major bacterial pathogens frequently involved in foodborne outbreaks. Control of the two pathogens in foods especially Ready-to-Eat foods is essential to ensure food safety. It is of great interest in the use of antimicrobial compounds naturally present in edible plants to control foodborne pathogens. Wasabi (*Wasabia japonica*) is such an edible plant containing antimicrobial compounds.

Purpose: The purpose of this study was to evaluate the antibacterial effect of wasabi against *L. monocytogenes* and *Salmonella* Typhimurium.

Methods: Brain Heart Infusion (BHI) broth media supplemented with wasabi powder at varying concentrations were used in the study. The media were inoculated with fresh culture of *L. monocytogenes* or *Salmonella* Typhimurium to obtain the initial bacterial concentration approximately 10^5 CFU/ml. For comparison, a pure chemical, isothiocyanate (AITC, a major antimicrobial compound present in wasabi) was also tested at different concentrations in BHI broth against *L. monocytogenes*. Each experiment was carried out for 12 h at 37°C with agitation. The viable bacterial concentrations were determined by plate count method using *Listeria* agar and *Salmonella Shigella* agar.

Results: The data showed that wasabi at 0.5% to 2% effectively prevented the growth of both *L. monocytogenes* and *Salmonella* Typhimurium as evidenced by the lower cell counts compared with the control. At least 5-log unit reduction in cell counts of either bacterium was achieved when the media contained 2% wasabi. In contrast, 2.5- and 7.5-log unit reductions in listerial cell counts were obtained when the media contained 93 ppm and 930 ppm AITC, respectively.

Significance: The results showed that wasabi has high antibacterial activity against *L. monocytogenes* and *Salmonella* Typhimurium, suggesting that wasabi has a high potential to be used to effectively control the two pathogens in foods, especially at the point of ingestion of many Ready-to-Eat foods.

P3-113 Bioactive Extracts of Berry Pomace and Probiotic *Lactobacillus* Synergistically Inhibit Pathogenic *Salmonella*

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Introduction: As the role of colonic microfloral composition in farm animal health are getting more attention, the improvement of gut flora with a combination of organic prebiotic-like substrates and probiotics is a critical area of interest. Berry pomaces are of interest to researchers because of their richness in several phytochemicals including proanthocyanidins, anthocyanins and other flavonoids. Partial degradation of phenolics by probiotics can increase bioavailability of these derivatives, hence combine effect of probiotic and its degradation activity of phenolic components will reduce pathogenic bacterial colonization in animal gut.

Purpose: To modulate the gut flora with synbiotic culture, we evaluated the role of bioactive extracts of berry pomace in growth of beneficial bacteria such as *Lactobacillus plantarum* and their inhibitory role against *Salmonella* Gallinarum and S*almonella Pullorum*.

Methods: Growth performance of pathogens and probiotics were carried out in broth and fecal-medium. Co-culture of pathogen and probiotic was done in fecal medium in presence of berry pomace extract.

Results: We found that 2.0 mg GAE/ml of blackberry or blueberry pomace extract reduced the growth of *Salmonella* Gallinarum and *Salmonella* Pullorum by > 5 logs at 24 h in broth. In semisolid poultry fecal-medium, which represents simulated gut environment, 1.0 mg GAE/ml of both extracts reduced the growth of these pathogens by > 2 logs at 24, 48 and 72 h time points. The growth of probiotic *L. plantarum* was unaffected in the presence of berry pomace extracts in broth but growth stimulation was observed in fecal-medium at 72 h. Moreover, *L. plantarum* completely inhibited *Salmonella* Gallinarum and *Salmonella* Pullorum when co-cultured in fecal-medium in the presence of 1.0 mg GAE/ml of both pomace extracts.

Significance: This study indicated that bioactive extracts from berry pomace are potential antimicrobials for organic producers which act synergistically with probiotic to reduce pathogen in simulated poultry gut condition.

P3-114 Efficacy of a Citric Acid-based Organic Sanitizer against *Salmonella enterica* and *Escherichia coli* K-12 on Organic Leafy Greens during Wash Water Recycling

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Introduction: Post-harvest treatment with sanitizers is an effective way to reduce pathogenic and background microorganisms on organic leafy greens.

Purpose: The objective of this study was to investigate the efficacy of a citric acid-based organic sanitizer (organic Chicowash™) against *Salmonella enterica* serovar Newport on organic leafy greens in laboratory scale and surrogate *Escherichia coli* K-12 on iceberg lettuce in a large scale with recycling of wash water.

Methods: In the laboratory scale test, ten grams of organic iceberg or romaine lettuce, baby or mature spinach samples were inoculated with *Salmonella* Newport and treated with 200 ml of 1:20 Chicowash for 1 or 2 min. In the large scale test, seven kilograms of organic iceberg lettuce were inoculated with *E. coli* K-12, treated with 75 liters of 1:20 Chicowash for 2 min, and 4 additional batches of lettuce samples (7 kilograms each) were washed consecutively. Samples were stored at 4°C and enumerated for surviving *Salmonella* or *E. coli* K-12 at day 0, 1 and 3. De-ionized water and tap water were used as controls for laboratory and large scale tests, respectively.

Results: In the laboratory scale test on 4 leafy green types, Chicowash caused 0.1 - 1.3 and 0 - 1.2-log reduction in *Salmonella* population at day 0 for 1 and 2 min treatments, respectively; at day 1, reductions of 1.6 - 2.7 and 1.4 - 2.1 logs were observed for 1 and 2 min treatments, respectively. The

greatest reductions were seen at day 3; 2.1 - 2.5 and 2.3 - 2.9 logs for 1 and 2 min treatments, respectively. In the large scale test, Chicowash showed similar reductions in *E. coli* K-12 population on iceberg lettuce after each wash; 1.6 - 1.9, 2.3 - 2.6, and 2.9 - 3.5 logs for day 0, 1 and 3, respectively. The reduction was storage time dependent.

Significance: The results could provide the organic produce industry with more options to select alternate natural sanitizers for post-harvest treatments.

P3-115 Efficacy of a Low pH Acid Antimicrobial on Pork Products Inoculated with Generic *E. coli* and *Salmonella*

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Introduction: Food safety and reduction of bacteria on food products decrease bacteria, especially pathogens, is important to the food industry. Low pH acid antimicrobials have been used in the food market with varying success.

Purpose: This study was designed to look at the application of a low acid pH antimicrobial (sold under the trade name Citrilow™) to pork products.

Methods: Bone-in, boneless and pork trim products were inoculated with generic *Escherichia coli* (EC) and *Salmonella* (SLM). For the bone-in and boneless products, ten samples of each type were individually weighed and the top surface was individually swabbed twice for the control samples. The pieces were flipped over and treated with the antimicrobial (pH 1.2, flow rate 2 gallons per minute) for 5 s. The pieces drained for 20 s and then the treated side was swabbed twice. The samples were then reweighed. The swab samples were tested for Aerobic Plate Count (APC), EC, and quantitative SLM.

Results: The bone-in samples had a reduction of 3.21 logs of APC, a reduction of 3.75 logs of EC and a reduction of 2.24 logs of SLM. The boneless samples had a reduction of 3.53 logs of APC, a reduction of 4.54 logs of EC and a reduction of 2.45 logs of SLM. The weight difference of the average green weight and the average treated weight for the bone-in product was 0.39% and for the boneless product was 0.28%.

Significance: As shown in the above data, the use of a low pH antimicrobial showed a reduction of greater than three logs of APC, almost a four-log reduction of EC, and a greater than two-log reduction of SLM without adding greater than 0.49% moisture pick-up. The reduction of bacteria including pathogenic bacteria provides the consumer with a safer product.

P3-116 Efficacy of a Commerical Antimicrobial Agent on Organic Produce against *Escherichia coli* O157:H7 and *Salmonella enterica*

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Introduction: Produce has potential for contamination by bacterial pathogens on the farm, during harvest and during handling. Post-harvest washing of produce creates the potential for cross-contamination from contaminated to non-contaminated produce. Methods for controlling cross-contamination need to be developed that meet requirements of organic growers.

Purpose: The purpose of this study was to evaluate the antimicrobial efficacy of a novel commercial sanitizing solution (CX) for preventing cross-contamination of USDA-certified organic produce including, cherry tomatoes, baby spinach, and romaine lettuce during washing.

Methods: Pathogens used were cocktails of *Escherichia coli* (EC) O157:H7 or *Salmonella enterica* (SE) resistant to nalidixic acid (NAR). A microbroth dilution assay was used to determine minimum inhibitory concentration (MIC) of CX against EC or SE. Wild type and NAR strains were compared to confirm similar resistance characteristics to CX. Produce samples were inoculated with EC or SE and were placed into an aqueous solution at 0%, 0.5% and 0.75% CX (v/v) with and without 1.0% organic load (OL). To determine if cross-contamination occurred in the treatment liquids, un-inoculated produce was introduced to the same CX solutions after inoculated produce. After washing, produce was hand-massaged and rinsate plated on CT-SMAC or TSAN (TSA + Nalidixic acid) to enumerate survivors.

Results: The MICs for all strains of EC and SE were the same at 0.75%. For all produce samples at the initial sampling, CX solutions had little effect on reducing survival of EC or SE. However for the un-inoculated produce, cross contamination by EC or SE was eliminated (< 2 log CFU/ml rinsate) by CX compared to the control (ca. 4 - 5 log CFU/ml of rinsate). OL had no effect on CX inactivation.

Significance: Results indicate that the commercial sanitizer at 0.5% and 0.75% had efficacy in eliminating cross-contamination on organic produce in a model wash system.

P3-117 Probiotic Properties and *Salmonella* Inhibition Using Bacteriocin-producing Lactic Acid Bacteria Isolated from U.S. Kimchi and Broiler Chicken

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Introduction: Salmonellosis associated with consumption of contaminated meats, especially poultry products, is a significant concern due to the major outbreaks in the US. Although it has been known that lactic acid bacteria (LAB) could reduce *Salmonella* infections, there was no report that focuses on isolating LAB from US Kimchi for inhibiting the growth of *Salmonella* spp.

Purpose: The present study screened LAB, which showed antimicrobial effects against four clinically important serotypes of *Salmonella*. Additionally, we selected LAB that had additional probiotic properties.

Methods: Using an agar diffusion test, LAB strains isolated from US Kimchi and broilers produced bacteriocin-like substances, which showed antimicrobial effects against *Salmonella* Enteritidis, Heidelberg, Newport, and Typhimurium. These strains were investigated with regard to additional probiotic properties such as tolerance to gastric juice and bile, resistance to enzymes, and antibiotics susceptibilities.

Results: Out of 488 LAB strains isolated, 30 LAB samples including *Lactobacillus casei* or *paracasei* Cab-18, *Lb. saniviri* Cuc-1, and *Leuconostoc mesenteroides* Com-54 isolated from kimchi and *Lb. johnsonii* F-6 and *Lb. crispatus* F-59 isolated from broiler chicken demonstrated promising probiotic properties with the ability to produce bacteriocin-like substances with relatively strong antimicrobial activity against *Salmonella*.

Significance: The LAB strains isolated in this study could be applied as feed supplements to poultry for the purpose of reducing colonization of *Salmonella* in poultry as well as contamination during processing.

P3-118 Antimicrobial Resistance and Virulence Genes of Extraintestinal Pathogenic *Escherichia coli* (ExPEC) Isolates from Retail Poultry Meats

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Introduction: Extraintestinal pathogenic *Escherichia coli* (ExPEC) can cause urinary tract infections and neonatal meningitis. Antimicrobials used in poultry can promote antibiotic resistance, however little is known about the genotype of ExPEC from retail poultry meats.

Purpose: The objective of this study was to analyze the resistance and virulence genotypes of ExPEC isolated from retail poultry meats to assess their food safety risks.

Methods: Fifty-seven ExPEC isolates from retail chicken (43) and turkey (14) meats were investigated for their susceptibility to 14 antibiotics using Sensititre system according to the CLSI's guidelines. A DNA-microarray designed to detect 264 virulence genes and 96 antibiotic resistance genes as well as PCRs were used to determine their genotype.

Results: The most common resistance pattern was amoxicillin-clavulanic acid-ampicillin-cefoxitin-ceftiofur-ceftriaxone-tetracycline (43%). Resistance to sulfisoxazole, streptomycin and gentamicin was found in 16 (28.0%), 15 (26.3%) and 7 (12.3%) isolates, respectively. Except for gentamicin, resistance was more prevalent in chicken than in turkey. A total of 25, 16 and 9 isolates harbored 1-5, 6-10 and 11-17 resistance determinants, respectively. The β-lactamase bla_{CMY-2}, bla_{OXA27}, bla_{OXA2b} and bla_{TEM} genes were detected in 24 (42.1%), 16 (28.0%), 17 (29.8%) and 6 (10.5%) isolates, respectively. Other detected genes include those conferring resistance to aminoglycosides (*aadA, aphA, aph3/strA*), quinolone (*aac6-1b-CR, qnrA*) trimethoprims (*dhfr-1, dhfr-15*), tetracyclines (*tetA, tetB, tetR*) and sulfonamides (*sul1, sul2*). Class 1 integrons were found in 8 isolates. The number of detected virulence genes varied from 29 to 62. All 57 isolates harbored the following virulence genes: *b(1121), csgA, csgE, gad, EcSf, hlyE, ibeB1a, ibeB1b, iucD, iut(A2), iut(upec), iutA, ompA, sitA* and *sitD*.

Significance: This study shows that ExPEC from retail poultry meats can harbor multiple antibiotic resistance and virulence genes of clinical importance. The presence in retail poultry meats of such isolates harboring class D β-lactamases (bla_{OXA}) with potential carbapenem hydrolyzing activity is of concern and raises a potential food safety risk.

P3-119 Evaluating the Re-usability of Organic Sanitizers in Reducing *Escherichia coli* O157:H7 on Organic Leafy Greens

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❖ ***Developing Scientist Competitor***

Introduction: In recent years, organic leafy-greens have been implicated in *Escherichia coli* O157:H7 related outbreaks. Approved antimicrobials for organic produce are limited, resulting in investigations into plant-derived alternatives. Oregano and cinnamon essential oil (EO) and their primary constituents, carvacrol and cinnamaldehyde, respectively, have proven to be effective against *E. coli* O157:H7. Flume-tank washing of organic greens, prior to packaging, is common practice where wash water is re-used multiple times before being discarded. It is therefore important to evaluate the re-usability of antimicrobials during flume-tank washing.

Purpose: Evaluate the re-usability of essential oils and their primary constituents for flume-tank washing of organic leafy-greens to reduce *E. coli* O157:H7.

Methods: Oregano and cinnamon EO and carvacrol and cinnamaldehyde were tested at 0.5% concentration. Hydrogen-peroxide, water and phosphate buffered saline were used as controls. Organic leafy greens, baby and mature spinach and romaine and iceberg lettuce, were inoculated with *E. coli* O157:H7 (10^6 CFU/g). Each antimicrobial was re-used five times to wash (for 1 min) five separate batches of inoculated leafy greens that were stored at 4°C and surviving bacteria enumerated on days 0, 1, and 3. Wash water was enumerated for *E. coli* O157:H7 after each use and pH and turbidity measured.

Results: Tested antimicrobials showed significant ($P < 0.05$) reduction of *E. coli* O157:H7 over five washes. Carvacrol and oregano EO were the most effective, reducing pathogen populations to undetectable levels on day 0 in all leafy greens except mature spinach where undetectable levels were achieved on day 3 with carvacrol. Cinnamon EO and cinnamaldehyde were able to reduce pathogen populations to undetectable levels in all leafy greens by day 1. Wash water resulting from the antimicrobial washes did not show any growth of *E.*coli O157:H7.

Significance: This study provides evidence that plant-derived compounds could serve as effective sanitizers that retain their antimicrobial activity with continued use.

P3-120 Reduction of Shiga Toxin-producing *E. coli* and *Salmonella* Typhimurium on Cattle Hides by Spray Treatment with Levulinic Acid Plus Sodium Dodecyl Sulfate

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Introduction: Animal hides are a significant source of zoonotic pathogens which contaminate carcasses at beef slaughter. Studies revealed that 76% and 67% of animal hides entering processing plants can be contaminated with *E. coli* O157 and non-O157 STEC, respectively.

Purpose: The purposes of this study was to determine the efficacy of levulinic acid plus sodium dodecyl sulfate (SDS) to inactivate STEC and *Salmonella* Typhimurium on cattle hides as a surface spray treatment at different concentrations in vitro.

Methods: A mixture of six isolates of STEC, including serovars O26, O45, O103, O111, O121, and O157 (10^8 CFU/ml) and a mixture of 5 strains of *Salmonella* Typhimurium (10^8 CFU/ml) were sprayed on the surface of 10 x 10-cm sections of cattle hide. The inoculated hides were dried under a hood at either 21°C or 4°C for 72 h. The hides were treated by surface spray with a microbicide comprised of levulinic acid plus SDS at different concentrations at 45 psi for 15 s. Water only was used as the negative control. Sponge samples of the hides were collected at 1, 3, and 5 min after treatment and enumerated for STEC and *Salmonella*.

Results: For STEC-contaminated hides, 3% levulinic acid plus 0.5% SDS for 5 min reduced STEC populations by 2.3 log/cm^2, compared to the water only treatment. For *Salmonella* Typhimurium-contaminated hides, treatment with 2% levulinic acid plus 0.2% SDS reduced the *Salmonella* population by 3.2 log/cm^2. Scrubbing hides with a brush processing for 30 s followed by the microbicide spray treatment further reduced *Salmonella* contamination by 0.5 log CFU/cm^2. However, for wet hides, a spray treatment with 4% levulinic acid plus 2% SDS for 5 min reduced *Salmonella* by only 1.3 log CFU/cm^2.

Significance: These in vitro studies provide needed for designing validation studies for this live cattle hide intervention treatment to reduce STEC and *Salmonella* contamination.

P3-121 Delivery of Antimicrobials via an Air-assisted Electrostatic Spraying System (ESS) or the Sprayed Lethality In Container (SLIC) Method on the Surface of Beef Subprimals to Control Shiga Toxin-producing *Escherichia coli*

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Introduction: Innovative applications for delivering antimicrobials to beef are being researched to reduce costs, improve coverage, and enhance safety.

Purpose: Evaluate the efficacy of an air-assisted electrostatic spraying system (ESS) and/or the Sprayed Lethality in Container (SLIC®) method to deliver antimicrobials on surface of beef subprimals to reduce levels of Shiga Toxin-producing *Escherichia coli* (STEC).

Methods: Beef subprimals were surface inoculated (lean side; ca. 5.5 log CFU/subprimal) with a cocktail comprised of single strains of STEC (STEC-8; O111:H, O45:H2, O103:H2, O104:H4, O121:H19, O145:NM, O26:H11, and O157:H7). Inoculated subprimals were then surface treated with lauric arginate (LAE; 50 ppm), peroxyacetic acid (PAA; 400 ppm), or cetylpyridinium chloride (CPC; 400 ppm) by passing each subprimal through an ESS cabinet or via SLIC. Next, subprimals were vacuum-packaged and stored at 4°C for up to 7 days. One set of subprimals was sampled after 2 h of storage, whereas the other set, was retreated with the above antimicrobials via SLIC after 3 days of storage and then sampled after 2 h or 4 days of additional storage at 4°C.

Results: Single/initial application of LAE, PAA, or CPC via ESS or SLIC resulted in reductions of ca. 0.6 to 2.0 log CFU/subprimals over 7 days of storage at 4°C. However, when subprimals were initially treated with LAE, PAA, or CPC via ESS or SLIC and then retreated with the same antimicrobials via SLIC on day 3, additional reductions of 0.1 to 1.6 log CFU/subprimals in pathogen numbers were observed after an additional 4 days of storage at 4°C.

Significance: Application of LAE, PAA, or CPC, alone or in combination, via ESS or SLIC is effective for reducing low levels of STEC on the surface of beef subprimals.

Poster

P3-122 Evaluating the Efficacy of Lauric Arginate Followed by a Peroxyacetic Acid Spray for Reducing Shiga Toxin-producing *Escherichia coli* (STEC) on Beef Subprimals

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Introduction: STEC serotypes O157, O145, O121, O111, O103, O45 and O26 (STEC-7) are considered adulterants in non-intact/ground raw beef by the USDA. Regulatory mandates to control these STEC have led to numerous intervention strategies throughout the beef processing system.

Purpose: This study validated the efficacy of two lauric arginate (LAE) solutions [Mirenat-GA (M) and Cytoguard LA20 (C)] as an in-bag subprimal treatment, followed by a peroxyacetic acid (PAA; 200 ppm solution) spray upon re-opening vacuum bags for mechanical tenderization, to reduce STEC-7 populations.

Methods: Beef striploins were inoculated with a STEC-7 cocktail to achieve 5 log CFU/cm^2. Four primary treatments (M+tap water, M+deionized water, C+tap water, and C+deionized water) were evaluated using seven application concentrations (0, 50, 100, 150, 200, 250 and 500 ppm) per kilogram of meat treated. Treatments were applied to loin surfaces within Cryovac bags immediately before vacuum sealing, allowing the natural purge of the vacuum-packaged subprimal to distribute the antimicrobial. Packaged product was stored at 4°C for 24 or 48 h, then removed from bags and sprayed with PAA. STEC populations were determined post-inoculation before treatments, post-chill period, and post-PAA treatment. The statistical analysis was done using SAS 9.4 and was a repeated measures analysis.

Results: No differences ($P > 0.05$) were found among the primary LAE treatments; thus, observations were pooled to evaluate concentration effects. STEC reductions greater ($P \leq 0.05$) than the 0 ppm control were seen at ≥ 200 ppm LAE, with no differences observed between solution sources. Overall STEC-7 reductions were ~1.2 log CFU/cm^2 at ≥ 200. No additional STEC population reductions were achieved by applying 200 ppm PAA to chilled subprimals.

Significance: This research validated an in-bag intervention strategy that could be applied by the beef industry to help reduce STEC risk in enhanced/tenderized beef products.

P3-123 Antimicrobial Effect of Pomegranate Juice on *Listeria innocua* and *Escherichia coli* in Different Culture Systems

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Introduction: The edible part of pomegranates (*Punica granatum* L.) contains organic acids, sugars, vitamins, polysaccharides, polyphenols and minerals with unique flavor, taste and heath promoting characteristics. Natural antimicrobials developed from pomegranates (seeds and peel) have potential application in food preservatives.

Purpose: The objective of this study was to investigate the antimicrobial effects of pomegranate juice at different concentration against *Listeria innocua* and *Escherichia coli*.

Methods: The experiments were conducted in the distilled water (DW) and bacterial culture broth systems. *L. innocua* or *E. coli* at 10^5 CFU/ml was inoculated into sterile test tubes containing five different concentrations of pomegranate juice and then incubated at 4, 25 or 37°C, respectively. The bacterial population and pH value were monitored at 0, 6, 24 and 48 h.

Results: The antimicrobial effects of pomegranate juice were dependent upon the concentrations of juice, culture conditions (temperature, time and matrix), as well as bacteria species tested. Overall, increased juice concentration and incubation temperature resulted in increased antibacterial effects. Both bacterial species were more effectively killed in DW system than in culture broth, while *L. innocua* was more sensitive to pomegranate juice compared with *E. coli*. In DW system, pomegranate juice regardless of concentrations reduced the population of *L. innocua* to undetectable levels at 4, 25 or 37°C for 48 h while *E. coli* was reduced by 3.6 log when treated with 10% juice at 37°C for 48 h. The population of *L. innocua* and *E. coli* were also significantly inhibited by 20% pomegranate juice ($P < 0.05$) in the culture broth system at 37°C for 48 h. By monitoring the pH changes, it was suggested that both high acidity and polyphenols in pomegranate juice contributed to the antimicrobial effect.

Significance: This study provides useful information on potential application of pomegranates or pomegranate extracts as food additives in food systems.

P3-124 Evaluating the Efficacy of Three USDA-approved Antimicrobial Sprays for Reducing Surrogate Shiga Toxin-producing *Escherichia coli* (STEC) on Bob Veal Carcasses

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❖ ***Developing Scientist Competitor***

Introduction: STEC serotypes O157, O145, O121, O111, O103, O45, and O26 are adulterants in non-intact/ground raw beef and veal. Veal has been recognized as an important contributor to STEC positive regulatory samples, leading to regulatory actions and costs for processors.

Purpose: This study evaluated the efficacy of approved antimicrobial sprays (4.5% lactic acid, Citrilow™, and Beefxide®) for reducing STEC surrogates on processed bob veal carcasses under commercial conditions, and determined their impact on carcass color.

Methods: Bob veal calves (< 3 weeks of age and < 150 lbs) were harvested using USDA approved practices and were inoculated with a 5-strain mixture of surrogate *E. coli* bacteria. Levels of surrogate *E. coli* were determined post-inoculation, post-final water wash, post-antimicrobial spray, post-24-h chill, and post-2nd antimicrobial spray. Carcass color was measured using a Hunter MiniScan at each sampling point. Three experimental replications were conducted, each using four bob veal calves. Statistical analysis was done using SAS 9.4 and was a repeated measurement analysis with the repeated measurements over treatments.

Results: Water washing alone decreased ($P < 0.05$) *E. coli* levels by 0.88 log CFU/cm^2. There were no differences in the effectiveness of the three antimicrobial treatments. Antimicrobial sprays applied to pre-rigor carcasses provided an additional 0.5 log cycle reduction compared to water alone. Carcass *E. coli* counts declined further (~0.4 log cycles) during the 24-h chilling period (total reductions of ca. 1.8 log CFU/cm^2). A second antimicrobial spray to chilled carcasses provided no additional reductions. Chilled carcass color values (L*, a* and b*) were similar ($P > 0.05$) among all antimicrobials applied and the water only control.

Significance: These findings validate three USDA-approved antimicrobial sprays at the hot carcass level, providing the veal industry with practical tools to reduce their STEC positive rates, and in return, reducing the public health risk associated with STEC contamination of veal products.

P3-125 Antibacterial Effectiveness of Cinnamaldehyde against *Escherichia coli* O157:H7 and *Salmonella enterica* in Carrot and Blackberry Juice Blends Held at 4°C

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Introduction: Increased consumer concerns over adverse health effects of eating foods with synthetic preservatives are driving food processors to use naturally-derived antimicrobials for controlling foodborne pathogens.

Purpose: The present study was conducted to determine the antimicrobial effect of cinnamaldehyde, a plant-based extract, against *E. coli* O157:H7 and *Salmonella enterica* in refrigerated carrot juice (CRJ) and blackberry juice (BBJ).

Methods: Carrot juice (pH 6.25, 8.5°Brix) and BBJ (pH 3.59, 12.3°Brix) with added cinnamaldehyde (0.25, 0.5, and 1.5 µl/ml) were inoculated with a 5-strain cocktail of *E. coli* O157:H7 or *S. enterica* to give an initial viable count of 5.0 log CFU/ml. Inoculated juices without cinnamaldehyde served as control. Survival of pathogens in the juices (4°C) for 24 hours was monitored by surface plating diluted (10-fold) juice samples on sorbitol MacConkey agar (*E. coli* O157:H7) and xylose lysine tergitol 4 agar (*S. enterica*) and counting bacterial colonies on agar media following incubation (35°C, 48 h).

Results: No growth of the pathogens was observed in juices with or without added cinnamaldehyde. Numbers of viable pathogens in control juices were reduced by about 1.0 to 1.4 log CFU/ml at 24 h. Both pathogens were less sensitive to the lethal effects of cinnamaldehyde in CRJ compared to BBJ. At 24 h, initial viable counts of *S. enterica* and *E. coli* O157:H7 in CRJ with 2.0 µl/ml cinnamaldehyde were reduced by ~4.5- and 5.0 log, respectively ($P < 0.05$). In contrast 1.5 µl/ml cinnamaldehyde in BBJ resulted in a 5-log reduction of *S. enterica* and *E. coli* O157:H7 as early as 2 and 8 h, respectively ($P < 0.05$).

Significance: Cinnamaldehyde has good potential for use as a natural antimicrobial to decrease the viability of bacterial pathogens in refrigerated fruit and vegetable juice blends.

P3-126 Novel Inhibitors of Virulence Gene Expression in Shiga Toxin-producing *Escherichia coli*

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Introduction: A series of low molecular weight chemical compounds (LMWC) capable of inhibiting Shiga Toxin gene expression has been identified, which could diminish the virulence of this pathogen.

Purpose: The aim of this study was to determine the influence of LMWC on growth and expression of the Shiga Toxin genes, *stx*1 and *stx*2, by *Escherichia coli* O157:H7.

Methods: STEC O157:H7 strains were grown in tryptic soy broth (TSB) or Luria Bertani broth (LB) containing 0.1% DMSO (control) and 50 µm LMWC at 37°C. Bacterial growth was monitored by measuring optical density ($OD_{600\ nm}$) and viable cell count over 10 h. qPCR was performed on cDNA generated at mid-logarithmic (MLP) (3.5 h), late logarithmic (LLP) (6.5 h) and stationary (SP) (20 h) growth phases to monitor gene expression. Primers were designed for *gap*A (reference), *arc*A (housekeeping), *stx*1, and *stx*2 genes.

Results: No significant difference in viability was detected when the bacteria were treated with LMWC CCG-203592. In TSB, the expression of *stx*1 and *stx*2 in strain c7927 decreased by 51 ± 7.5% ($P \leq 0.007$) and 39 ± 9.8% ($P \leq 0.02$) at the LLP, respectively. The expression of *stx*2 by strain 3178-95 decreased by 26 ± 40.6% ($P \leq 0.38$) at the MLP, by 30 ± 19.4% ($P \leq 0.11$) at the LLP and by 40 ± 18.6% ($P \leq 0.19$) at the SP. In LB broth, *stx*1 and *stx*2 expression by strain 3178-95 decreased by 34.5 ± 3.5% ($P \leq 0.05$) and 85.7 ± 1.4% ($P \leq 0.008$) at the LLP, respectively, while *stx*2 expression decreased by 87.1 ± 1.4% ($P \leq 0.008$) at the SP.

Significance: The use of LMWC could be an alternative way to inhibit virulence gene expression in STEC without causing cell death, indicating a promising starting point for development of novel antimicrobial agents against STEC.

P3-127 Host Autophagy Diminishes *Escherichia coli* O157:H7 Epithelial Adhesion

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Introduction: *Escherichia coli* O157:H7, is a leading Shiga Toxin-producing *E. coli* that has low infectious dose and causes serious illness in humans. *E. coli* O157:H7 gut colonization is the central problem leading to beef, dairy and green vegetable contamination, while adhesion to epithelial cells is the first step for *E. coli* O157:H7 to colonize, proliferation, and further develop infection and tissue damage in the gut. Autophagy is a pivotal catabolic process that has the ability to degrade unnecessary proteins and intracellular pathogens by activating autophagosomes.

Purpose: To study the impact of host autophagy and associated signaling on *E. coli* O157:H7 epithelial cell adhesion.

Methods: HT-29 cells were cultured in DMEM complete media, which were pre-treated with 10 ng/ml tumor necrosis factor (TNF)-α or subjected to starvation for 12 h. Then, cells were infected with *E. coli* O157:H7 for 4 h, and attached bacteria were serial diluted, plated and enumerated. Protein samples were further collected post *E. coli* O157:H7 infection for autophagy signaling analyses.

Results: Both starvation and TNF-α pre-treatment enhanced autophagy in HT-29 cells as indicated by increased LC3B II/I ratio ($P \leq 0.05$), which, on the other hand, decreased the adhesion of *E. coli* O157:H7 to HT-29 cells ($P \leq 0.01$). Stress-activated protein kinases (SAPK)/Jun amino-terminal kinases (JNK) and endoplasmic reticulum stress sensor Inositol-requiring enzyme 1α (IRE1-α) regulate autophagy activation. Consistently, both of them were up-regulated by starvation but inhibited by *E. coli* O157:H7 infection.

Significance: Host autophagy plays a positive role in preventing *E. coli* O157:H7 from adherence to the host cells.

P3-128 Inhibition of *Listeria monocytogenes* by Buffered Vinegar-Based Antimicrobials in Ready-to-Eat, Low-sodium, Uncured Turkey

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Introduction: Due to increased consumer demand for natural products, development of clean label antimicrobials for Ready-to-Eat (RTE) meat products is a high priority for the meat industry.

Purpose: The objectives of this study were to validate the inhibition of *Listeria monocytogenes (Lm)* on RTE, low sodium (1.4% sodium chloride), uncured turkey manufactured with different antimicrobial treatments and stored at 4°C for 12 weeks; and to determine the effect on sensory, color and cook loss.

Methods: Treatments tested were – an untreated control; 0.4%, 0.6% and 0.8% dry buffered vinegar; and 0.5%, 0.7% and 0.9% dry buffered vinegar-low sodium. The treatments were surface inoculated with a five strain mixture of *Lm* to provide approximately 5 log CFU per 100g package (equivalent to 3 log CFU per ml of rinse material when using 100 ml rinse for testing). Inoculated products were vacuum packaged and stored at 4°C for 12 weeks. Triplicate inoculated samples were tested for *Lm* growth by plating on modified Oxford agar and incubated at 37°C for 48 h. The study was conducted in three replications.

Results: Results showed that, dry vinegar at 0.6% and 0.8%, and dry vinegar-low sodium at 0.7% and 0.9%, significantly ($P < 0.05$) inhibited the growth of *Lm* compared with the untreated control by showing <1 log increase for 12 weeks. Untreated control showed >1 log and >3 log CFU/ml rinse by the end of 2 and 4 weeks, respectively. The 0.4% dry vinegar and 0.5% dry vinegar-low sodium showed >1 log increase at the end of 8 and 11 weeks, respectively. Cook loss, color and informal sensory results showed no significant differences between the antimicrobial treatments.

Significance: These results demonstrated that buffered dry vinegar ingredients were effective in inhibiting the growth of *Lm*; thus offering a promising solution for *Listeria* control in RTE meats.

P3-129 *Salmonella* Typhimurium Growth in Maillard Reaction Product-mediated Acidic and Thermal Stress Conditions

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❖ ***Developing Scientist Competitor***

Introduction: Due to increasing consumer demand for healthy foods, naturally derived antimicrobials are highly desired as alternatives to chemical preservatives in the food industry. Maillard Reaction products (MRP) have displayed antimicrobial effects on foodborne pathogens such as *Escherichia coli* and *Listeria monocytogenes in vitro*. Therefore, investigation of MRP as an antimicrobial may contribute to development of novel strategies for pathogen control.

Purpose: The objective of this project is to evaluate the antimicrobial effect of lysine-derived MRP prepared with various carbohydrates [fructose (FL), glucose (GL), ribose (RL), sucrose (SL), and xylose (XL)] on *Salmonella enterica* serovar Typhimurium (ST) under acidic and thermal stress conditions.

Methods: Water-soluble fraction of crude MRP was used in this study. ST was inoculated in brain heart infusion broth (BHIB) and acidified BHIB (pH 5.5) at 37°C. In the thermal stress assay, bacterial cells in BHIB were subjected to 42°C. All matrices were supplemented with MRP [Optical density (OD) at 420 nm = 1.03 (FL) 1.69 (GL) 0.58 (RL), 1.49 (SL), 0.81 (XL)] and the growth of ST was monitored by measuring OD_{600nm} for 24 h with a 96-well plate reader.

Results: In acidified BHIB, the GL significantly reduced the duration of lag phase duration (λ) of ST growth compared to the control ($P < 0.05$). However, under thermal stress environment, all MRPs except FL exerted significantly shorter λ of ST growth ($P < 0.05$). Maximum growth rate was significantly decreased in all MRP treatments compared to the control, but there were no significant differences between the MRP samples ($P < 0.05$).

Significance: These findings suggest that MRP may influence the growth of ST under acidic and thermal stress conditions through complex mechanisms that require further elucidation. This can be attributed to the diversity of compounds derived from Maillard Reaction.

P3-130 Efficacy of Buffered Vinegar in Controlling *Listeria monocytogenes* and Extending Shelf Life of Frozen Vegetable Products

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Introduction: *Listeria monocytogenes* (LM) is known to be an environmental contaminant in food processing areas and its ability to grow at 40°F makes it a pathogen of concern in frozen and refrigerated products. Handling of vegetables during packaging may introduce LM into the product and it can proliferate under abuse or elevated temperatures during storage of these products.

Purpose: This study evaluated the effectiveness of buffered vinegar (V) in controlling growth of LM and extending shelf life of frozen vegetable products.

Methods: A cold-adapted, five-strain cocktail of LM was used to inoculate (2 log CFU/g) frozen corn, edamame, and broccoli. After cell attachment for 2 h at 40°F, vegetables were sprayed with three treatments V (0.4%), DI water, and control (no spray) using a prototype sprayer. Six sub-treatments

in this test included: inoculated DI, V, and control; un-inoculated DI, V, and control. Bacterial counts (25 g, n = 2) were determined (LM on MOX, shelf life on TSA, MRS and YM) after overnight freezing (day 1) and then during storage (45°F, 10 days) on days 3, 5, 8, and 10.

Results: LM counts did not increase (showed slight decrease 0.3 - 0.8 log CFU/g) throughout the 10 day abuse storage; control samples (DI and no treatment) showed an increase of 1.3 - 1.5 log CFU/g. Treatment with V also allowed shelf life extension to 10 days at 45°F. Total aerobic counts increased by 2.2 - 2.3 as compared to significant increase of 3.3 - 4.3 log CFU/g in controls. V treatment allowed yeast and mold counts to stay below 4 log CFU/g throughout the 10 day storage, while in controls, the counts were reported too numerous at day 8.

Significance: If there is a potential for temperature abuse, V can be used as a control measure to inhibit growth of LM in frozen vegetables post-thawing and extend shelf life.

P3-131 Effect of Buffered Vinegar on Growth of *Staphylococcus aureus* and Enterotoxin Production

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Introduction: *Staphylococcus aureus* commonly associated with humans and handling has potential to be a pathogen of safety concern in canned tuna products; produced from eviscerated tuna which is pre-cooked to facilitate separation of meat from skin and bones. *S. aureus* cells can be thermally inactivated but enterotoxins produced cannot be eliminated during canning process.

Purpose: This study evaluated the effect of buffered vinegar (V) on growth and enterotoxin production by *S. aureus* in broth and simple food model.

Methods: Buffered vinegar (0.15%, 0.3%, 0.5%) was evaluated using Bioscreen to determine efficacy against four strains and cocktail of *S. aureus* (6 log CFU/ml). Testing was done to study growth (Baird Parker) and enterotoxin formation (3M SETVIA Staph Enterotoxin Kit) of *S. aureus* (4 log CFU/ml, n = 2) in 5% and 7.5% V solution in TSB and 0.15% and 3% (attached concentration on food sample upon spraying solution with 3% pick-up) tuna slurry (50 g pre-cooked tuna in 150 ml DI water) at 37°C (0 h, 4 h, 6 h, 8 h, 12 h) and 21°C (0 h, 12 h, 24 h, 32 h, 48 h).

Results: Bioscreen results indicated growth reduction (turbidity) in *S. aureus* with V; 7.35%, 14.87% and 36.34% with 0.15%, 0.3% and 0.5% V, respectively. V showed an inhibitory effect, *S. aureus* were 1.1 - 2.6 and 1.2 - 2.8 log CFU/ml lower than controls in treated tuna slurry and broth at 37°C and 21°C, respectively. Bacterial counts in control increased to over 6 log CFU/ml within 8 h at 37°C and in 32 h at 21°C and toxin formation was observed; while all treated samples showed negative results in the toxin test. Testing is in progress to determine spray application of V on pre-cooked tuna meat to control *S. aureus*.

Significance: V can be used as a potential intervention method to control growth of *S. aureus* and prevent enterotoxin formation during canned tuna processing.

P3-132 Inhibition of *Listeria monocytogenes* in Cured Deli-style Turkey Formulated with Commercial Blends of Propionate, Diacetate, Lactate, and Acetate-Based Antimicrobials

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Introduction: Manufacturers of Ready-to-Eat meat and poultry products continue to expand their portfolio of antimicrobials to inhibit growth of *Listeria monocytogenes* during long-term refrigerated storage.

Purpose: To determine the antilisterial effect of commercial ingredients containing various combinations of propionate, diacetate, acetate, and lactate in cured turkey stored at 4 and 7°C for up to 12 weeks.

Methods: Eight cured deli-style turkey breast treatments (75 - 76% moisture, pH ~6.3, ~1.95% NaCl, 156 mg/kg $NaNO_2$, 547 mg/kg sodium erythorbate) were formulated with 0.2, 0.4 or 0.65% Provian®D (sodium acetate, lactate and diacetate; proprietary blend) or 0.25, 0.5, or 0.75% Provian®K (potassium acetate and diacetate; proprietary blend). Control treatments included turkey formulated with no antimicrobials or with 2.5% potassium lactate-sodium diacetate (56-4%) blend. Sliced cooked products were surface inoculated with 3 log CFU/g of *L. monocytogenes* (5-strain mix), vacuum packaged (100 g/package), and stored at 4°C and 7°C. Triplicate samples per treatment were assayed at 0, 2, 4, 6, 8, 10 weeks for both storage temperatures and an additional 12th week at 4°C by enumerating on Modified Oxford Agar.

Results: Treatments containing no antimicrobials or with 0.2% Provian®D supported > 1-log increase of *L. monocytogenes* by 6 and 10 weeks at 4°C, respectively; the remaining six treatments inhibited growth (< 0.5-log increase) through 12 weeks. In contrast, only treatments containing ≥ 0.25% Provian®K and ≥ 0.4% Provian®D inhibited growth (< 0.5-log increase in *L. monocytogenes*) for the 10 weeks storage at 7°C, whereas the two control treatments and the 0.2% Provian®D treatment supported > 1-log increase of *L. monocytogenes* within 4 to 10 weeks. Both control treatments supported growth at times within ranges predicted by a commercially-available model for *L. monocytogenes* inhibition.

Significance: Results from this study validate the efficacy of additional commercial antimicrobials to inhibit growth of *L. monocytogenes* on Ready-to-Eat meat and poultry products.

P3-133 Evaluation of Vinegar against *Listeria monocytogenes* in Ready-to-Eat (RTE) Deli Ham Stored at 4°C

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Introduction: Consumption of RTE meat and poultry products contaminated with *L. monocytogenes* has been related to different foodborne outbreaks. Intervention methods to control *L. monocytogenes* in deli meats exposed to the environment are needed to reduce the risk of foodborne illnesses. Consumer demand for natural antimicrobials is increasing. Verdad® N8 and Verdad® N9 are liquid vinegars based on fermentation that can provide necessary antimicrobial performance to control the outgrowth of *L. monocytogenes* in RTE meat and poultry products.

Purpose: The objective is to validate the efficacy of Verdad® N8 and N9 against *L. monocytogenes* in RTE deli ham products stored at 4°C.

Methods: RTE ham formulation contained lean, inside and outside muscles, water (24.12%), salt (1.8%), sugar (1.7%), sodium phosphate (0.4%), sodium erythorbate (0.055%), sodium nitrite (0.250%), carrageenan (0.250%) and antimicrobial at different levels. RTE ham was sliced, inoculated with a five-strain *Listeria monocytogenes* cocktail at ca. 2.50 log CFU/g, vacuum packed and stored at 4°C for 120 days. Twenty-five g samples were homogenized in 0.1% peptone water and plated out onto MOX agar. Plates were incubated at 35°C for 48 h and typical *L. monocytogenes* colonies were counted.

Results: Ham control samples had the fastest *Listeria monocytogenes* growth reaching 7.98 log CFU/g at the 25th day of storage. All tested concentrations of Verdad® N8 (2.10%) and Verdad® N9 (2.64%) resulted in < 1 log CFU/g outgrowth of *L. monocytogenes* over the shelf life (120 days).

Significance: This research substantiates the antimicrobial performance of Verdad® N8 and Verdad® N9 against *L. monocytogenes* in RTE deli ham. The research provides meat industry with natural antimicrobials for ensuring food safety of RTE meat products.

P3-134 Shelf-life Extension of Pork Patties Stored at 4°C under Modified Atmosphere Packaging Using Cultured Corn Sugar and Vinegar

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Introduction: Fresh pork patties stored at 4°C are susceptible to support the growth of spoilage microorganisms which can be detrimental for the shelf life of the product. The incorporation of antimicrobial compounds which can inhibit or slow down the growth of spoilage organisms is important. Verdad® N16 is a corn sugar fermentation-based product which contains organic acids, small peptides and residual sugars, and vinegar and has excellent antimicrobial properties.

Purpose: To evaluate the performance of Verdad®N16 on the shelf life extension of fresh pork patties packed under Modified Atmosphere conditions (MAP) and stored at 4°C.

Methods: Ground pork shoulder (95%), water (5.0%) and Verdad® N16 were mixed for 10 min. One hundred g of the mixed pork was formed into a patty and MAP packaged (80% O_2, 20% CO_2) and stored at 4°C. Samples were analyzed on days 0, 7, 9 and 12 for aerobic plate, lactic acid bacteria, Psychrotrophs and *Enterobacteriaceae* counts individually using conventional plating methods.

Results: Fresh pork patty with no antimicrobial exhibited fastest microbial growth. Addition of Verdad® N16 resulted in APC log CFU/g increases of 2.67, 1.68 and 2.10 log CFU/g for 2.0%, 2.5% and 3.0% concentrations, respectively. Verdad® N16 addition suppressed the growth of *Enterobacteriaceae* at all the tested concentrations while in the control treatment, *Enterobacteriaceae* counts reached > 7 log CFU/g counts on day 9. Verdad® N16 at all tested concentrations slowed down the growth of psychrotrophic bacteria to < 6 log CFU/g at 12th day of storage. LAB counts exceeded > 5 log CFU/g at the 12th day of storage for all the treatments including the control sample.

Significance: This research substantiates the antimicrobial performance of Verdad® N16 in fresh pork patties. This research provides meat industry with an excellent antimicrobial to incorporate in the fresh meat for shelf life extension.

Poster

P3-135 Antibacterial Activity and Mechanism of Action of Punicalagin against *Staphylococcus aureus in vitro*

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Introduction: Punicalagin, the main active compound in pomegranate peel, has been reported to possess many properties, including antioxidant, antimicrobial, anti-proliferative, apoptotic, antiviral and immune-suppressive activities

Purpose: The aim of this study was to investigate the antibacterial effect of punicalagin against *Staphylococcus aureus* (*S. aureus*) and to elucidate its mechanism of action.

Methods: Inhibition zone diameter was measured using agar diffusion method. Agar dilution method was taken to determine the minimum inhibitory concentration (MIC). The effects of punicalagin on cell membrane of *S. aureus* were assessed by measuring potassium efflux, pH_{in}, scanning electron microscopy, and transmission electron microscopy.

Results: Punicalagin showed clearly antistaphylococcal effect. The inhibition zone diameter was 11.1 (± 0.4) mm in the presence of 1 mg/ml of punicalagin and the MIC was determined as 0.25 mg/ml. An increase of potassium efflux and pH_{in} was observed when cells were treated with punicalagin at 2 × MIC. Moreover, punicalagin induced significant morphological damage to the cell membrane.

Significance: The findings indicate that punicalagin has the potential to be developed as an alternative to control *S. aureus* and prevent the diseases associated with this microorganism.

P3-136 Effects of Blueberries on the Biofilm Formation of *Vibrio parahaemolyticus*

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Introduction: *Vibrio parahaemolyticus* is a leading cause of seafood-derived food poisoning throughout the world. The formation of its biofilm on the surface induces serious food safety issues. Our previous studies indicated that blueberries have antimicrobial activity against *V. parahaemolyticus*.

Purpose: The aim of this study was to evaluate the effects of blueberries on the biofilm formation of *V. parahaemolyticus.*

Methods: Wild blueberries (*Vaccinium angustifolium* and *Vaccinium uliginosum*) and cultivar blueberries (DingFeng) were chosen for this study. *V. parahaemolyticus* strains ATCC33847, ATCC17802 and O3:K6 were selected. Crystal violet staining assay was used to measure the biofilm production of *V. parahaemolyticus* in different time periods. Planktonic susceptibility and biofilm susceptibility were measured under different concentrations of blueberry crude extract treatment. Susceptibilities were determined by minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs), minimum biofilm inhibitory concentrations (MBICs) and minimum biofilm bactericidal concentrations (MBBCs), and the plate count method. Fluorescence microscope was also used to observe the effects of blueberry on biofilm formation in 24 h, 48 h, 72 h and 96 h, compared with the control group (without blueberry).

Results: The results showed that the most biofilm production of *V. parahaemolyticus* resulted from incubation of 30 h. The MICs and MBICs were 15.625 mg/ml and 31.25 mg/ml, respectively. The MBCs and MBBCs were 62.5 mg/ml and 125 mg/ml, respectively. These results indicated that biofilms were more resistant to the blueberry treatments when compared to the bacterial cells. Through fluorescence microscope, we found the biofilm formation was reduced by blueberry treatments when compared with no blueberry controls.

Significance: This study suggests that blueberries may reduce the biofilm formation of *V. parahaemolyticus*, indicating they have the capability to detach and kill existing biofilms. The results of this study lay the foundation for future characteristics and mechanism research.

P3-137 Prevention of Biofilm Formation on Food Contact Surfaces by a Nanoscale Plasma Coating

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❖ ***Developing Scientist Competitor***

Introduction: Microbial biofilm formation can lead to great food safety issues in the food industry because, once formed, the cells are more resistant to common sanitizing methods. Low temperature plasma coating technology is a method that can effectively prevent biofilm formation on food contact surfaces.

Purpose: Aim of this study was to investigate and compare the anti-biofilm efficacy of a nanoscale plasma coating of trimethylsilane (TMS) and TMS+O_2 (1:4) on stainless steel (SS) and high density polyethylene (HDPE) against biofilms of important foodborne pathogens.

Methods: SS or HDPE wafers (1 cm × 1 cm) were coated with TMS or TMS+O_2 plasma to an approximate thickness of 30 nm. A 3-5 strain-cocktail each of *Listeria monocytogenes*, *Escherichia coli* O157:H7, non O157 Shiga Toxin-producing *E. coli* (STEC), and *Staphylococcus aureus* was allowed to form biofilms on the wafer surfaces for 48 h. The biofilms were rinsed with peptone water, followed by ultrasonication to release the cells. The pour-plate method was conducted to determine bacterial counts. Laser confocal scanning and scanning electron microscopy were performed to determine the effects of the plasma coating on the cells.

Results: On SS coated with TMS+O_2, *L. monocytogenes* numbers dropped from 10^7 to 10^5 CFU/wafer. For *E. coli* O157:H7, the TMS treatment resulted in a 1-log reduction, which was better than the TMS+O_2 treatment. TMS also showed better efficacy than TMS+O_2 on *S. aureus* (10^6 to 10^4 CFU/wafer). However, for STEC, neither plasma coating was effective. On HDPE wafers, TMS and TMS+O_2 worked similarly (1-log reduction for *L. monocytogenes* and STEC, and 2-log reduction for *E. coli*O157:H7).

Significance: Results of this study showed TMS and TMS+O_2 coating on SS surfaces could significantly reduce biofilm formation. However, the efficacy of the treatment depends on the strain, growth conditions, coating surface and material tested.

P3-138 Generation of Chlorine By-products Haloacetic Acid and Trihalomethanes in Simulated Produce Wash Water with High Organic Loads

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✦ ***Undergraduate Student Award Competitor***

Introduction: Free Chlorine (FC) is easily consumed by organic matter during commercial produce washing process. Repeatedly adding chlorine into wash solution with high organic loads promotes the formation of toxic chlorine by-products. There is limited research quantitatively evaluating chlorine by-products generation in produce wash water.

Purpose: This study evaluated the dynamic impact of FC and organic load on the generation of haloacetic acids (HAAs) and trihalomethanes (THMs) in simulated wash water with water quality changes.

Methods: Lettuce juice (60 ml) was sequentially added into sodium hypochlorite solution (initial FC~80 mg/l, pH 6.7-6.8) with free chlorine periodically replenished when the FC concentration dropped below 2.0 mg/l. Water samples were collected 1 min after each lettuce juice addition with gentle agitation to measure water qualities including FC concentration, oxidation-reduction potential (ORP), pH, chemical oxygen demand (COD), and determine HAAs and THMs. HAAs and THMs' concentrations were quantified by Gas Chromatography/Electron Capture Detection and Capillary Column Gas Chromatography/Mass Spectrometry using US-Environmental Protection Agency (EPA) methods. Data (four replicates) were analyzed using the 2-factor model and Pearson product-moment correlation test of SPSS.

Results: Results indicate that 88-2103 µg/l of total HAAs and 20.79-859.47 µg/l of total THMs were detected during dynamic water quality variances. These concentrations exceed US-EPA and European Union drinking water standards. Monobromoacetic acid (19.5 to 1348.9 µg/l), tribromoacetic acid (6.7 to 529.7 µg/l), chlorodibromoacetic acid (36.5 to 89.2 µg/l), and trichloroacetic acid (2.3 to 148.6 µg/l) are the major HAAs components. Chloroform (Trichloromethane, 20.12 to 858.0 µg/l) is the primary THMs present. A significant ($P < 0.001$) correlation of HAAs with COD, and THMs with FC was observed.

Significance: Optimizing wash water sanitizing systems to limit organic matters and maintain minimal effective FC concentration is critical. Results are important in produce safety, environmental and occupational health risks for produce washers, and regulatory agencies charged with setting limits for compounds of concern.

P3-139 A Microbial Investigation of Florida Tomato Packinghouse Processing Efficacy

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Introduction: Tomatoes are widely consumed in the U.S and Florida was the nation's number one producer in 2013. During the 1990s and early 2000s, there were several foodborne outbreaks associated with tomato consumption, imposing public health and financial burdens. Most packers use a flume or 'dump tank' to transfer tomatoes from field bins to their processing lines as a measure to prevent microbial cross-contamination. However, more studies are necessary to better understand the positive and negative impacts of this process.

Purpose: This project was designed to investigate the bacterial levels on tomatoes by testing field fruit before and after processing.

Methods: Green, round tomatoes were sampled from five different packinghouses in Florida from Feb. 2013 until Dec. 2014. At each sampling period 20 composite samples of 5 tomatoes were collected from field bins (pre-processing) and 20 composite samples of 5 after washing (post-processing). Samples were transported on ice and screened via traditional culture methods and molecular techniques for total aerobic mesophiles, total coliforms, and generic *Escherichia coli* within 24 h. Parametric and nonparametric tests were used to compare results of before/after processing, seasons and years.

Results: Five packinghouses across Florida were sampled 18 times during a two-year span. The packinghouse processing significantly reduced ($P < 0.0001$) total aerobic mesophiles on tomatoes (n = 360) when comparing with the unwashed field tomatoes (n = 360). Tomatoes collected in 2014 (n = 320) had significantly higher ($P = 0.0002$) total aerobic mesophiles than 2013 samples (n = 400). When categorizing harvested tomatoes in spring (n = 320), summer (n = 120), fall (n = 160) and winter (n = 120), summer tomatoes had significantly higher ($P < 0.0001$) total aerobic mesophiles and total coliforms than other seasons. *E. coli* levels were below the detection limit of 1.3 log CFU/tomato in 701 out of 720 samples collected.

Significance: These data will provide quantitative information for tomato producers to evaluate and improve their product quality and safety.

P3-140 How to Combine Temperature of Action and Concentration of Active Chlorine to Reach Targeted *Pseudomonas aeruginosa* Population Reduction

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Introduction: The processing environment and decontamination treatments used in minimally-processed vegetables (MPV) are limited. *Pseudomonas* species are common and persistent contaminants reported by industrial surface samplings and chlorine treatment was reported to be more effective for bacterial inactivation in wash water rather than removal and inactivation of pathogens from fresh produce.

Purpose: This study aims at quantifying the impact of chlorine concentration on *P. aeruginosa* planktonic cell inactivation to reach targeted population reduction in MPV washing bath.

Methods: The quantification of bacterial population decrease throughout lethal chlorine exposure (25 - 100 ppm) was determined on *P. aeruginosa* ATCC15442 at 4 temperatures (4 - 37°C) under continuous stirring conditions. Free active chlorine was generated using commercial household bleach and concentration was manually adjusted using colorimetric chlorine test trips. After sampling, immediate neutralization was performed using sodium thiosulfate and survivors were enumerated on nutrient agar to acquire inactivation kinetics or kill-curves. Kinetics were performed for three biological replicates and fitted using a Weibull-like model to determine a 3-log population reduction. The quantified impact of temperature on the population reduction during chlorine exposure was further determined with the 95% confidence intervals using a Bigelow-like model.

Results: Experimental kill curves underlined a slightly concave curve with a slight effect of temperature on bacterial reduction. At 8°C, the 3-log population reduction is reached after 95 min at 80 ppm. Note that the presence of by-product that could be generated with the use of chlorine and organic matter on bacterial destruction was not investigated.

Significance: Within the frame of SUSCLEAN European project on sustainable cleaning and disinfection in fresh cut food industries, a MatLab-based app was developed to quantify and quickly identify the combination of conditions ensuring no growth or inactivation of *P. aeruginosa* to enhance industrial outreach and further *in silico* test industrial relevant scenario in a few seconds.

P3-141 An Investigation into Alternative Antifungal against Citrus Black Spot

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❖ ***Developing Scientist Competitor***

Introduction: Citrus black spot (CBS) is an important fungal disease that is currently threatening the South African citrus industry resulting in a ban on exporting citrus fruits to other citrus-producing countries such as European Union Countries. Currently available method used to eradicate CBS is synthetic fungicides. However their excessive use has resulted in numerous drawbacks such as damage to the environment and humans. Consequently this has increased demands to reduce the use of synthetic fungicides.

Purpose: The purpose of this research was to use essential oils as alternative antifungal against CBS.

Methods: Essential oils (EOs) thyme, geranium, citronella, lavender and eucalyptus were characterized using GC/MS and their effectiveness was tested *in vitro* against *Guignardia citricarpa* the causative agent for CBS using the agar diffusion Bio-Assay. Scanning Electron microscopy (SEM) was used to evaluate morphological changes occurred due to EOs. While the XTT colorimetric assay was used to measure the metabolic state of treated cells by determining the activity of mitochondrion dehydrogenases enzyme.

Results: Results from the bio-assay showed that EOs inhibited growth; this was indicated by an inhibition zone (no growth), minimal growth and maximum growth zone towards the edge of the plate. This occurs due to antifungal anti-mitochondrial action of the essential oils. This we believe occurs through inhibition of structures with increased mitochondrial activity, indicated by the bio-assay results, Scanning Electron microscope (SEM) and XTT colorimetric assay

Significance: Essential oils used in the study, especially thyme oil had great antifungal activity by affecting the mitochondria, resulting in cell death. Therefore EOs can be used as alternative antifungal agents to replace synthetic fungicides currently used in the control CBS pathogen. However these preliminary findings need to be tested in the field for the successful EOs, subsequently environmentally-friendly spraying programs will be implemented in consultations with growers.

P3-142 Studies on Antibacterial Activity of Ovomucin

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Introduction: Ovomucin shares genetic similarities with mammalian gel-forming mucins. It has been reported that mucins have antimicrobial activity.

Purpose: This paper investigated the antimicrobial activity of ovomucin against the common bacteria in eggs such as *Staphylococcus aureus, Saprophyticus subsp, Bacillus subtilis, Escherichia coli, Salmonella*, and the decay-causing *Streptococcus mutans*.

Methods: Growth curve and Oxford cup methods were used to characterized the antimicrobial activity of ovomucin.

Results: The minimum inhibitory concentration (MIC) was measured by doubling dilution method. On this basis, the influence of the thermal treatment, metallic ion on antimicrobial stability was studied by Oxford cup. Results indicated the MIC of ovomucin against *Staphylococcus aureus, Saprophyticus subsp, Streptococcus mutans* was 62.5 μg/ml, 125 μg/ml and 1000 μg/ml, respectively. However, ovomucin had poorly inhibitory effect on *Bacillus subtilis, Escherichia coli, Salmonella* .The thermal treatment (20°C, 40°C) had no obvious influence on the antibacterial . The effects of metal ions on ovomucin antimicrobial activity were discussed. 0.05 mol/l to 0.20 mol/l of Na^+, K^+, Ca^{2+}, Mg^{2+} didn't show any positive effect on antimicrobial activity of ovomucin, and a little positive effect was observed when solution was Cu^{2+}, Zn^{2+}, Mn^{2+}, Fe^{3+} with concentration from 0.15 mol/l to 0.20mol/l. While these metal ions strengthened antimicrobial effect of ovomucin when their concentration was 0.05 mol/l, and in this concentration the inhibition zones increased 19.97 mm, 14.98 mm, 7.03 mm and 12.47 mm, respectively, compared with the control.

Significance: Ovomucin had antimicrobial effects on bacteria existing in eggs, which revealed the role of ovomucin in egg preservation and added its application in natural preservative resources of food industry.

P3-143 Effects of Antibacterial Coatings on the Shelf Life of Fresh Eggs Stored at Ambient Temperature

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Introduction: Antimicrobial coatings incorporating natural antimicrobials, such as chitosan (CH), lysozyme (LYZ), or oregano essential oils (OEO), can protect eggs against microbial contaminations and preserve their internal qualities for extending shelf life of fresh eggs.

Purpose: This study investigated the feasibility of antibacterial coatings for controlling the microbial growth and improving qualities of fresh eggs stored at ambient temperature

Methods: Prior to coating application onto whole eggs, the antibacterial effect of prepared coating solutions (10% LYZ-3% CH, 0.5% cellulose nanofiber (CNF)-0.5% CH, and 0.5% CNF-0.1% OEO) was investigated against *E. coli* using agar-based disc diffusion method. Eggs were then coated by three coating solutions using an air-spray gun (~25-30 psi) and dried at the ambient temperature under an air-flow fan. Coated eggs were stored at

ambient conditions (~25 °C) for 5 weeks. Total bacterial counts (TPC) and weight loss of whole eggs and egg grade were evaluated during the storage. Data were analyzed via analysis of variance (ANOVA) with the Tukey method ($P < 0.05$).

Results: The 10% LYZ-3% CH and 0.5% CNF-0.5% CH coatings significantly inhibited *E. coli* growth in comparison with 0.5% CNF-0.1% OEO coating. TPC of eggs coated with 10% LYZ-3% CH, 0.5% CNF-0.5% CH, and 0.5% CNF-0.1% OEO were 7.30, 11.34 and 11.98 log CFU/g egg after 5 week of storage, respectively, which was significantly lower than that of non-coated eggs (12.82 log CFU/g egg). Weight loss of coated eggs was also significantly lower (6.57-8.17%) than that of uncoated ones (9.18%) after 5 weeks of storage. The 10% LYZ-3 %CH coated eggs maintained B grade after 5 weeks of storage, but non-coated ones rapidly changed from AA to C grade within 3 weeks at ambient storage.

Significance: This study proved that lysozyme and chitosan based coatings can protect eggs from microbial contamination and improve internal freshness, thus extending shelf life.

P3-144 Natural Antimicrobial System for Inhibition of Pathogens on Fresh Produce

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Introduction: There is a critical need for control of *E. coli* O157:H7 and *Salmonella* spp. on fresh produce. Although chemical sanitizers such as chlorine and hydrogen peroxide are widely used, industry trends toward "greener" or more "natural" interventions continue to gain momentum. Natural antimicrobial systems capable of inactivating bacterial pathogens in complex matrices would provide attractive clean-label solutions for enhancing produce safety.

Purpose: To develop an effective multicomponent antimicrobial system for fresh produce based on rational combinations of interactive natural components.

Methods: Antimicrobials examined included Grape Seed Extract (GSE; Kikkoman), long-chain sodium polyphosphate (BEKAPLUS FS; ICL Performance Products) and various organic acids, including citric, malic and tartaric acids. The efficacy of individual antimicrobials and their combinations against *E. coli* O157:H7 ATCC 35150 and *S. enterica* ser. Typhimurium ATCC 14028 was evaluated using a Bioscreen C Microbiological Reader (Growth Curves USA). One antimicrobial system was also evaluated against *Salmonella* Typhimurium in a lettuce extract system with plating on selective (XLT-4) and non-selective (Tryptic Soy Agar) media after 12 and 24 h exposure.

Results: A combination of GSE (0.5%), BEKAPLUS FS (1%) and tartaric acid (0.125%) showed the best inhibitory effect against two pathogens in our Bioscreen tests. This system reduced *Salmonella* Typhimurium by ~4 logs within 12 h, and by > 6 logs after 24 h in the lettuce extract system. Selective and non-selective plating revealed cellular injury by GSE. Higher levels of BEKAPLUS FS (5%) resulted in buffering and reduced system efficacy.

Significance: Our work indicates that natural antimicrobial systems composed of phenolic compounds, metal chelators and organic acids may have promise for control of *E. coli* and *Salmonella* on minimally processed fruits and vegetables. By using a systems approach, effective levels of individual components may be minimized, potentially reducing negative organoleptic impacts of the treatment on produce.

P3-145 Antifungal Effect of Thymol and Carvacrol Added on Starch Coatings on Tropical Fruits (Mango and Papaya)

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Introduction: The incorporation antimicrobial compounds to edible coatings, such as essential oils and its derivates, can notably improve antimicrobial properties for coatings in tropical fruits. In this case, coating applications can also affect consumer sensory appreciation and adequate design of the composition, including the antimicrobial agents, needs to be considered, taking into account the compatibility of the compound with each food application.

Purpose: The aim of this work was to analyze the antifungal effect of thymol and carvacrol added to starch coatings on mango (*Mangifera caesia*) var. ataulfo and papaya (*Carica papaya L.)* var. maradol throughout 15 days of storage at 20ºC.

Methods: Four different treatments were performed on mangoes and papayas: fruits without treatment as a control, only starch coating fruits, Formulation 1: starch coated fruit with 1125 ppm of carvacrol and 375 ppm of thymol, and Formulation 2: starch coated fruit with 750 ppm of carvacrol and 750 ppm of thymol. After coating fruits were stored at 20ºC on a dry place for 15 days, every day fungal growth (log CFU/cm^2) was measured on fruits surfaces. Growth curves were adjusted to Gompertz parameters.

Results: There were significant differences ($P < 0.05$) in fungal growth for all coatings in relation to control; there is evidence that coatings with thymol and carvacrol are able to controlfungal development. Formulation 1 was the most efficient in delaying surface fungal growth and therefore deterioration, lag phase are increased.

Significance: Edible starch coatings added with thymol and carvacrol are a good alternative for mango and papaya preservation, improving shelf life with a fungistatic effect.

P3-146 Development and Characterization of Geraniol-loaded Polymeric Nanoparticles with Antimicrobial Activity against Foodborne Bacterial Pathogens

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❖ ***Developing Scientist Competitor***

Introduction: Geraniol (3,7-Dimethyl-2,6-octadien-1-ol) is an essential oil component known to be highly effective antimicrobial. The hydrophobic nature of the plant essential oil (EO) component geraniol limits its utility for microbial pathogen control due to low solubility in the aqueous fraction of many foods.

Purpose: This study aimed to synthesize geraniol-loaded polymeric nanoparticles (NPs) with enhanced anti-pathogenic properties.

Methods: A rapid nano-precipitation method was used for the preparation of nanoparticles. Geraniol-loaded NPs were characterized for size, encapsulation efficiency (EE), storage stability at different pH (4.0, 7.0, and 10.0) and temperature (4, 25, 37, and 50°C) conditions, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against *Escherichia coli* O157:H7 and *Salmonella enterica* Typhimurium.

Results: Nanoparticles had a mean EE of 57.5 + 5.5 wt.%. Storage at 25°C or pH 7.0 did not impact NP diameter over 60 days ($P > 0.05$); > 7 h were required for 50% EO release from NPs stored at 25°C. Antimicrobial NPs inhibited *Salmonella* Typhimurium and *E. coli* O157:H7 growth at 0.4 and 0.2

wt%, respectively, whereas MICs of free geraniol against *Salmonella* and *E. coli* O157:H7 were 0.8 and 0.4 wt%, respectively. The nanoparticles showed better efficiency at inhibiting growth of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium than the efficiency of pure drug (geraniol).

Significance: Nano-encapsulation of hydrophobic antimicrobial could improve their solubility, stabilization, and antimicrobial activity. In addition, hydrophilic nanoparticles are safer and more environmentally friendly than hydrophobic agent since water is used as a solvent rather than acetone, ethanol, etc. Geraniol nanoparticles of lower geraniol concentration were significantly more effective than pure drug in inactivation of both bacteria. Antimicrobial NPs may be useful for the post-harvest decontamination of foods, such as fresh produce, from cross-contaminating microbial pathogens.

P3-147 Possible Skin Residues Exceeding Maximum Residual Level after 200 mg/l of Doxycycline Applied in Drinking Water to Broilers for Five Consecutive Days

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Introduction: The recommended use of doxycycline (DC) is 100 mg/l via drinking water with a 7-day withdraw time (WDT). However, commonly applied concentration in field practice is doubled, yielding concern of possible tissue residues exceeding the maximum residual level (MRL).

Purpose: The study was aimed to evaluate tissue depletion of DC in broiler chicken when 200 mg/l of DC in drinking water were applied. The serum DC concentrations during and 24 h after medication were also studied.

Methods: Two hundred mg/l of DC in drinking water was applied to chickens for 5 consecutive days. Fresh water was supplied after daily dose reached 40 mg/kg body weight. Skin/fat, liver, kidney, heart, gizzard and muscle tissues were analyzed for DC level on the 3rd, 5th, and 7th day of withdraw. Blood samples were collected during and up to 24 h post medication.

Results: The skin/fat residue on the 7th day was 240 ± 45 ng/g (n = 3), and the 95% upper distribution limit (383 ng/g) exceeds its MRL (300 ng/g). All other tissues contained DC levels below their respective MRLs on the 3th day of withdraw. The serum DC level reached a steady state after the 3rd day of medication and the C_{max} was 2.9 ± 0.2 µg/ml (n = 4). After medication ceased, the serum level dropped to 0.4 µg/ml in 24 h.

Significance: These data suggested a possible violation in skin/fat but not other tissues under common field medication practice. Longer WDT may be necessary if the recommended DC concentration is doubled in drinking water.

Poster

P3-148 Tridecaptin A_1: A Novel Antimicrobial to Inhibit Foodborne Pathogens

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❖ ***Developing Scientist Competitor***

Introduction: Tridecaptin A_1 ($TriA_1$), an antimicrobial produced by *Paenibacillus terrae* NRRL B-30644, has potential to control the growth and survival of enteric pathogens. A synthetic analogue with an N-terminal octanoyl chain was synthesized (8-$TriA_1$).

Purpose: The objective was to assess the antimicrobial activity of 8-$TriA_1$ and a partially purified preparation of the native peptide.

Methods: The spectrum of activity was determined with 8-$TriA_1$ against the following organisms: *Bacillus* spp. (2 strains), *Carnobacterium maltaromatcium*, *Clostridium botulinum* (3 strains), *Enterococcus faecalis* (2 strains), enterohemorrhagic *E. coli* (11 strains), *Lactobacillus casei*, *Lb. curvatus*, *Lb. sakei*, *Listeria monocytogenes* (4 strains), *S. enterica* Enterica (5 strains), *Staphylococcus aureus* (3 strains) and *Weissella confusa*. The MIC of 1 mM 8-$TriA_1$ was determined against *C. botulinum, L. monocytogenes, S. aureus* AT-1, and *W. confusa*. The supernatant of a 48 h culture of *P. terrae* NRRL B-30644 was passed through an Amberlite XAD16N column and an antimicrobial preparation (AMP) was eluted with 80% isopropanol containing 0.1% trifluoroacetic acid. After concentration, antimicrobial activity was confirmed using *S. enterica* and *E. coli* as indicators.

Results: The activity of the 8-$TriA_1$ was greatest among Gram-negative indicators, including species of *E. coli* and *Salmonella* Enterica, with less activity against *Bacillus* spp., *Carnobacterium*, *C. botulinum*, *L. monocytogenes* and *S. aureus.* Regardless of concentration, no activity was observed against *Enterococcus*, *Lactobacillus,* and *Weissella.* The MIC ranged from 23.8 - 380.0 mg/l, depending on the target organism. The AMP had activity against *S. enterica* and *E. coli.*

Significance: Evidence of antimicrobial activity was observed with 8-$TriA_1$. Partial purification of $TriA_1$ provided a crude antimicrobial preparation that was active against Gram-negative pathogens and could be used to control pathogens in meat.

P3-149 Evaluation of Antimicrobial Activity of Chitosan Nanoparticles in Different Matrices from Animals with Disease

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Introduction: The essential role of antibiotics has been challenged by the occurrence of antimicrobial resistance (AR). Metritis and mastitis are commonly treated by antibiotics, but the treatment failure rate is about 30% in dairy cattle due to AR. Chitosan nanoparticles (CN) have been developed as a natural antimicrobial agent, and *in vitro* results provide promising use for disease treatment. However, antimicrobial activity of CN *in situ* matrices remains unclear.

Purpose: The purpose of this study was to evaluate antimicrobial activity of CN in fluids from animals with metritis and mastitis.

Methods: CN was prepared by cross-linking of chitosan solution, and the size of CN was measured by nanoparticle analyzer. For antimicrobial activity of CN, cow uterine fluids and milk samples were collected from animals with metritis and subclinical mastitis, respectively. Antimicrobial activity of CN was evaluated by the enumeration of naturally infected pathogens in the fluids.

Results: CN treatment effectively reduced the concentrations of pathogens in the matrices of cow uterus and milk. The antimicrobial activity varied depending on matrices and CN concentrations. In LB broth, 0.1% CN completely killed *E. coli* O157:H7 during 2 h of incubation. In milk, naturally infected pathogens were completely killed in 4 h with 0.1% CN. In cow uterine fluid, although the growth of naturally infected pathogens was inhibited at 0.1%, higher concentration (0.6%) of CN was required to kill pathogens, suggesting the antimicrobial activity of CN is inhibited in the uterine fluid. In addition, CN resistance was not detected in *E. coli* O157:H7 after treatment, suggesting CN provides insight for potential use for antimicrobial resistant microorganisms.

Significance: The data demonstrate that a natural antimicrobial agent CN retains antimicrobial activity in different matrices that provides encouraging solution to enhance animal and public health, especially targeting antimicrobial resistant microorganisms.

P3-150 Effects of Organic Acid-surfactant Mixtures on Levels of Bacteria and Beef Quality Trait

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Introduction: Organic acid efficacy as an antimicrobial treatment of beef carcass surfaces may be increased through the addition of surfactants. However, the effects of antimicrobial-surfactant mixtures on beef quality traits such as flavor and color stability may make their use unacceptable.

Purpose: To identify organic acid-surfactant mixtures that can reduce surface contamination of beef carcasses and that do not adversely affect beef quality.

Methods: The immediate and 48 hour residual efficacy of 2% Citric Acid + 0.05% sodium dodecyl sulfate (SDS; *CS*); 2% levulinic acid + 0.05% SDS (*LS*); and 2% Lactic acid + 0.5% Caprylyl/Myristyl Glucoside (*LG*) were assessed for reduction of aerobic plate counts (APC), *Enterobacteriaceae* (EB) and inoculated pathogens (*E. coli* O157:H7, *Salmonella*, and Shiga Toxin-producing *E. coli* of serogroups O26, O103, O111, O121 and O145) on beef surfaces (n = 20 each treatment). Flavor profile analysis, and color-life during simulated retail display were assessed for steaks cut from non-intact (blade-tenderized) beef subprimals that were treated with commercial food grade mixtures of *CS* and *LS*.

Results: The addition of a surfactant only increased the antimicrobial efficacy of organic acids by approximately 10%. Immediate post-treatment reductions of APC were 0.7, 1.5 and 1.5 log CFU/cm^2, and EB were 1.0, 1.3 and 1.2 log CFU/cm^2 for *CS*, *LS* and *LG,* respectively. The mean reductions of all pathogens combined were 0.56, 1.46 and 2.02 log CFU/cm^2 for *CS*, *LS* and *LG,* respectively. Residual chilled 48-hour reductions were generally an additional 0.2 log CFU/cm^{2}greater than the immediate effect. Statistically significant, but very small in magnitude, differences detected in flavor profiles were not likely to be of practical importance. Overall color change during simulated retail display did not differ from controls.

Significance: The addition of a surfactant to commonly used organic acids is an inexpensive option that moderately improves antimicrobial efficacy without negatively impacting beef quality.

P3-151 The Trojan Horse: Development of Antimicrobials that Inhibit Bacteria from Within

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Introduction: Genomic analysis demonstrates the presence of temperate phages within the genomes of foodborne pathogens. Cell lysis through induction of temperate phages represents a novel approach to control bacterial pathogens in foods.

Purpose: The objective of this study was to investigate prophage induction as a way to control foodborne bacteria on fresh produce.

Methods: Lysogenized *Escherichia coli* isolates were treated with phage inducers (mitomycin C and streptonigrin, at concentrations of 0, 0.2, 0.5, 1 and 2 µg/ml), in broth culture. OD600 nm readings were taken at 0, 1.5, 3, 8 and 18 h during incubation at 37°C. PCR of prophage integrase genes was used to confirm the release of induced phages. In another experiment, the stem scar of fresh red greenhouse tomatoes was inoculated with 7.0 x 10^9 CFU/ml of an overnight culture of *E. coli* strain 185. After drying, mitomycin C (4 and 6 µg/ml) was sprayed on tomatoes, while control tomatoes were sprayed with water. Following overnight incubation, the tomatoes were immersed in PBS, the cells were collected by centrifuging the PBS, washed to remove residual inducer, and plate counts were performed.

Results: Beginning at 3 h for mitomycin C and 8 h for streptonigrin, all concentrations significantly reduced the OD of the broth cultures relative to the control in a concentration dependent manner. PCR confirmed bacterial release of prophages, and also showed differential prophage induction from the compounds. One log (4 µg/ml) and three log (6 µg/ml) reductions in *E. coli* cells were observed on tomatoes sprayed with mitomycin C compared to those sprayed with water.

Significance: These results demonstrate the feasibility of using prophage induction to control foodborne pathogens on fresh produce. The differential induction of phages means that multiple inducers will need to be included in an antimicrobial based on this concept.

P3-152 Effects of Different Particle Sizes on the Antibacterial Activity of Catechin-Zn Complex Entrapped β-Chitosan Nanoparticles

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Introduction: Catechin derivatives chelating with Zn can improve the antibacterial activity of either catechins or Zn alone. Chitosan (CS) nanoparticles (NPs) exhibits higher antibacterial activity due to larger surface area and affinity with bacteria cells, and can encapsulate functional compounds for improving their stability in various conditions.

Purpose: This study investigated the antibacterial activity of catechin-Zn complex entrapped β-CS nanoparticles (NPs) prepared at different particle sizes.

Methods: Catechin-Zn complex entrapped β-CS NPs were prepared with particle sizes of 208.0, 479.3, and 590.7 nm under different ratios of CS and catechins-Zn complex of 1:1, 1:3, and 1:5. Catechin-Zn complex were encapsulated by β-CS NPs using ionic gelation technology with sodium tripolyphosphate. Polydispersity index (PDI) and Zeta-potential were investigated for evaluating the homogeniousity of prepared nanoparticles. Strain growth curves, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) against *L. innocua* and *E. coli* were also evaluated for catechin-Zn complex entrapped β-CS NPs prepared at different particle sizes. One-way ANOVA and *post-hoc* least significant difference (LSD) test and Student-Newman-Keuls test (S-N-K) were used to determine significant treatment differences among treatments using SPSS program ($P < 0.05$).

Results: Prepared catechin-Zn entrapped β-CS NPs had PDI of 0.377-0.395, indicating good distribution of the nanoparticles, and positive Zeta-potential of 39.17-45.62 mV. The growth rate of *L. innocua* and *E. coli* treated by catechin-Zn complex entrapped β-CS NPs was slower than that without any treatment. MIC and MBC of catechin-Zn complex entrapped β-CS NPs at the smallest particle size of 208.0 nm against *L. innocua* and *E. coli* were 0.0625 and 0.125 and 0.03125 and 0.0625 mg/ml, respectively, which were significantly lower than those prepared at larger particle sizes.

Significance: This study demonstrated that catechins-Zn complex entrapped β-CS NPs can be used as a potential antibacterial substance for food and other applications, and their antibacterial activity can be enhanced by reducing particle size.

P3-153 Assessment of *Listeria monocytogenes* Growth Inhibition by Two GRAS Compounds

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Introduction: Generally recognized as safe or GRAS compounds are most commonly used as food additives. Many of these compounds are also used as preservatives to control microbial growth. During previous studies with *L. monocytogenes*, we identified two GRAS compounds, deoxycholic

acid and trans-aconitic acid, that inhibited growth as well as or better than other GRAS compounds typically used to inhibit *L. monocytogenes*, e.g., nisin.

Purpose: To determine if the extent of growth inhibition by deoxycholic acid and trans-aconitic acid was generalized across serotypes of *L. monocytogenes* strains.

Methods: A panel of 12 *L. monocytogenes* (Lm) strains consisting of the major disease causing serotypes was evaluated. Growth was tracked in a Bioscreen C at 37°C and at 5°C in brain heart infusion broth (BHI) or BHI supplemented with 7% NaCl. Nisin was also assessed as a control. The area under the curve was calculated for the various curves and a percent inhibition was determined using parallel controls.

Results: Our results indicate increased average inhibition of Lm growth in BHI across all 12 strains by both trans-aconitic acid (9% at 37°C; 18% at 5°C) and deoxycholic acid (29% at 37°C; 22% at 5°C). Nisin, at 0.1%, however showed no inhibition, regardless of temperature. The addition of 7% NaCl to BHI enhanced the effectiveness of all three compounds. However, the effect was most pronounced for deoxycholic acid which showed nearly 100% inhibition of all 12 strains, regardless of temperature. This is notable as both nisin and trans-aconitic acid showed variability across all the serotypes that was not seen for deoxycholic acid in the presence of salt.

Significance: These data indicate that deoxycholic acid may be a potential candidate for use in controlling Lm growth. Further work is necessary to evaluate the interplay between with NaCl and different foods to achieve optimal growth inhibition.

P3-154 Effectiveness of PRO-SAN, a Biodegradable Antimicrobial, for Killing *Salmonella enterica* and *Escherichia coli* O157:H7 on Parsley and Green Onions Used as Fresh Garnishes

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Poster

Introduction: Fresh garnishes undergo no further intervention kill steps for pathogens beyond chlorination and are eaten raw. Therefore, they may pose food safety risks to consumers due to the potential of contaminating pathogens to survive chlorination treatment.

Purpose: This study investigated the effectiveness of PRO-SAN compared to that of chlorine for killing *Salmonella enterica* and *Escherichia coli* O157:H7 on parsley and green onions.

Methods: Each vegetable (25 g) was inoculated with 5 strains of nalidixic acid resistant *S. enterica* or *E. coli* O157:H7 and held at 23 ± 1°C for 16 to 18 hours. The vegetables were subsequently immersed (2 min) in: distilled water (H_2O), chlorine (CHL; 150 ppm), 1% (vol/vol) PRO-SAN (PRO1) or 2% (vol/vol) PRO-SAN (PRO2). Samples that were not immersed in solutions served as controls. The immersed vegetables were rinsed (3 s) in distilled water and then pummeled in a stomacher bag containing 50 ml of sterile 0.1% (w/v) peptone. Aliquots (0.1 ml) of peptone wash solution were surface-plated on xylose lysine tergitol 4 agar or sorbitol MacConkey agar each containing nalidixic acid (50 µg/ml). The agar plates were incubated at 35°C and bacterial colonies were counted after 48 h.

Results: Immersion of parsley in water reduced *E. coli* and *S. enterica* by 1.30 and 1.07 log, respectively. Log reductions of pathogens on green onions, immersed in water, were 0.43 (*E. coli*) and 0.58 (*S. enterica*). CHL and PRO1 reduced initial numbers of the pathogens by approximately 2.5 log. PRO2 [2% (vol/vol)] consistently killed more than 5.0 log of each pathogen ($P < 0.05$). These results demonstrate the effectiveness PRO2 for killing *S. enterica* or *E. coli* O157:H7 on parsley and green onions.

Significance: The results indicate that the PRO-SAN (2%) has good potential to replace chlorine solution (150 ppm) for killing enteric pathogens on parsley and green onions.

P3-155 Efficacy of Cetylpyridinium Chloride for the Reduction of *Campylobacter* on Poultry Cacasses

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Introduction: Poultry processors are researching effective antimicrobials for the reduction of *Campylobacter* on poultry carcasses.

Purpose: This study evaluated the reduction of *Campylobacter* incidence and numeration on cetylpyridinium chloride (CPC) treated poultry carcasses (antimicrobial solution sold under the trade name Cecure®).

Methods: Whole pre-chill carcasses were treated with four different concentrations of CPC (0.1%, 0.2%, 0.3% and 0.4%). Two carcasses were collected for each CPC treatment and four were collected for the control samples. Each control carcass was individually bagged and rinsed using United States Department of Agriculture/Food Safety Inspection Service (USDA/FSIS) standard methodology for collecting a whole bird carcass rinse. Each CPC treated carcass was treated with 0.5 gallons of the specified CPC concentrations. After CPC treatment, each carcass was allowed to drain for 30 s, and then treated with a cursory water rinse. After treatment, each carcass was individually bagged and rinsed using the same methodology for the collection of a whole bird carcass rinse. The rinsates were collected in labeled sterile containers and placed in a refrigerator and held at 2 - 4°C until time of microbiological evaluation (< 4 h). Samples were evaluated for *Campylobacter* incidence and enumeration using USDA Microbiological Laboratory Guidebook (MLG), pre-enrichment and modified plating procedures.

Results: The control carcasses had an average of 0.34 logs of *Campylobacter* and 100% incidence. All four CPC treatments showed no *Campylobacter* recovery on direct plating and no positive samples on the pre-enrichment.

Significance: The study results show the use CPC is an effective microbial intervention for reducing the *Campylobacter* levels on poultry carcasses.

P3-156 Survival and Transfer of *Salmonella* on Netted and Unnetted Cantaloupe

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Introduction: Netted surfaces of cantaloupes have proven to be a very advantageous site for foodborne pathogen attachment and harborage.

Purpose: Determine survival and transfer of *Salmonella* onto netted and unnetted cantaloupe varieties in a post-harvest wash scenario.

Methods: To evaluate chlorine post-harvest wash efficacy, a commercially available netted variety and an experimental unnetted variety of cantaloupe (n = 12) were inoculated with 100 µl of a cocktail of five prominent *Salmonella* serotypes resistant to 50 ppm nalidixic acid and dried. Nine fruit were immersed in 100 ppm free chlorine for two minutes, while three served as no wash controls. Each cantaloupe was rinsed with phosphate buffer saline (PBS) with 0.2% Tween 80. Remaining *Salmonella* populations were enumerated onto Tryptic Soy Agar with Nalidixic Acid (TSAN). Additionally, nine cantaloupes of each variety were placed in ten liters of water containing 10^7 CFU/ml of *Salmonella* cocktail for two minutes each, simulating a contaminated dump tank. After rinsing with PBS with 0.2% Tween 80, *Salmonella* populations were enumerated on TSAN.

Results: Surviving populations of *Salmonella* on netted and unnetted cantaloupe, when exposed to 100 ppm free chlorine were, 6.83 and 4.24 log CFU/cantaloupe, respectively ($P < 0.05$). The lack of netting had a significant effect on the efficacy of the chlorine treatment ($P < 0.05$), resulting in a 1.83 and 4.08 log reduction on netted and unnetted surfaces. When evaluating transfer by contaminated water, unnetted melons had an average of 5.42 log CFU/cantaloupe and netted averaged 6.17 log CFU/cantaloupe, but these populations were not significantly different ($P > 0.05$).

Significance: Chlorine treatment of contaminated unnetted cantaloupe was significantly more effective than that of netted cantaloupe. However, there was no difference in populations transferred to netted and unnetted cantaloupe when immersed in contaminated water. Further studies with unnetted cantaloupe varieties should be undertaken to better understand advantages over netted varieties with respect to food safety.

P3-157 Assessment of Irrigation Practices and Water Treatment for Risk Reduction in Organic Farming

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Introduction: Contaminated open surface water has contributed to produce outbreaks. Treatment of irrigation water has been proposed as a method to reduce risk from open surface water sources.

Purpose: This study evaluated overhead and drip irrigation as well as chlorine dioxide water treatment on pathogen presence (*E. coli* O157 and *Salmonella*) and indicator organism levels (fecal coliforms and generic *E. coli*) within an organic farming system.

Methods: Irrigation water from a creek was sampled at the source, prior to filtration and chlorine dioxide treatment. Replicate lettuce plots, amended with broiler litter, were randomly assigned: untreated overhead or drip irrigation and chlorine dioxide treated overhead or drip irrigation. Samples from agricultural inputs (288 water, 128 soil, 44 potting mix, 43 transplants) and lettuce (n = 275) were quantified for indicator organisms and for *E. coli* O157 and *Salmonella* isolation.

Results: In both years of the study, fecal coliform levels were reduced (at least 1 log MPN/100 ml) at the point of irrigation water application when a chlorine dioxide treatment system was used. However, fecal coliforms and generic *E. coli* levels were similar in the soil and from lettuce harvested from untreated and treated plots. Several *Salmonella* serovars (Oranienburg; Livingstone; Tennessee, and Infantis) and *E. coli* O157:H7 were isolated from potting soil and transplant plugs (*Salmonella* Mbandaka, Minnesota, Ealing). *E. coli* O157:H7 was detected in creek samples (1/54) and in the irrigation line after the filter (1/25). One untreated drip irrigation sample (1/37) and two untreated overhead irrigation samples (2/36) were positive for *E. coli* O157:H7. *E. coli* O157:H7 was detected on lettuce from treated drip (1/64) and untreated (3/128) overhead and drip irrigation plots.

Significance: Use of chlorine dioxide water treatment appeared to reduce indicator levels in water and may have reduced pathogen contamination in lettuce.

P3-158 Antagonistic Activity of Bacteria Recovered from Spinach against *Salmonella* Typhimurium and *Escherichia coli* O157:H7 on Spinach Surface

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Introduction: Interventions that prevent transmission of pathogens on produce surfaces by exploiting epiphytic pathogen-antagonizing native microbes could potentially assist in produce safety.

Purpose: The research objective was to evaluate the ability of naturally occurring microorganisms isolated from spinach leaves to antagonize *Escherichia coli* O157:H7 ATCC 700728 and *Salmonella enterica* Typhimurium LT2 and prevent adherence on the surface of spinach.

Methods: Hand harvested spinach was purchased and washed. After drying for 1 h, three 10 cm^2 spinach samples were spot-inoculated with a suspension of bacteria showing in-vitro antagonistic activity against *S. enterica* Typhimurium LT2 and *E. coli* O157:H7 ATCC 700728 then stored at 25°C for 24 h. Then, each sample was spot-inoculated with a suspension including both pathogens and stored at 25°C. At 0, 6, 12, and 24 h of storage, loose and strong attachment of pathogens on the surface was determined. Pathogens were enumerated on lactose-sulfite-phenol red-rifampicin agar and isolates on de Man Rogosa and Sharpe Agar or tryptic soy agar, incubating at 35°C for 24 h. The proportion of the total bacterial population which was physically attached to the surface was calculated.

Results: Populations on spinach leaf surface at 0 h of *E. coli* O157:H7 and *S.* Typhimurium LT2 in the presence of the antagonistic isolates were 3.7 ± 0.2 and 3.5 ± 0.2 log CFU/cm^2, respectively, and after 24 h, ranged from 4.9 ± 0.6 to 6.3 ± 0.4 and 3.8 ± 0.6 to 5.8 ± 0.3 log CFU/cm^2, respectively. Populations of the antagonistic isolates at 0 h were 5.7 ± 0.8 log CFU/cm^2. The strength of attachment in the presence of the isolates after 24 h for *E. coli* O157:H7 and *S.* Typhimurium LT2 ranged from 0.02 ± 0.03 to 0.26 ± 0.28 and 0.09 ± 0.12 to 0.49 ± 0.10, respectively.

Significance: These data suggest that although the native microorganisms on the surface may not inhibit pathogen growth, there might be potential in their utilization for decreasing attachment strength.

P3-159 Bacterial Populations Present on Various Leafy Greens and Their Antagonistic Activity against Enteric Pathogens

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Introduction: The composition of epiphytic bacteria on leafy greens may affect the possible pathogen presence. Bacteria showing pathogen-antagonizing effects can be useful biocontrol agents.

Purpose: To determine the bacterial loads on leafy green surfaces and identify epiphytic bacteria antagonistic to the enteric pathogens *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Saintpaul.

Methods: Spinach, lettuce, and parsley (50 samples each) were collected from different fields in Texas. Mesophiles, lactic acid bacteria (LAB), coliforms and psychrotrophs were enumerated. One to 10 colonies per sample and bacterial group were tested for antagonistic effects against *E. coli* O157:H7 and *Salmonella* Saintpaul using the agar spot method. Antagonistic isolates were then identified using a Vitek-2 system.

Results: Mean bacterial populations on parsley, lettuce, and spinach were 3.0 ± 0.7, 5.2 ± 0.6 and 6.0 ± 0.6 log CFU/g for coliforms, 3.0 ± 0.4, 4.6 ± 0.5 and 5.2 ± 0.8 log CFU/g for LAB, 4.6 ± 0.4, 6.1 ± 0.4, and 6.9 ± 0.5 log CFU/g for mesophiles and 4.2 ± 0.8, 5.0 ± 0.5, and 6.4 ± 0.6 log CFU/g for psychrotrophs, respectively. Spinach presented the highest overall bacterial populations, while lowest populations were found on parsley ($P < 0.05$). Antagonists of enteric pathogens from lettuce were *Bacillus licheniformis* and *pumilus*, *Gemella bergeri*, *Leuconostoc mesenteroides*, *Listeria grayi*,

Pantoea spp., *Pediococcus pentosaceus*, *Staphylococcus sciuri*, *Streptococcus sanguini*, *mutans* and *alactolyticus*, and *Vagococcus fluvialis*. Antagonistic isolates from spinach included *Cupriavidus pauculus*, *Enterococcus cecorum*, and *Kocuria kristinae*. In parsley, pathogen antagonists identified were *Gemella morbillorum, Staphylococcus intermedius* and *gallinarum, Pseudomonas paucimobilis, Serratia plymuthica,* and *Providencia rettgeri*. Antagonistic isolates recovered from multiple commodities included *Enterococcus casseliflavus*, and *Pseudomonas pseudoalcaligenes* (spinach, parsley), *Enterococcus gallinarum*, *Myroides* spp. and *Staphylococcus lentus* (parsley, lettuce), *Aerococcus viridans* and *Alcaligenes faecalis* (all commodities).

Significance: The populations of epiphytic bacteria on leafy greens were affected by commodity type, and included bacteria showing antagonism against *Salmonella* and *E. coli* O157:H7. These bacteria may be useful biocontrol agents for controlling enteric pathogens.

P3-160 Bio-mitigation Strategies to Reduce the Survival and Persistence of *Salmonella enterica* in Soils under Vegetable Production Systems

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Introduction: Practical, time and cost sensitive, and sustainable bio-mitigation strategies are needed in vegetable production systems to minimize the persistence of pathogens in soil to expedite planting or replanting following a contamination incident.

Purpose: To assess the influence of sustainable farming practices (cover crops, solarization and dry-wet events) on the survival of *Salmonella* in soils.

Methods: Microcosm, mesocosm, and field studies were established using clay and loam soils. Avirulent (field) and pathogenic (greenhouse) strains of *Salmonella* Typhimurium were inoculated into un-composted chicken litter at log 2, 4, and 6 CFU/g and incorporated into soil. Survival was assessed after growing+discing spinach, followed by cover cropping, solarization, and dry/wet events. The survival of *Salmonella* was assessed after each inoculation and intervention event for up to 90 days. The influence of secondary cover crop metabolites on the survival of *Salmonella* was assessed under the experimental systems.

Results: Microcosms: Significant negative correlations were established between the population of *Salmonell*a, temperature, and time ($P < 0.05$) while positive correlations were found between *Salmonella* populations and soil moisture content ($P < 0.05$). Greater death rates were observed in loam than clay soils ($P < 0.05$). Inhibition tests: Sinigrin associated with mustard cover crops did not significantly influence the survival of *Salmonella* ($P < 0.05$); however plant slurries from mustard significantly reduced *Salmonella* populations by 1.5 log CFU/ml when compared to other cover crops ($P < 0.05$). Field Trials: Time to non-detection of *Salmonella* varied between soil types (clay: 22 days, loam: 52 days) ($P < 0.05$). No significant decrease of *Salmonella* was observed after tilling spinach and leaving the field fallow for 15 days. *Salmonella* was not detected after growing a cover crop, solarization (1 month) and dry-wet events (3 cycles).

Significance: Development of sustainable bio-mitigation strategies to remediate soils contaminated with *Salmonella* will minimize delays in the accessibility of arable land for safe production of fresh produce.

P3-161 Lactic Acid Bacteria (LAB) Effect on Mineral and Vitamin C Content of Fresh Strawberries

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Introduction: Since 1990, approximately 600 outbreaks associated with fresh produce have occurred. Lactic acid bacteria cultures can be an effective intervention to reduce foodborne pathogens.

Purpose: Evaluation of lactic acid bacterial cultures and their effect on nutritional value of strawberries.

Methods: Fresh strawberries were obtained for this study. A control sample was treated with deMan, Rogosa and Sharpe (MRS) broth. Three LAB treatments with MRS broth were applied as a dip as follows: 1) *Lactococcus lactis* FS56, 2) *Lactobacillus plantarum* C28, and 3) *L. acidophilus* NP51. Treated strawberries were stored at 4°C for five days before analysis of selected minerals and Vitamin C was performed.

Results: Data was compared with values from the USDA National Nutrient Database. Strawberries treated with MRS broth showed an increase in sodium (Na), phosphorus (P), and potassium (K). Reductions in calcium (Ca), iron (Fe), and zinc (Zn) were observed. All three LAB cultures decreased Ca by 3.5 mg/100 g. Fe decreased by 0.051 mg/100 g with *L. lactis* FS56 and 0.095 mg/100 g with *L. plantarum* C28 and *L. acidophilus* NP51. P increased approximately 5.0 mg/100 g in all of the LAB cultures. K was affected the most of all of the minerals that were evaluated. *L. plantarum* C28 and *L. acidophilus* NP51 increased K values by 33 mg/100 g while *L. lactis* FS56 increased it by 40.7 mg/100 g. *L. lactis* FS56 culture treatment increased Na levels by approximately 3.5 mg/100 g and 5.5 mg/100 g by *L. plantarum* C28 and *L. acidophilus* NP51. Zn levels increased by 2.0 mg/100 g in all three LAB cultures.

Significance: These data suggest that the LAB cultures can impact the nutritional value of strawberries. This knowledge is relevant to decide whether to use LAB treatment for pathogen control in this type of product.

P3-162 Screening of Biocontrol Agents against *Listeria monocytogenes*, *Salmonella*, and *Escherichia coli* O157:H7 and Antimicrobial Efficacy on Iceberg Lettuce

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Introduction: Foodborne disease outbreaks continue to plague the produce industry, indicating a need for alternative or additional microbial control methods. The use of biological control agents postharvest could provide an additional hurdle to challenge pathogen growth. Many bacterial species, including Lactic Acid Bacteria (LAB) and *Bacillus* species, have demonstrated antimicrobial activity and thus are candidates for postharvest biocontrol agents.

Purpose: The purpose of this study was to screen 22 bacterial isolates for antimicrobial activity against *Listeria monocytogenes*, *Salmonella* species, and *Escherichia coli* O157:H7 *in vitro*, then to assess antimicrobial efficacy of select isolates against *L. monocytogenes* on iceberg lettuce.

Methods: The antimicrobial activity of the LAB isolates was determined using a seeded-overlay method and all other isolates were evaluated by spot inoculating the isolate on pathogen-seeded TSA; antimicrobial activity was determined by the size of the clearing around the isolate. Antimicrobial efficacy on iceberg lettuce was assessed by spraying a cocktail of the three LAB isolates (10^7 - 10^8 CFU/g) onto lettuce spot-inoculated with *L. monocytogenes* (10^2 - 10^3 CFU/g), then incubating at 10°C for 14 days.

Results: Three LAB isolates and six *Bacillus* isolates suppressed *L. monocytogenes*, *Salmonella*, and *E. coli* O157:H7 *in vitro*. LAB isolates *Lactobacillus plantarum*, *Pediococcus acidilactici*, and *Pediococcus pentosaceus* were chosen for use in the iceberg lettuce challenge study. *L. monocytogenes* levels were 1.84 logs lower on lettuce treated with LAB cocktail than untreated lettuce after 14 days incubation at 10°C.

Significance: This study has identified nine bacterial isolates capable of inhibiting *L. monocytogenes*, *Salmonella*, and *E. coli* O157:H7 *in vitro*. Three LAB isolates suppressed *L. monocytogenes* on iceberg lettuce and merit additional testing to determine commercial applicability.

P3-163 *Bacillus subtilis* Increases Crop Yield and Reduces Contamination by *Listeria monocytogenes* on Cantaloupes

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Introduction: Cantaloupes serve as one of the major crops in the state of Delaware accounting for approximately $850,000 in estimated annual sales. Preliminary data has shown the ability of *Bacillus subtilis* UD1022 to help increase crop yield through complex plant-microbe interactions that increase plant defenses toward plant pathogens. Growth of *L. monocytogenes* is inhibited in culture and on Romaine lettuce plants when UD1022 is inoculated onto the roots of plants.

Purpose: The purpose of this project is to evaluate the use of a plant growth promoting rhizobacteria, *Bacillus subtilis* UD1022, to reduce the contamination of cantaloupes by *L. monocytogenes* in the pre-harvest environment as well as in the packinghouse environment.

Methods: In this study, pieces of cantaloupe rind (3.20 cm x 2.50 cm) were treated with UD1022 supernatant or sterile water immediately following inoculation with *L. monocytogenes* (9.05 ± 0.7 log CFU/ml) or after an 8 hour incubation at 37°C, 22°C or 4°C. Three cantaloupe rind pieces were pooled into one sample (n = 6 samples). Bacterial enumeration was performed by plating samples onto Brilliant Listeria Agar.

Results: At 22°C, recovery of *L. monocytogenes* was ~2.5 log lower when treated with UD1022 supernatant at 8 hours post-inoculation (hpi) and ~3 log lower at 24 hpi compared to controls, which was statistically significant ($P = 0.0024$). Recovery of *L. monocytogenes* was also ~2.5 log lower at 22°C when UD1022 supernatant was applied at 8 hpi and *L. monocytogenes* was enumerated at 24 hpi. Although recovery of *L. monocytogenes* was lowest for all temperatures when treated with UD1022, this reduction was not significant at 37°C or 4°C.

Significance: These results indicate that UD1022 may be used as a natural biocontrol agent to reduce the risk of contamination by *L. monocytogenes* on cantaloupe rind during times of temperature abuse in the packinghouse, storage, or transport.

P3-164 A Pilot Scale Evaluation of a Spray Cabinet with Commercial Antimicrobials against *Escherichia coli* O157:H7 Surrogate Survival on Jalapeno Peppers

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Introduction: Jalapeno peppers have been an unrecognized and under-researched food vehicle associated with foodborne pathogens. Limited data address the efficacy of commercial antimicrobials against *Escherichia coli* O157:H7 on jalapeno peppers, especially in semi-commercial small produce processer settings.

Purpose: This experiment aims to validate the use of antimicrobial solutions in a spray cabinet to inactivate *E. coli* O157:H7 surrogate organisms on jalapeno peppers.

Methods: Jalapeno peppers, inoculated with a 5-strain mixture of rifampin-resistant (100 µg/ml) *E. coli* O157:H7 pathogenic surrogates (7.0 log CFU/ml, ATCC BAA-1427, BAA-1428, BAA-1429, BAA-1430, BAA-1431), were passed through a commercial antimicrobial cabinet containing a top and bottom bar spraying (20 psi and 2 l/min) water, sodium hypochlorite (SH; 50 ppm), SH with pH adjusted to 6.7 (ASH), peroxyacetic acid (PAA; 80 ppm), PAA with pH adjusted to 6.7 (APAA), a lactic and citric acid blend (LCA; 1%), and chlorine dioxide (CD; 5 ppm). Surviving bacteria were recovered in 0.1% buffered peptone water (60 s shake) followed by spread plating onto tryptic soy agar plus rifampicin (100 µg/ml). Data (2 replicates/3 samples per replicate) were analyzed using the ANOVA of SAS.

Results: There were no significant difference ($P \geq 0.05$) in recovered *E. coli* populations between untreated peppers (3.9 ± 0.3 log CFU/g) and those treated with water and CD (3.7 ± 0.3 and 3.6 ± 0.4, respectively). Significantly fewer ($P < 0.05$) *E. coli* were on the peppers after treatment with all other antimicrobials applied in the commercial antimicrobial cabinet with the fewest *E. coli* recovered (2.9 log CFU/g) on peppers sprayed with PAA and APAA. Interestingly in a single spray application when applying SH or PAA to peppers, pH adjustment made no difference ($P \geq 0.05$) in recovered *E. coli* populations.

Significance: These results validate the use of a commercial antimicrobial spray cabinet and are useful for developing application protocols for antimicrobials to control *E. coli* O157:H7 during post-harvest processing of jalapeno peppers.

P3-165 Antagonistic Activity of a *Bacillus subtilis* Strain against *Salmonella* during Alfalfa Seeds Sprouting

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Introduction: Alfalfa sprouts are eaten raw and have been associated with multiple outbreaks of foodborne illness. A strategy that could be implemented to assess its safety is the application of secondary metabolites from antagonistic microorganisms such as *Bacillus* genera. However, studies are needed to determine antagonistic activity as these metabolites have been used only against phytopathogens.

Purpose: To determine antagonistic activity of secondary metabolites produced by a *Bacillus subtilis* strain against *Salmonella in vitro* and during alfalfa seeds sprouting.

Methods: A *Bacillus subtilis* strain isolated from biofertilized soil of blackberry crops in Queretaro, Mexico showing antagonistic capacity was grown in tryptic soy broth (4 days at 35°C, 180 rpm). Supernatant was recovered and used to determine antagonist effect against five strains of *Salmonella* using well diffusion test on tryptic soy agar (TSA) by triplicate. Secondary metabolites were precipitated by acidification with HCl (pH 2) for 24 h, resuspended in sterile water and used to irrigate alfalfa seeds inoculated with a *Salmonella* cocktail (~6 log CFU/g) during germination for 5 days. Aerobic mesophilic bacteria (AMB) and *Salmonella* were enumerated periodically in TSA and TSA supplemented with rifampicin, respectively.

Results: *In vitro* test of supernatant showed inhibition halos against all *Salmonella* strains (range 11.17 - 19.91 mm). After five days, alfalfa sprouts irrigated with secondary metabolites, *Salmonella* population was reduced by 5.26 ± 0.09 log CFU/g, whereas sprouts irrigated with sterile water only

showed a 1.21 ± 0.05 log CFU/g reduction. AMB did not showed significance differences (Tukey, $P < 0.05$). Additionally, growth of alfalfa was promoted with secondary metabolites showing a final length of 13.8 cm ± 0.8 compared to 7.13 cm ± 0.63 in those sprouts irrigated with water.

Significance: Application of secondary metabolites produced by this *Bacillus subtilis* strain could be a strategy to eliminate *Salmonella* on alfalfa sprouts and reducing sprouting time.

P3-166 Treatment of Alfalfa Sprout Seeds Contaminated with *Escherichia coli* O157:H7 Using Supercritical Carbon Dioxide and Peracetic Acid

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Introduction: Recent sprout-implicated outbreaks, relative to their consumption levels, suggest that both sprout production and consumption present challenges for ensuring consumer safety. Seeds are a source of contamination and 30°C and humid conditions required for germination are conducive to growth of low levels of pathogens.

Purpose: In an attempt to suggest a method for producing safe sprouts for human consumption, supercritical carbon dioxide ($SC\text{-}CO_2$) was applied in combination with a food grade sanitizer, peracetic acid (PAA), to alfalfa sprout seeds.

Methods: Alfalfa seeds inoculated with a five strain cocktail of *Escherichia coli* O157:H7 were exposed to treatments of $SC\text{-}CO_2$ at 500, 630, 710, 740, and 780 kg/m^3 with and without pre-soaking in 80 parts per million PAA. Inoculum was recovered using TSA with ampicillin and confirmed with UV fluorescence of GFP. Following treatments, percent germination was determined by incubating seeds at 30°C in the dark for 2 days, watering twice daily and counting the number of seeds with the appearance of at least 2 mm of visibly protruding radicle. All experiments were run in biological triplicate.

Results: $SC\text{-}CO_2$ treatment alone had less than 1 log reduction of *E. coli* O157:H7. Similarly, treatment with 80 ppm PAA for 15 min achieved less than 1-log reduction. The most effective treatment was the use of 80 ppm PAA for 5 min with $SC\text{-}CO_2$ at 780 kg/m^3 for 15 min, achieving greater than 4-log CFU/g reduction. The most effective decontamination treatment above decreased percent germination of alfalfa seeds to less than 60%.

Significance: While the combined use of $SC\text{-}CO_2$ and surface sanitizer can result in significant log reduction of *E. coli* O157:H7 on alfalfa seeds, the recommended safety level of 5-log reduction is not met and the negative effect on the utility of the seed remains a limitation for practical implementation.

Poster

P3-167 Development of Combined Thermal and Chlorine Dioxide Gas Treatment with Mechanical Mixing for Inactivation of *Salmonella* Montevideo on Mungbean Seeds

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Introduction: Foodborne outbreaks have been associated with the consumption of fresh sprouted beans. The use of a kill step on the seeds prior to sprouting step would enhance the safety of fresh sprouts.

Purpose: The objective of this work was to evaluate the effectiveness of the combined thermal and chlorine dioxide gas (ClO_2) treatment with mechanical mixing (tumbling) to eliminate *Salmonella* on artificially inoculated mungbean seeds.

Methods: Mungbean seeds were artificially inoculated with *Salmonella* Montevideo and stored at 4°C for 24 h. The effectiveness of inactivating *Salmonella* cells on the inoculated seeds using wet or dry heat with or without gaseous ClO_2 (3.5 mg/l air) was investigated. The role of tumbling (mechanical mixing) during treatments on inactivating *Salmonella* cells was also investigated.

Results: Although no viable *Salmonella* was recovered from seeds treated in hot water at 60°C for 2 h, these treated seeds failed to germinate. Dry heat treatments (55, 60, or 70°C) for up to 8 h reduced *Salmonella* populations in excess of 3 log CFU/g. The use of tumbling while treating the seeds resulted in up to 1.6-log CFU/g reduction in *Salmonella* populations as compared to no tumbling. The combined treatment (70°C-ClO_2-tumbling for 4 h) reduced *Salmonella* cells by 5 log CFU/g. All dry heat treated seeds were capable of germinating as well as the non-treated controls.

Significance: The data presented here suggested that thermal and ClO_2 gas treatments were capable of penetrating and inactivating cells which are attached to inaccessible sites and/or are within biofilms on the seed surface. The increased reductions in pathogenic populations on the seeds with the use of tumbling could be attributed to increased uniformity of heat transfer and exposure to chlorine dioxide gas. The results presented here suggest that the combined treatment with tumbling would be a viable process for enhancing the safety of fresh sprouts.

P3-168 Decontamination of Sprout Seeds by Pulsed Light

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❖ ***Developing Scientist Competitor***

Introduction: There is a crucial need for identifying alternative sprout decontamination techniques due to the limitations of existing techniques. Pulsed light processing is an emerging technology which is proved to inactivate myriad microorganisms on various foods. Therefore, pulsed light might potentially be used for decontamination of sprout seeds.

Purpose: The purpose of this study was to evaluate the efficacy of pulsed light for decontaminating sprout seeds in both dry and wet environments. Wet environment is expected to reduce the temperature buildup during pulsed light treatment and protect germination of seeds.

Methods: Alfalfa seeds were inoculated with 4-strain *Salmonella* cocktail (Tennesse, Cubana, Muenchen and St. Paul). For dry treatments, 1 g of alfalfa seeds forming a 1-mm thick monolayer was treated with pulsed light. For wet treatment, 1 g of seeds was immersed in 1 ml of water during pulsed light treatment. Temperature profile was recorded using an infrared camera for both dry and wet treatments.

Results: The microbial reduction increased with the increased treatment time (up to 80 s) and shorter distances from the pulsed light lamp (3.26 to 5.26 in). However, increased microbial reduction typically resulted in decreased germination rate. An 80 s pulsed light treatment at 4.26 in from the window face reduced the *Salmonella* level by 2.18 ± 0.70 log CFU/g. The corresponding seed germination rate was 28 ± 2%. In wet environment, temperature profile showed that 1:1 (volume to mass) water was able to reduce the surface temperature of seeds by >20°C. An 80 s pulsed light treatment in the wet environment at 4.26 in resulted in 1.44 ± 1.01 log CFU/g reduction of *Salmonella* on seeds while maintaining the germination rate at > 80%.

Significance: Pulsed light technology can potentially be used for decontamination of sprout seeds. There is a potential to combine the existing sanitizer treatment with pulsed light for increased efficacy.

P3-169 New Proprietary Post-harvest Treatment Reduces *Listeria monocytogenes* and *Salmonella* Species on Fresh Grape Tomatoes

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Introduction: Fresh produce, often consumed raw, has been repeatedly linked to foodborne illness and accounts for some of the most deadly outbreaks. *Salmonella* spp. (*S*.spp.) and *Listeria monocytogenes* (*L.m.*) are two of the most critical pathogens of concern for produce safety.

Purpose: Ansera Analytics is developing a new proprietary post-harvest treatment for fresh produce to protect the commodity from biological hazards during distribution. The purpose of this study was to explore the treatment's ability to reduce *L.m.* and *S*.spp. on fresh grape tomatoes.

Methods: Fresh grape tomatoes (n = 12) were soaked in a 10^6 CFU/ml *L.m.* or *S*.spp. solution for one minute, air-dried, and then soaked in the treatment solution for one minute. Samples were air dried to allow the material to dry. Bacterial swabs (n = 3) were collected from the tomatoes at day 0, 3, 6, and 9. All cultures were grown on nutrient agar and CFUs were enumerated at 24 h. Plates were held for 7 days.

Results: Preliminary data suggests a clear trend towards a significant reduction in *L.m.* and *S*.spp. on treated tomatoes. Adulterated, non-treated tomatoes (control) showed confluent growth of pathogens for the majority of swabs during the 9-day sampling period, while treated tomatoes showed a 3 - 6 log CFU/ml reduction. Some swabs contained no visible colonies after 24 h but started to grow up after 2 - 4 days. This demonstrates the treatment's inhibitory effect. Swabs from day 6 and day 9 grew less *L.m.* than swabs from day 3, but contained more molds.

Significance: The post-harvest treatment currently in development demonstrates the ability to reduce *L.m.* and *S*.spp. populations on the surface of fresh grape tomatoes and inhibits the pathogen's growth for several days. The treatment has also effectively been tested on adulterated cantaloupes.

P3-170 UV-C Inactivation of Bacteria Artificially Inoculated on Apricot Fruit in Laboratory and Commercial Settings

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Introduction: Washes with chemical sanitizers such as chlorine are commonly used by the fresh produce industry to sanitize wash water and fresh produce. Some soft fruit, such as tree-ripened apricots, cannot be washed with aqueous sanitizers, due to their innate softness and delicate surfaces. Non-aqueous interventions are needed to enhance microbial safety of this type of fruit.

Purpose: The objective of this study was to investigate the efficacy of ultraviolet-C (UV-C) light in inactivating *Escherichia coli* O157:H7, *Salmonella* spp. and *E. coli* ATCC 25922 on apricots in the laboratory, and in inactivating an *E. coli* surrogate at a commercial setting.

Methods: Robada apricots were dip-inoculated with cocktails of 5 strains of *E. coli* O157:H7, 4 strains of *Salmonella* spp., and *E. coli* ATCC 25922. UV-C treatment was conducted at an intensity of ~13 mW/cm^2. Fruit was rotated during the UV-C treatment. The efficacy of a UV-C treatment system (two treatment chambers connected by an inclined belt to rotate apricots between chambers) was also tested in a commercial setting.

Results: Results showed that in the laboratory setting using the rotating device, UV-C at a dose of ~125 mJ/cm^2 reduced populations of *E. coli* O157:H7, *Salmonella* spp. and *E. coli* ATCC 25922 by 1.5, 2.1, and 1.8 log CFU/fruit, respectively. Further increases in UV-C doses achieved higher reductions of the bacteria. In the commercial setting, the reductions of *E. coli* ATCC 25922 were only 0.5 - 0.7 log CFU/fruit even though similar or higher average doses of UV-C were used. Further evaluation using film dosimetry revealed that there were large variations in UV-C doses among varying apricot surface locations at the commercial trial.

Significance: This study demonstrates the need of using a better rotation device more capable of delivering uniform UV-C dosage to the surface of apricots for inactivating human pathogens in commercial settings.

P3-171 Evaluation of Cinnamic Aldehyde Emulsion on the Removal of Foodborne Pathogens from Inoculated Tomato Surfaces

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Introduction: Essential oils are secondary hydrophobic metabolites which are produced by wide range of plants. Its analgesic, antioxidant and antimicrobial properties are well known since long time ago so they constitute an alternative for been used in food safety and preservation.

Purpose: This study investigated the antibacterial activity of cinnamaldehyde emulsions (3-fenil 2-propenal) extracted from cinnamon bark oil against three important foodborne pathogens: *Salmonella enterica*, *Staphylococcus aureus* and *Listeria monocytogenes*.

Methods: Two nonionic emulsifiers (Tween 85 and Span 20) were used to stabilize the essential oil in water. Antimicrobial activity of emulsions was tested by well diffusion agar test. Minimum Inhibitory Concentration (MIC) was determined for cinnamaldehyde emulsified with Tween 85 and Span 20. The reduction of inoculated *S. enterica, L. monocytogenes,* and *S. aureus* on tomatoes with cinnamic aldehyde emulsion was evaluated at low density of pathogens on tomato surface (4 log CFU/tomato). Differences among treatments were determined by Duncan test at significant level of 0.05.

Results: Inhibition zone diameters from 16-36 mm were obtained at concentrations of 0.5 to 1.5% of cynnamaldehyde emulsions prepared with tween 85, and from 14 - 49 mm for emulsions prepared with span 20. For emulsions prepared with span 20, the MIC were below 0.25% for pathogen suspensions at 4 log CFU/ml and up to 0.5% for pathogen suspensions at 6 log CFU/ml. Reductions between 95% and 98%, equivalent to 2 log CFU of the inoculum were obtained on inoculated tomatoes treated with 0.5% cinnamaldehyde emulsion by immersion for five min, which were significantly different than the results showed on inoculated tomatoes immersed in water ($P < 0.05$).

Significance: More research is necessary in order to determinate the feasibility of the industrial use of these emulsions.

P3-172 Evaluation of *Salmonella* Transfer and *E. coli* Removal from Wood and Plastic Crates Used for Tomato Transportation in Central America

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Introduction: *Salmonella* spp. is a ubiquitous organism and its presence in fruits increases the risk of illness. The containers used in the transportation of fruits and vegetables play an important role in the contamination of fresh produce. In Central America wood crates are commonly used for harvest, storage and transportation of tomato for regional commerce.

Purpose: The purpose of this study was (i) to evaluate the transfer and cross contamination of *Salmonella,* and (ii) to evaluate the process of cleaning of wood and plastic crates used for tomato transportation in Central America.

Methods: *Salmonella* Poona and *Salmonella* Typhymurium strains were used to inoculate tomatoes at 7.0 log CFU/tomato. Ten inoculated tomatoes were passed on clean plastic and wood crates, followed by passing ten uninoculated tomatoes on the contaminated surfaces. Transfer coefficients were determined by estimating log CFU/tomato of *Salmonella* recovery from uninoculated tomatoes. For the sanitation, two treatments of cleaning were applied on inoculated crates with *E. coli*: washing with water and washing with chlorine solution (200 ppm) and detergent. Transference coefficient between plastic and wood crates was compared using *t*-test, and efficiency of washing treatments was compared by ANOVA. Significant differences among treatments were determined by Duncan test at significant level of 0.05.

Results: Cross-contamination of tomato at levels of 2.5 log CFU/tomato occurred on both type of surfaces. There was not significant difference between transfer coefficients on wood or plastic crates ($P > 0.05$). Sanitation with chlorine solution showed *E. coli* reduction of 99.999% in plastic crates and 98.38% in wood crates ($P < 0.05$).

Significance: Regardless the material of crates, both materials may suffer cross contamination. It is strongly recommended to substitute the use of wood for plastic crates and perform correct Standard Sanitation Operating Procedures to reduce risk contamination of fresh produce.

P3-173 Disinfection Methods to Mitigate Food Safety Risks Associated with Contaminated Irrigation Water on Drip-irrigated Tomatoes

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Introduction: Water used for irrigating fresh produce is a common source of pathogen contamination. Testing of an irrigation water source can become costly and is a poor control when irrigating from surface sources. In lieu of post-contamination corrective actions, irrigation water can be treated before distribution to mitigate pathogen contamination risk.

Purpose: Evaluate the ability of in-line water disinfection techniques to mitigate the risk of Shiga Toxigenic *E. coli* (STEC) and *Salmonella* contamination on drip-irrigated tomatoes.

Methods: Tomatoes were cultivated on bare ground or plastic mulch raised beds. Plots were drip irrigated with water from 1) a municipal water supply (negative control) or 2) a pond harboring STEC and *Salmonella* (positive control). The surface water was also treated with 3) ultraviolet light to achieve a dose of 10,000 μW s cm^{-2}, 4) 20 ppm free chlorine, or 5) 20 ppm peroxyacetic acid (PAA). Irrigation water was enumerated for *Salmonella* and STEC. Tomatoes were harvested, rinsed in BPW containing 0.2% Tween 80 and plated onto Chromagar STEC and XLT4. The remaining rinsate was used in selective enrichments of *Salmonella* in Tetrathionate Broth and STEC in mTSB with 8 mg/l sodium novobiocin, and pathogen presence was confirmed using confirmation testing.

Results: Over seven weeks, populations of STEC and *Salmonella* in untreated source irrigation water were found to fluctuate between 0.6 to 2.3 log CFU/100 ml and 0.07 to 2.5 log CFU/100 ml, respectively. Chlorine and ultraviolet water treatment methods were successful at reducing levels of both *Salmonella* and STEC to below detectable levels before application. None of the sampled tomatoes were contaminated with STEC post-harvest, and one sample of tomatoes from chlorine-treated irrigation water was positive for *Salmonella* post-harvest.

Significance: Economically feasible irrigation water disinfection systems may provide a way to lower the food safety risk associated with using a surface water irrigation source.

P3-174 Efficacy of Peroxyacetic Acid and Aqueous Chlorine Dioxide Treatments to Reduce *Salmonella* and *E. coli* O157:H7 on Frozen Berries and Mangoes

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Introduction: Minimally processed fruit products, such as frozen fruit blends intended for raw consumption, pose a potential risk to consumers. Proposed Food Safety Modernization Act (FSMA) regulations mandate implementation of preventive approaches to reduce microbiological food safety hazards in these products. The primary option to control microbiological hazards is to employ sanitizers; however, limited data is available on their efficacy against foodborne pathogens under industry-relevant conditions.

Purpose: Evaluate efficacy of peroxyacetic acid and aqueous chlorine dioxide to reduce *Salmonella* and *E. coli* O157:H7 on frozen berries and mangoes.

Methods: Frozen fruit pieces (berries or mangoes) were spot-inoculated with a five-strain cocktail of *Salmonella* spp. or *E. coli* O157:H7 to achieve 5 - 6 log CFU/g. Inoculated fruit was and held at -20°C for 24 - 48 h prior to treatment with sanitizer. Peroxyacetic acid (Tsunami 200, 40 - 80 ppm) and chlorine dioxide treatments (Selectrocide, 5 ppm) were performed by agitating frozen fruit in a thermostatically-controlled circulating water bath (10°C - 30°C) for industry-relevant timeframes (15 - 60 seconds). Immediately following treatment, fruit samples were neutralized by diluting and gently rinsing in buffered peptone water (BPW; 1:2). Rinses were serially diluted with BPW and plated on tryptic soy agar and Hektoen Enteric Agar and incubated (37°C, 24 - 48 hours) prior to enumeration.

Results: Physical agitation and rinsing associated with all treatments resulted in significant reduction of both pathogens compared to inoculated, unwashed fruit ($P < 0.05$). Mean log reductions of 1.16 ± 0.46 to 1.61 ± 0.52 achieved by peroxyacetic acid treatments (40 and 80 ppm; 25°C) were significantly greater ($P < 0.05$) than treatments without sanitizer (0.78 ± 0.37). Chlorine dioxide (5 ppm) was not significantly more effective than water to reduce *Salmonella* spp. or *E. coli* O157:H7 on frozen fruits.

Significance: Peroxyacetic acid rinses (40 and 80 ppm) significantly reduce survival of foodborne pathogens on frozen berries and mangoes compared with water only.

P3-175 Comparative Efficacy of Gas Phase Sanitizers for Inactivating *Salmonella*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* on Intact Lettuce Heads

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❖ ***Developing Scientist Competitor***

Introduction: It is now acknowledged that the control of human pathogens associated with fresh produce requires an integrated approach of several interventions as opposed to relying on post-harvest washes to remove field acquired contamination. To this end, current research is directed towards identifying such interventions that can be applied at different points in leafy green processing.

Purpose: In the following the efficacy of different gas phase treatments to decontaminate whole lettuce heads during pre-processing storage were evaluated.

Methods: Whole Cos lettuce heads were spot inoculated with *L. monocytogenes, E. coli* O157:H7 *or Salmonella* spp. The inoculated lettuce heads were then placed in a treatment chamber and exposed to ozone, chlorine dioxide or hydroxyl radicals at different time periods under a range of relative humidity. Survivors of the treatments were enumerated along with sensory analysis performed on the treated lettuce.

Results: Ozone gas reduced *L. monocytogenes* by 2-log after 10 min of exposure with *Salmonella* and *E. coli* O157:H7 being decreased by 0.66 and 0.56 log CFU, respectively. Chlorine dioxide gas treatment reduced *L. monocytogenes* and *Salmonella* on lettuce heads by 4 log CFU but only supported a 0.8 log CFU reduction in *E. coli* O157:H7 numbers. In comparison, hydroxyl radicals supported a 2.9 - 4.8 log CFU reduction of model human pathogens inoculated onto lettuce heads but required extended exposure times and relative humidity < 0.8.

Significance: From the gas phase sanitizers tested, chlorine dioxide and hydroxyl radicals are the most effective. The latter process holds most promise based on the ease of delivery, worker safety and preservation of lettuce sensory characteristics, Although expose times for hydroxyl radicles was relatively long (24 h) this should not be considered a limitation given the intervention is applied in store rooms or in transport containers during transit.

P3-176 Inactivation of *Salmonella enterica* and *Listeria monocytogenes* in Cantaloupe Puree by High Pressure Processing

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Introduction: Cantaloupe is mostly eaten raw or as puree. Cantaloupe being the most netted varieties of melons presents a greater risk of pathogen transmission during food preparation. Method is needed that can inactivate pathogens in cantaloupe products with minimal alteration of quality.

Purpose: The objective of this research was to explore the potential application of high pressure processing (HPP) to reduce or eliminate inoculated *Salmonella enterica* and *Listeria monocytogenes* in fresh cantaloupe puree (CP). Effects of HPP on background microbial loads were also examined.

Methods: Freshly prepared CP containing no and 0.1% ascorbic acid (AA) was inoculated with a bacterial cocktail composed of three serotype mixture of *S. enterica* (*S.* Poona*, S.* Newport H1275 and *S.* Stanley H0558) and a mixture of three strains of *L. monocytogenes* (Scott A, 43256 and 51742) to a population of ca. 10^7 CFU/g. Inoculated CP (ca. 5 g) was placed in individual sterile stomacher bags, double sealed and double bagged. Samples were pressure treated at 300, 400 and 500 MPa at 8°C and 15°C (initial temperatures) for 5 min. Treated samples were stored at 4°C and subjected to various analyses.

Results: Data indicated increased inactivation of both *Salmonella* and *Listeria* with higher pressure. Log reduction (LR) for samples containing 0.1% AA at 300 MPa, 8°C was 3.4 ± 0.6 and 1.2 ± 0.15 log CFU/g for *Salmonella* and *Listeria,* respectively, which was significantly lower ($P < 0.05$) compared to LR values of 6.6 ± 0.4 and 6.2 ± 0.2 log CFU/g for *Salmonella* and *Listeria,* respectively, at 500 MPa, 8°C. Initial sample temperatures had minor influence on LR values. *Listeria* exhibited higher resistance to HPP than *Salmonella*.

Significance: Study highlighted a method to enhance the safety and quality of cantaloupe puree. Results from this work suggest efficacious treatment strategy for CP indicating 6 or higher log reduction for pathogens with minimal impact on quality.

P3-177 Attachment of *E. coli* O157:H7 on Produce and Damage of Bacterial Cell Envelope by Dry Pulsed Light (PL), Wet PL and PL-Surfactant Combinations

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Introduction: It has been showed in our previous study that pulsed light (PL) could potentially be used for decontamination of *Salmonella* or *E. coli* O157:H7 on green onions as well as raspberries. It has been observed that the decontamination efficacy varied on different food matrixes. Also, the mechanism of PL to inactive pathogenic bacteria was not clear.

Purpose: 1) To investigate the inactivation mechanisms of PL and PL-surfactant on *E. coli* O157:H7 attached on glass coverslips, and 2) observe the bacterial attachment on green onions (stems and leaves) and raspberries as well as decontamination effect of dry PL.

Methods: 1) To investigate the mechanism of PL, *E. coli* O157:H7 cells were attached on glass coverslips and treated by dry PL, wet PL and PL-surfactant. The ones without treatment served as control. Scanning electron microscopy (SEM) has been used to observe the bacterial morphological change before and after the treatments. 2) To compare two different fresh produce, SEM and Cryo-SEM were used to observe the surface structure of green onions (stems and leaves) and raspberries. The attachment of *E. coli* O157:H7 on the produce as well as their morphological change after PL treatments have also been compared.

Results: The results showed that 1) dry PL caused intracellular liquid evaporation and led to flatten out on the edge of the cells. Wet PL and PL-surfactant treated bacterial cells showed more membrane disruption; and 2) different topology of the food matrixes affected bacterial attachment.

Significance: 1) PL was proved by the SEM image to have photo-thermal effect on *E. coli* O157:H7 which caused a change of cell morphology, membrane disruption, and shrinking of intracellular tissues. 2) Surface structure of green onions (stems and leaves) as well as raspberries affected the attachment of bacterial cells and the surface roughness related with bacterial cell envelope structure after PL treatment.

P3-178 Combination of Ultrasound and Near Neutral Electrolyzed (NEO) Water in Reducing *Escherichia coli* O157:H7 and *Salmonella* Typhimurium DT 104 Inoculated on Romaine Lettuce Leaves

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❖ ***Developing Scientist Competitor***

Introduction: Increased consumption of fresh produce has led to increased reports of foodborne illnesses associated with them, which may largely be attributed to the lack of effective cleaning treatment before consumption. Leafy greens (lettuce, cabbage, etc.) form a significant proportion of these reported cases.

Purpose: The aim of the study was to ascertain the effects of a combined ultrasound and NEO water (155 mg/l chlorine at pH 6.5) treatment on the reduction of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium DT 104 inoculated on romaine lettuce leaves.

Methods: Whole lettuce leaves (80 g) were spot-inoculated with 100 µl of a mixture of 5 strains of either *E. coli* O157:H7 or *Salmonella* Typhimurium DT 104 and treated in a beaker containing 1.6 l of deionized (DI) or NEO water with ultrasound. Different treatment times (5, 10 and 15 min) and ultrasound power (130 and 210 W) were tested. Treatments without ultrasound served as a control and six samples of each treatment were analyzed.

Results: NEO water use (compared to DI water), longer duration of treatment and higher ultrasound power resulted in significantly increased reductions of the pathogens ($P < 0.05$). The 15 min-210 W, NEO water combination led to the highest reductions, with 4.4 and 4.3 log reductions of *E. coli* O157:H7 and *Salmonella* Typhimurium, respectively, compared to < 2.8 log reductions for treatments without ultrasound for both pathogens. Treatments with NEO water completely inactivated pathogens in wash solutions however washing with DI water did not.

Significance: Results obtained in this study suggest that applying ultrasound to NEO water treatments led to additional reductions in the pathogens of concern inoculated on romaine lettuce and therefore this technique can be applied to ensure produce safety.

P3-179 Food Hygiene Knowledge and Practices of Food Handlers in the Convenience Food Industry in Gauteng, South Africa

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Introduction: Accessing safe, good quality food has been humankind's main endeavor from the earliest days of existence. Food safety remains a critical issue in the light of outbreaks of foodborne illnesses that result in substantial cost to individuals, the food industry and the economy.

Purpose: Eight convenience food manufacturing plants were randomly selected for the purpose of conducting interviews with food handlers who prepare Ready-to-Eat foods. The purpose of this study was to present data on the food hygiene knowledge and practices of these food handlers.

Methods: Interviews based on structured questions were completed by a target population (88) that represents 100% of the population of food handlers & management in eight convenience food manufacturing plants, predominantly supplying Ready-to-Eat products to retailers. The interviews were conducted on a one-on-one basis with the food handlers before they commenced their shift, but after they had entered their working environment.

Results: An average of only 82% of respondents always washed their hands as a standard hygiene practice, 66% had received some form of food hygiene training and only 34% received training from the chemical supplier on how to use their products. None of the plants had an adequate supply of hot water for cleaning. All the plants used chemicals and/or detergents for washing, but only 47.7% used cold water, soap, pressure and sanitizer, which comprise the best option. All plants frequently send product samples for microbiological analysis and 25% of the plants reported samples that tested positive for *Staphylococcus aureus,* 50% for *Listeria* and 37.5% for *Escherichia coli*.

Significance: Food handlers had an unacceptable knowledge level of good food hygiene practices, as well as poor implementation thereof. Although food safety training programs are essential, behavioral changes will not occur merely as a result of training, thus should be further developed through continuous programs, workplace-specific courses and skills development.

P3-180 The Effect of Cattle Diet on Fecal Shedding of *Escherichia coli:* A Meta-Analysis

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Introduction: Some strains of Shiga Toxin-producing *Escherichia coli* (STEC) can cause severe foodborne illness. Cattle are typically the reservoir for STEC, and the impact of diet on shedding remains unclear with studies showing conflicting results. Meta-analysis is a statistical method that systematically synthesizes the results of independent, but related, studies.

Purpose: A meta-analysis was conducted to summarize and estimate the effect of roughage-based diet on shedding of generic *E. coli* and colonic pH in cattle compared to grain-based diets.

Methods: A literature review was conducted using PubMed and articles were screened for relevance. References from selected publications were reviewed to identify additional work. Studies of the effects of roughage versus grain diets in cattle on pre-slaughter fecal colonic pH and total generic *E. coli* counts were included in the meta-analysis. Studies using composite fecal samples or inoculated cattle were excluded. Standardized mean differences in generic *E. coli* counts and colonic pH were analyzed using Likelihood and Bayesian approaches. Due to significant between-study variation, a random effects model was fitted for each outcome assuming alternate underlying distributions. Sensitivity analyses were conducted by eliminating one study at a time from the analysis.

Results: Five studies involving 181 presumably uninfected cattle were included in the meta-analysis. Based on the best estimate from the aggregate studies, diets consisting of roughage alone, roughage and molasses, and modified grain diets reduced generic *E. coli* counts by -3.08, -2.79, and -1.09 log/CFU, respectively and increased colonic pH by 4.67, 3.64 and 2.62 units, respectively.

Significance: Meta-analysis can be a useful tool in summarizing the effectiveness of existing food safety interventions. Given the conflicting research on the effect of cattle diet on fecal shedding of STEC and the small sample sizes in existing studies, a meta-analysis is warranted.

P3-181 Microbiological Hazard Analysis of Mulberry Farms at the Harvest Process

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Introduction: Mulberry fruits have abundant nutrients and anthocyanin content. So interest in mulberry fruits has increased as a raw ingredient in health functional food industry.

Purpose: This study was conducted to investigate the microbial hazards on mulberry fruits at the harvesting process in farm.

Methods: Samples (n = 63) were collected from environments (soil and irrigation water), harvest baskets, personal hands and berries (before harvesting, after harvesting, and after freezing) on three farms in Jeonbuk Province. We analyzed sanitary indicator bacteria from the collected samples (total aerobic bacteria, coliform bacteria and *E. coli*).

Results: Total aerobic bacteria were detected at the level of 5.78 - 8.27 log CFU/g from berry samples and ND - 7.14 log CFU/g (or ml, 100 cm^2, hand) from environments and postharvest facilities. Coliform bacteria were detected at the level of ND - 3.83 log CFU/g from berry samples and ND - 4.38 log CFU/g (or ml, 100 cm^2, hand) from environments and postharvest equipment. As a result of the qualitative analysis, *E. coli* were detected from 21 samples (33%) of 63 samples.

Significance: According to the results, the microbial population on the mulberry was higher than other kinds of berries. Therefore, it is necessary to prevent cross-contamination of foodborne pathogens.

P3-182 Contamination Patterns of *Listeria monocytogenes* in a Frozen-vegetable Processing plant

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❖ ***Developing Scientist Competitor***

Introduction: *Listeria monocytogenes* is a foodborne pathogen that can prevail inside frozen-vegetable processing environment. To understand contamination patterns throughout the entire process is necessary to identify sources and pathways of contamination to generate effective control and prevention strategies to avoid contamination of final product.

Purpose: The main goal of the present study was the molecular characterization of *L. monocytogenes* strains isolated from a frozen-vegetable processing plant to determine contamination pathways of the pathogen.

Methods: Six samplings were conducted during one year in a frozen-vegetable processing plant. A total of 574 samples (contact-surfaces, non-contact surfaces and product) were collected and *L. monocytogenes* was detected in 49 samples (8.54%). From positive samples, 61 strains of *L. monocytogenes* were isolated and subtyped using pulse field gel electrophoresis (PFGE) technique following the CDC standardized PulseNet protocol using ApaI as the restriction enzyme. PFGE patterns were analyzed and a dendrogram UPGMA (unweighted pair group method with averages) was constructed using Dice coefficient method with a 1.0–1.5% tolerance window using PAST program.

Results: *L. monocytogenes* isolated strains were differentiated in 11 pulsotypes and were identified with letters from A to K. Pulsotype B was found in 39 strains (63.93%) recovered from all samplings. The dendrogram showed that pulsotype C (1.64%), D (4.92%), and E (3.28%) were genetically related with pulsotype B. The persistent pulsotypes distribution inside the processing plant suggests a cross-contamination between product and contact surfaces (conveyor belts, dispenser) located at the packing area. Additionally, these pulsotypes were found on non-contact surfaces (strainer, floor, forklift skates, waste collector).

Significance: *L. monocytogenes* pulsotype B strains and related pulsotypes are established inside the frozen-vegetable processing plant. Strains characterization by PFGE technique allowed the identification of` the main sources and contamination pathways of the pathogen. This information should assist processors to implement effective *L. monocytogenes* control methods.

P3-183 Behavior of *Escherichia coli* in Field-inoculated Sweet Onions during Conventional Curing

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Introduction: In arid Western growing regions, bulb onions are harvested after uprooting and subsequent drying (curing) in the field for 2 - 3 weeks. The impact of conventional curing practices on the survival of foodborne pathogens introduced through contaminated irrigation water is not well characterized.

Purpose: To evaluate the survival of surrogate *Escherichia coli* on overhead-inoculated sweet onions during conventional field curing.

Methods: Two days after the final irrigation, sweet onions (*Allium cepa* var. *cepa* Walla Walla Sweet) (4-month-old transplants, i.e., 6-month-old plants) were overhead-inoculated with a suspension of non-pathogenic rifampicin-resistant *E. coli* (PTV 353, PTV 354, PTV 355) using a backpack sprayer. One week later, plants were lifted, roots were undercut, and bulbs were placed on the soil surface (cured) for another 2 weeks. *E. coli* was recovered from individual onions bulbs (n = 10 - 40 per time point) by massaging and shaking in 0.1% peptone for 30 s. *E. coli* survivors were enumerated on tryptic soy agar with rifampicin and cycloheximide and CHROMagar ECC with rifampicin or by a most-probable-number method. When counts were below the limit of detection, samples were enriched in tryptic soy broth with rifampicin.

Results: *E. coli* populations declined from 5.2 ± 0.3 to 3.8 ± 0.5 log CFU/onion plant within 2 h after inoculation. At subsequent sampling times (≥ 8 h), the amount of *E. coli* recovered from each onion was highly variable (range at 8 h: 1.9 to 6.9 log CFU/onion). At the end of curing (22 d postinoculation), *E. coli* was recovered from 50% of the onions (32% of samples ranged from 3.1 to 7.0 log CFU/onion). *E. coli* was not detected on any uninoculated onions.

Significance: When applied to onion plants (preharvest) by overhead application, *E. coli* can survive on sweet onion bulbs throughout the duration of conventional curing in the field.

P3-184 Efficacy of Conventional Curing Practices to Reduce Generic *E. coli* and *Salmonella* on Dry Bulb Onions

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Introduction: Implementation of the Food Safety Modernization Act (FSMA) has placed increased emphasis on microbial risks of irrigation water. The Treasure Valley area of eastern Oregon and western Idaho has the highest yield of dry bulb onions in the country. Unfortunately, their irrigation water is often of poor microbiological quality.

Purpose: Determine efficacy of conventional curing practices on reducing generic *E. coli* and *Salmonella* in dry bulb onions and soil to assist with mitigation strategies to comply with FSMA regulations.

Methods: Spanish yellow dry bulb onions (*Allium cepa* var. Ovation) were grown in two soil types from onion farms: silt loam (Treasure Valley) and muck (Willamette Valley). Onions were grown in the greenhouse and irrigated with contaminated water (200 ml) containing generic *E. coli* and *Salmonella* spp. (3.47 log CFU/ml) every 2 - 3 days. At maturity, irrigation was stopped for 2 weeks followed by two weeks of curing. Onion samples were collected, rinsed, and massaged with 0.1% peptone water (1:1) while soil samples (100 g) were mixed with 0.1% peptone water. Serial dilutions were plated onto Hektoen Enteric (HE) Agar and selectively enumerated following incubation (37°C, 24 - 48 hours) and as microbial levels decreased, a most-probable-number (MPN) was used in lieu of plating.

Results: The irrigation period resulted in a final contamination level of 3.69 ± 0.34 log CFU/g onion of both *Salmonella* and generic *E. coli*. On day 124, 13 days after irrigation, generic *E. coli* and *Salmonella* spp. were reduced to < 1 CFU/g. *E. coli* and *Salmonella* spp. levels were stable throughout the remainder of the curing process.

Significance: Reduction of generic *E. coli* and *Salmonella* levels in onions during conventional curing demonstrates the low risk of contaminated irrigation water used in onion production. This data supports extended irrigation-to-harvest intervals as an effective strategy to mitigate risk associated with irrigation water.

P3-185 Quantification of Bacterial Transfer from Cucumber Skin to Flesh and Peeler during Peeling

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❖ ***Developing Scientist Competitor***

Introduction: Fresh fruit and vegetables have been recognized as a source of infection in many foodborne disease outbreaks. In 2013, a multistate outbreak of *Salmonella* linked to cucumber imported from Mexico occurred. Microorganisms on the surface of cucumbers can contaminate interior flesh during slicing or peeling.

Purpose: Little is known about the degree to which bacteria can transfer from the surface of fresh produce items during peeling. This study quantified the transfer of the pathogen surrogate *Enterobacter aerogenes* from the surface of fresh cucumber to flesh during peeling.

Methods: A food-grade strain of nalidixic acid resistant *E. aerogenes* that has similar attachment characteristic to *Salmonella* was grown in 10 ml of tryptic soy broth containing 50 µg/ml nalidixic acid at 37°C for 24 h and re-suspended in phosphate-buffered saline after centrifugation. The cucumber was surface inoculated with 10 ml of *E. aerogenes* (~10^9 CFU/ml) in a zip top plastic bag by massaging for 30 seconds. Percent transfer was determined between the cucumber skin and the peeled flesh and between the cucumber skin and the peeler over 24 h.

Results: Transfer of *E. aerogenes* from inoculated cucumber skin to flesh and peeler occurred during peeling, although a majority of *Enterobacter* remained on the peel. The percent transfer of *Enterobacter* from cucumber skin to flesh during peeling was between 0.52 to 7.56% and transfer rate decreased as drying time after inoculation was extended to 24 h. The percent transfer to the peeler ranged from 0.11 to 4% but did not change in a consistent pattern. A statistically significant difference was only observed in freshly inoculated cucumber at 0 h ($P < 0.0001$ and $P = 0.0004$ for flesh and peeler, respectively).

Significance: This study quantifies the effect of peeling on removal of bacteria from the surface of cucumbers, and subsequent transfer to interior flesh and peeler.

Poster

P3-186 *Listeria monocytogenes* Transfer during Slicing of Cucumbers, Zucchini Squash and Onions as Impacted by Product Density

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❖ ***Developing Scientist Competitor***

Introduction: Computed tomographic (CT) imaging can be used to indirectly assess the density and internal structure of fruits and vegetables, which has been hypothesized to impact the transfer rate of foodborne pathogens during slicing.

Purpose: Our objective was to assess the relationship between produce density, as determined by a CT scanner, and transfer rates for *Listeria monocytogenes* during mechanical slicing of cucumbers, zucchini squash and onions.

Methods: CT scans were performed using a GE BrightSpeed™ Elite CT Scanner (General Electrics Healthcare, Buckinghamshire, UK). Two-dimensional CT images were acquired every 0.625 mm, at a voltage and current of 120keV and 240mA, respectively. Mean densities for each product were calculated from the CT images using MATLAB V2012a. In the slicing experiments, the products were dip-inoculated in a 3-strain avirulent *L. monocytogenes* cocktail (M3, J22F and J29H) to contain ~ 5 log CFU/g, air-dried and sliced using a NEMCO hand slicer to contaminate the slicer. Thereafter, *Listeria* transfer from the slicer to an uninoculated product of the same type was assessed by generating 15 additional slices. The 1st and 15th slices were collected and analyzed for *Listeria* by surface-plating on Modified Oxford Agar.

Results: The 1st and 15th slices of zucchini yielded average *L. monocytogenes* populations of 3.9 and 3.3 log CFU/g, respectively, which were significantly higher ($P > 0.05$) than onions (3.7 and 1.6 log CFU/g) and cucumbers (3.5 and 2.2 log CFU/g). Based on the CT scans, the mean density of zucchini (0.491 g/cm^3) was significantly lower ($P < 0.05$) than onions (0.656 g/cm^3) and cucumbers (0.604 g/cm^3), indicating that bacterial transfer is inversely related to product density.

Significance: The extent of cross-contamination of fresh produce during slicing correlated with product composition. These findings should prove useful in developing improved predictive models for bacterial transfer and expanding current risk assessments across a wider range of products.

P3-187 The Response of Verotoxin-producing *Escherichia coli* (VTEC) to Cold Stress on Plants and Fresh-cut Romaine Lettuce

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Introduction: Verotoxin-producing *Escherichia coli* (VTEC) have emerged as important foodborne pathogens with the majority of non-O157 VTEC infections attributed to six serotypes: O26, O45, O103, O111, O121 and O145. Compared to O157, little is known about the fitness and stress response in non-O157 serotypes rendering it difficult to draw conclusions about their behavior under adverse conditions.

Purpose: The objective of this study was to evaluate the impact of cold stress on the behavior of non-O157 VTEC on Romaine lettuce plants (16°C) and fresh-cut lettuce (4°C).

Methods: In duplicate trials, nine week old Romaine lettuce (*Lactuca sativa* cv. Parris Island) plants (n = 3, average weight 145 g) were spot inoculated with 10^9 CFU of VTEC serotypes O26, O44, , O103, O111, O145 and O157 and maintained at 16 + 2.0°C for 7 days in a controlled environment chamber. Plants were then harvested and leaves were cut into 5 mm strips across the midrib and stored in PD961EZ film bags at 4°C for 14 d. Samples (10 g) were taken on day 0, 3, 7, 10 and 14 and bacterial populations were determined by serial dilution plating on MacConkey agar.

Results: On Romaine lettuce plants at 16°C, serotype O103 was the most resistant to cold stress with populations increasing 0.67 log CFU/g over 7 d. Survival was variable ($P < 0.05$) among serotypes on fresh-cut lettuce stored at 4°C with a significant decline ($P < 0.05$) in numbers observed in serotypes O26 and O145 with 3.27 and 3.84 log CFU/g recovered on day 14, respectively.

Significance: Results indicate the variability in response to cold stress among VTEC serotypes. Specific information regarding the effects of cold stress on VTEC survival on leafy greens is essential to develop science-based food safety guidelines for the produce industry.

P3-188 Fate of *Salmonella enterica*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* in Mung Bean, Alfalfa and Radish Seed Stored at Three Levels of Relative Humidity

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Introduction: Recurrent outbreaks of foodborne illness associated with the consumption of sprouted vegetables suggest that enteric bacterial pathogens can persist on seed for extended periods of time. Little is known about factors that affect the survival of enteric bacteria during seed storage.

Purpose: The purpose of this work was to determine the influence of relative humidity on the survival of *Salmonella enterica, Escherichia coli* O157:H7 and *Listeria monocytogenes* during storage of mung bean, alfalfa and radish seed.

Methods: Mung bean, alfalfa and radish seed were inoculated with fresh cultures of *S. enterica, E. coli* O157:H7 and *L. monocytogenes* to achieve initial populations of 4 log CFU/g. The inoculated seeds were divided into three lots and were placed in desiccator jars containing saturated salt solutions to achieve relative humidity levels of 11, 51 and 75% at 21°C. Residual populations of each pathogen were estimated on selective media and by selective enrichment over a period of 16 weeks.

Results: Populations of each pathogen were reduced by 1 - 2 log CFU/g after 16 weeks at 11% RH. In contrast, selective enrichment was required to detect *S. enterica, E. coli* O157:H7 and *L. monocytogenes* in all seed types held at 51% RH. *E. coli* O157:H7 was not recovered from mung beans after 16 weeks at 75% RH.

Significance: The findings of this study indicate that the viability of *S. enterica, E. coli* O157:H7 and *L. monocytogenes* on sprouted vegetable seeds is enhanced by storage at low RH.

P3-189 Comparative Analysis of Growth Kinetics of *Listeria monocytogenes* Lineages I and II in Chopped Red Cabbage

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❖ ***Developing Scientist Competitor***

Introduction: In recent years, *Listeria monocytogenes* has been implicated in human foodborne illness outbreaks associated with fresh produce items. *L. monocytogenes* outbreaks are attributed to two main lineages (LI and LII). The majority of human Listeriosis is caused by LI strains, yet LII strains are more likely to be recovered from food and food processing environments. There is a lack of information reported on the growth kinetics of these two lineages in produce.

Purpose: To compare the persistence and growth kinetics of two *L. monocytogenes* lineages on freshly chopped red cabbage using predictive modeling.

Methods: Red cabbage was chopped into 100 g portions and inoculated with ca. 10^4 CFU/g of a cocktail of either LI (F2365, H7858, R2-503) or LII (LS814, J1-101, J1-067) antibiotic-resistant strains. Samples were stored in deli-style containers at 5°C or 10°C for 14 days, or 25°C for 7 days. At various intervals, samples were stomached and plated onto PCA with appropriate antibiotics. Data were modeled using DMFit from Combase and the USDA Pathogen Modeling Program. A *P*-value of less than 0.05 was considered significant.

Results: The growth rates ((log CFU/g)/h) of the *L. monocytogenes* LI cocktail in red cabbage were significantly higher at 5°C (0.006 ± 0.003), 10°C (0.017 ± 0.004), and 25°C (0.051 ± 0.017) than the LII cocktail according to the predictive models. The maximum populations (log CFU/g) attained by LI were also significantly higher at 5°C (3.95 ± 0.035), 10°C (4.23 ± 0.035), and 25°C (4.38 ± 0.049) than LII. Secondary models using the Ratkowsky equation presented r^2 values of 0.99 and 0.90 for LI and LII, respectively.

Significance: The information obtained from this study will aid in elucidating how *L. monocytogenes* survives and grows in chopped produce and the reasons why *L. monocytogenes* LI strains are overrepresented in human foodborne Listeriosis cases.

P3-190 Hygienic-Sanitary Condition of Tomatoes Marketed in the City of Rio de Janeiro Brazil

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Introduction: Consumer demand for healthy, fresh like and easy to prepare products is continuously increasing nowadays. So it is important to verify the microbiology of the tomato to determine the shelf life and/or temperature abuse that may influence its quality.

Purpose: To verify the microbiological hygienic-sanitary conditions on the surface of tomatoes collected at the final point of consumption in the city of Rio de Janeiro- Brazil

Methods: Two hundred fifty-one tomato samples were collected from supermarkets and open markets located in the city of Rio de Janeiro. Aerobic plate count, yeast/molds, total coliforms and *Escherichia coli* counts were performed, using the Food and Drug Administration's Bacteriological Analytical Manual (FDA- BAM) procedures.

Results: In relation to the hygienic-sanitary condition, 3 samples (1.2%) were confirmed as *Escherichia coli*. The total counting of mesophilic aerobic microorganisms was above 6 log CFU/g in 28% of the analyzed samples, as well the counting of total coliforms in 7.6% of tomatoes. The mold and yeast counts present values greater than 4 log CFU/g in 66% of the samples.

Significance: The data presented showed a high count of yeast and mold on the surface of tomatoes analyzed which can lead to a more rapid deterioration of the product. Only three samples showed the presence of *E. coli*, indicating possible fecal contamination.

P3-191 Influence of Retail Crisping on Cross-contamination of Romaine Lettuce

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Introduction: Contamination of fresh fruits and vegetables can occur during any processing or handling step from the farm to the table. Many retailers implement crisping, a rehydration process in which fresh produce is soaked in cool or warm water and then placed in a cooler to drain excessive water. Cross-contamination of product can occur during crisping the result of contaminated equipment, product, and workers.

Purpose: Crisping of lettuce was conducted with and without a sanitizer to determine control of *E. coli* O157:H7 in crisping water and prevention of cross-contamination.

Methods: Romaine lettuce (2 heads) were inoculated with *E. coli* O157:H7 and were soaked with six non-inoculated heads in 21°C water for 5 min. Treatment included potable tap water and tap water containing 30 ppm chlorine. The lettuce heads were removed and processed for microbiological analysis. Water samples were obtained immediately prior to and after treatment for determining pH and for microbiological analysis. The experiment was competed three times.

Results: *E. coli* O157:H7 was not detected following crisping in water containing 30 ppm chlorine. Based on enrichment of lettuce samples cross-contamination occurred in water alone and water plus 30 ppm chlorine. Reductions of 2 and 1.5 log CFU of *E. coli* O157:H7 and commensal flora, respectively, occurred on inoculated lettuce treated with 30 ppm chlorine.

Significance: Cross-contamination can occur during crisping. A reduction in pathogenic and native flora on inoculated lettuce can be achieved when a sanitizer is used. Including a sanitizer in the crisping water will control the level of foodborne pathogens and commensal/spoilage bacteria in the water. Addition of a sanitizer to water used for crisping is recommended and may improve product shelf life and reduce human health concerns.

P3-192 Occurrence of Extended-Spectrum Beta-lactamase- and AmpC Beta-lactamase-producing *Enterobacteriaceae* from Domestic and Imported Fresh Produce

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Introduction: Outbreaks of illnesses caused by contaminated produce have been reported over the last decade. However, little information is available on the occurrence of extended-spectrum and AmpC beta-lactamase-producing *Enterobacteriaceae* in produce. ESBL and AmpC enzymes are of great concern because they impede the treatment of diseases with beta-lactam antibiotics.

Purpose: The aim of this study was to investigate whether domestic and imported fresh produce play a role as a carrier of ESBL/AmpC-producing *Enterobacteriaceae*.

Methods: Three hundred and sixty produce were purchased and analyzed for ESBL/AmpC-producing *Enterobacteriaceae. Enterobacteriaceae* isolated from produce was screened with a cefotoxime (1 mg/ml). Selected isolates were screened for ESBLs and AmpC production by Kirby Bauer disk diffusion method and suspected isolates were subjected to double disk synergy. ESBL was tested by using cefpodoxime and ceftriaxone antibiotic discs. Amoxicillin plus clavulanic acid in combination with cefpodoxime and ceftriaxone were used. Cefoxitin discs and Amp C disk tests were used to test for Amp C producers and confirmation of presumptive isolates, respectively. Colonies from produce were also tested for resistance with other antibiotics: cefoxitin, amikacin, azrithromycin, erythromycin, streptomycin, vancomycin, cefotaxime, and ciprofloxacin.

Results: Twenty-five isolates (16.6%) and twelve (4.6%) isolates out of 150 were ESBL and AmpC producers, respectively. Our results indicate that domestic and imported produce were positive for ESBL/AmpC-producing *Enterobacteriaceae*. Majority of the isolates (82%) demonstrated resistance to streptomycin (95.3%), erythromycin (80%), vancomycin (63.5%), and were statistically higher ($P < 0.05$) than in other antibiotics evaluated. Other antibiotics indicated resistance of less than 20%.

Significance: These findings suggest that domestic and imported produce in grocery stores is contaminated with ESBL/AmpC- producing *Enterobacteriaceae*. Improving hygiene, limited use of antibiotics, and post-harvest controls for foodborne pathogens in farms would be a measure to control the dissemination of ESBL/AmpC- *Enterobacteriaceae*.

P3-193 Identification of Risky Food Safety Practices and Microbial Assessment of Produce at Southwest Virginia Farmers' Market

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❖ ***Developing Scientist Competitor***

Introduction:

The number of farmers' markets has increased significantly over the last decade. Fresh produce constitutes 82% of the food sold at farmers' markets. Between 1998 and 2008, produce accounted for 46% of the 4,589 foodborne illness outbreaks linked to a specific commodity. The high percentage of produce-related foodborne outbreaks and growing number of farmers' markets selling fresh produce highlights the need for a food safety focus within these markets.

Purpose:

The purpose of this study was to identify current food safety practices and assess the microbial quality of fresh produce sold at Southwest Virginia farmers' markets.

Methods:

Five farmers' markets across Southwest Virginia were visited from May – October 2014. Observational data collection was employed via a secret shopper model to identify and quantify behaviors considered to be high risk for food contamination. The microbial quality of produce sold at these markets was also evaluated. Nine different commodities were collected (385 total samples) from five markets and a chain grocery store.

Results:

Eighty-five percent of produce vendors had no temperature control for cut produce and no vendors used a thermometer. Additionally, there were significant differences ($p = 0.0130$) in the total aerobic bacteria from produce at farmers' markets and commercial produce with an average of 6.73 and 6.16 log CFU/g, respectively. There was no significant difference in the quantity of coliforms or *Enterobacteriaceae*isolated from produce at farmers' markets (4.91 and 6.01 log CFU/g respectively) and commercial samples (3.99 and 5.74 log CFU/g respectively) ($p = 0.2787$ and $p = 0.2478$, respectively).

Significance:

Farmers market vendors and managers could be benefit from food safety training tailored to their needs. Hands-on assistance specifically related to temperature control and sanitation could positively influence food safety behavior and reduce risk.

P3-194 Farmers' Market Booths: Does the Layout Affect Microbial Cross-contamination?

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Introduction: The most widely used layout and design for farmers markers in the U.S. are U-Shaped (US), L-Shaped (LS), and Square Shaped (SS) arrangements. Microbial (bacterial and viral) cross-contamination between produce items may occur.

Purpose: The purpose of this study was to determine if the risk of microbial cross-contamination between produce and non-produce items could be decreased at farmers' markets by vendor booth configurations.

Methods: Three common farmers' market configurations were identified from USDA guidelines (US, LS, and SS). Eighteen groups, consisting of three volunteers each (n = 108) participated in an LS, a US, or a SS mock markets. Cross-contamination patterns were studied using fluorescent-compound (FC; Glo Germ® lotion). A standard curve was created by dissolving known amounts of the FC into 200 proof ethanol and measuring

absorbance at OD_{370}. One gram of FC was spread on both hands of each participant. Once the participants walked through the mock markets, produce (tomatoes, green peppers, apples, onions, and cantaloupe) and non-produce items (plastic and glass containers) were swabbed using a 25 cm^2 plastic template. Swabs were placed in 7 ml of ethanol and vortexed for 20 s prior to analyzing absorbance at OD_{370} to enable quantification.

Results: The US market demonstrated the lowest level of average absorbance (OD_{370} = 0.049), with produce booths separated by non-produce booths. The highest average absorbance levels were observed in LS (OD_{370} = 0.128) and SS markets (OD_{370} = 0.118) when produce and non-produce items separated. Overall, the US market configuration demonstrated the lowest probability of cross-contamination.

Significance: This study indicates that the layout of farmers' markets can play a critical role in the risk of cross-contamination of produce and non-produce items. Overall, layout recommendations for farmers' markets should be considered to improve public health by enhancing the safety of fresh produce sold at market.

P3-195 Survival of *E. coli* on Microgreens during Growth

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Introduction: Microgreens, as yet, have not been associated with any foodborne illness outbreaks. Microgreens are recognized as being nutritional powerhouses and are gaining in popularity and filling the niche previously occupied by sprouts. However, they are still mainly grown on a very small scale and they do share some characteristics of sprouts; primarily youth. The vulnerability of plants to pathogen colonization seems to be greatest during and immediately following germination.

Purpose: This study was undertaken to investigate the persistence of enteric bacteria on or within seedlings.

Methods: Mizuna seeds inoculated with a 3-strain cocktail of *Escherichia coli* MW416, MW423, and MW425, strains known to have superior attachment to plant tissue, were grown in peat-based growth medium for up to 14 days. Plant trays were covered during germination and uncovered and exposed to light on different days to determine the impact of light and its consequent effects on the metabolic processes of the microgreens on *E. coli* survival. Seedlings (2 to 6 g) from each treatment group were harvested by cutting stems above the soil surface each sampling day and plated.

Results: Total *E. coli* populations declined over each of the three 2-week test periods. While light initially had a modest effect on *E. coli* population reduction, as treatments were exposed to light for more than one day, the rate of decline stabilized. *E. coli* cells on surface sterilized seedlings increased from extremely low levels to over 4 log CFU *E. coli* cells/g of plant tissue indicating growth of internalized cells. Scanning electron microscopy was used to observe the location of *E. coli* cells within the plant tissue.

Significance: This study is an important step towards learning what factors are involved in the die off of enteric pathogens frequently seen in plants and to determine whether the decline is sufficient to result in safe product.

P3-196 Proliferation of *Listeria monocytogenes* during Microgreen Production

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Introduction: Microgreens are young, tender vegetables that are produced in controlled indoor facilities. Although microgreens have becoming increasingly popular with consumers in recent years, there is a lack of data regarding the potentials of the microbial contamination by bacterial pathogens during microgreen production.

Purpose: The objective of this study was to examine the survival and proliferation of seed-borne *Listeria monocytogenes* and other native microflora on microgreen and plants and production media.

Methods: Radish seeds inoculated with *L. monocytogenes* were used to grow microgreens using a soil substitute and hydroponic production systems. The spatial and temporal distribution of mesophilic aerobic bacteria (APC), coliforms and inoculated *L. monocytogenes* were monitored during the production cycles by selective plating.

Results: During the course of microgreen growth for 10 days, *L. monocytogenes* counts on the seed coats increased by 0.7 to 1.3 log in comparison to the initial inoculation on the seeds, in hydroponic and soil substitute based production systems. Similar increases were observed on the edible portion of the microgreens. Seed coats, roots, and cotyledons were most heavily contaminated by *L. monocytogenes*. APC and coliform counts were generally stable and in most cases fluctuated less than 1 log during microgreen growth.

Significance: These results provide important information on the potential of *L. monocytogenes* proliferation in microgreen production and demonstrated the importance to minimize bacterial contamination of seeds for microgreen production.

P3-197 Survival and Transfer of Murine Norovirus within a Hydroponic System during Kale and Mustard Microgreen Harvesting

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❖ ***Developing Scientist Competitor***

Introduction: Hydroponically-grown microgreens are gaining in popularity, but there is a lack of information pertaining to the microbiological safety of microgreens. The potential risks associated with virus uptake through a hydroponic system have not been studied to date.

Purpose: This study aims to evaluate the capability of the huNoV surrogate (murine norovirus (MNV)) internalized from roots to edible tissues of microgreens, and virus survival in re-circulated water without disinfection.

Methods: After kale and mustard seeds growing on hydroponic pads (7-days, harvest at days 8 - 12), edible tissues (10 g) were cut 1 cm above the pads followed by collecting two pieces (3 × 3 cm^2) of pads containing only roots. Samples were collected from a newly contaminated system (circulated water inoculated with 3.54 ± 0.49 log PFU/ml MNV on Day 8), and from a previously contaminated system (upon harvest completion, a new set of microgreens was grown without disinfection). Samples were quantified by plaque assay and real-time RT-PCR, and data (3 replicates/3 samples each) were analyzed by ANOVA on JMP.

Results: The behavior of MNV was similar in kale and mustard microgreens ($P > 0.05$). Constant high levels of viral RNA were present in edible tissues (2.18 to 2.79 log copies/g) and roots (3.35 to 4.16 log copies/cm^2) from days 8 - 12; relatively low levels (1.49 to 0.83 log PFU/g and 1.19 to 0.20 log PFU/cm^2) of infectious viruses were found with a decreasing trend over time. Cross-contamination occurred easily; MNV remained infectious in previously contaminated hydroponic systems for up to 12 days (2.26 to 1.00 PFU/ml), and was translocated in edible tissues (1.10 to 1.61 PFU/g) via roots (0.34 to 0.71 PFU/cm^2). An uninocuated control system remained negative over the sampling period.

Significance: Viruses could be re-circulated in water, taken up through roots, and transferred to edible tissues. Ease of contamination shown here reinforced the need for proper sanitation.

P3-198 Potential Zoonotic Risks in Aquaponics

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❖ ***Developing Scientist Competitor***

Introduction: Aquaponics is the integration of aquaculture and hydroponics that is now being used as a model for sustainable food production. In a recirculating aquaponic system (RAS), liquid effluent rich in plant nutrients derived from fish manure, decomposing organic matter, and nitrogenous waste excreted from fish fertilizes hydroponic beds, providing essential elements for plant growth. Since waste products are used in this system, food safety concerns are on the rise.

Purpose: The purpose of this study was to assess the aquaponics industry by reviewing published manuscripts to better understand the zoonotic risks in this type of system and to determine areas of which still need to be investigated.

Methods: Search engines, for example PubMed and Web of Science, were used to compile the already published studies that have investigated the potential zoonotic risks in aquaculture systems. Key words to find these publications were, "aquaponics," "*Salmonella,*" "*Escherichia coli* O157:H7," "*Escherichia coli,*" "fish microbiota," "enteric commensal bacteria," and "zoonotic pathogens."

Results: Due to aquaponic's increasing popularity, food safety and proper harvesting procedures have only recently begun to be addressed for aquaponic producers. Levels of fecal coliforms and pathogenic bacteria, if present in aquaculture systems or fish, most likely originate from warm-blooded animals. It has been shown that fish intestinal flora and the survival rate of zoonotic pathogens can be related to the level of contamination of the water and/or food in their environment. Individuals who come in contact with fish or fish environments could be at risk for zoonotic infections with the possibility of antibiotic resistant.

Significance: Knowledge resulting from this investigative search suggests the areas of research that still need to be studied, especially with regards to experimental pathogen survival in aquaponic systems, including the potential for pathogen transfer from fish effluent to edible plant parts via root uptake or cross-contamination.

Poster

P3-199 Evaluation of Consumer Washing Techniques to Reduce Natural Microbiota on the Surface of Whole Cantaloupes

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❖ ***Developing Scientist Competitor***

Introduction: Foodborne illness outbreaks involving fresh produce, including cantaloupe, give cause to re-evaluate consumer produce handling recommendations. Consumers hold the responsibility to ensure that fresh produce is properly washed before serving in order to contribute to the safety and quality of the product prior to consumption. However, the effectiveness of cantaloupe surface washing in home settings for the reduction of surface microbial contamination is known to be negatively impacted by the naturally rough and ribbed surface of cantaloupe which provides physical protection against this type of treatment.

Purpose: This study evaluated four separate consumer washing methods and their efficacy in reducing natural microbiota on whole cantaloupe surfaces.

Methods: Cantaloupes were purchased from a local supermarket and subjected to one of the following four treatments: (1) running water at 25°C, (2) running water at 25°C and brushing, (3) running water at 25°C with dish washing soap and brushing, and (4) water at 100°C, with unwashed melons serving as controls. A 10.2 cm^2 rind sample was obtained from the resting spot of the melon, homogenized by stomaching for 2 min with 0.1% peptone water, serially diluted, then aerobic plate counts (APC) and total coliform counts (TCC) were determined using 3M petrifilm.

Results: Treatment 1 caused a 3-log/cm^2 reduction of APC and a 2-log log/cm^2 reduction of TCC, while treatment 4 resulted in a 2-log/cm^2 reduction for APC and a 1-log/cm^2 reduction for TCC. Both statistically lower ($P < 0.05$) than untreated. For treatments 2 and 3, a 2- and 1-log/cm^2 reduction of APC, respectively, and no reduction of TCC were achieved.

Significance: These results indicate that running water at 25°C and running water at 100°C contributed to a greater reduction of microbiological surface contamination. The use of cleaning aids, such as a brush or soap, did not considerably reduce microbial counts.

P3-200 Standardizing Produce Wash Water Models for Validation of Postharvest Sanitizers

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❖ ***Developing Scientist Competitor***

Introduction: The CDC reports over 45% of foodborne illnesses are produce related, making development of methods to reduce these outbreaks imperative. Adding sanitizing agents at adequate levels to the postharvest washing process has been shown to reduce contamination. Current research has used a variety of model wash water models to validate the efficacy of sanitizers, however, this makes comparing historical research challenging for manufacturers to select appropriate produce sanitizers for their operation. Therefore, development of a standardized model is critical to produce recommendations.

Purpose: Previous research has evaluated factors including Chemical Oxygen Demand and turbidity, however, there is little consistency between approaches. The purpose of this project is to understand what the common characteristics of industrial postharvest wash water are and identify appropriate measurable attributes to create a model system.

Methods: In this study, two types of were profiled: 1) water samples at the end of a postharvest washing 2) lab prepared produce purees diluted to 25 and 50 NTU and autoclaved before being treated with 50 ppm chlorine. Samples were analyzed for free residual chlorine using HACH kit, Total Organic Carbon (TOC), and Oxidation Reduction Potential (ORP).

Results: ORP, TOC, and turbidity results did not indicate correlation in free residual chlorine. For example doubling turbidity caused a change in free chlorine of 0.66 ppm, 1.17 ppm, and 4.00 ppm for the cucumber, yellow squash and red pepper, respectively. The ORP value changes were 90.57 mV, 70.30 mV, and 10.64 mV for the cucumber, yellow squash and red pepper, respectively. The same change in turbidity resulted in a change of TOC of 62.34 mg/l in our cucumber model.

Significance: It is imperative that a standard model be used to make comparisons between different sanitizers to help stakeholders implement best agricultural safety practices. This data suggests that individual models will have to be developed for quantifying sanitizer effectiveness.

P3-201 Application of Bacteriophage Cocktail in Leafy Greens to Control *Salmonella enterica* in Produce Wash Water

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❖ ***Developing Scientist Competitor***

Introduction: Produce is responsible for 46% of all foodborne illnesses in the USA, of which leafy greens is a leading contributor. *Salmonella enterica* causes 19,000 hospitalizations each year, and has been associated with produce. Presently, chlorine based sanitizers are recommended for disinfection of produce, but organic matter interferes with its activity. Bacteriophage treatments are an all-natural and target specific alternative method for pathogen inactivation.

Purpose: The objective of this study was to determine the impact of organic matter on the efficacy of bacteriophage and subsequent reduction of *S. enterica* in romaine lettuce.

Methods: To examine the impact of organic matter, simulated wash water was made by blending, straining, and diluting romaine lettuce into solution with turbidities of 0, 25, 50, and 100 NTU. Bacteriophage activity was measured after incubation with wash water solutions for 0, 30, 60, and 300 min at 20°C. To examine bacteriophage efficacy against *S. enterica*, 9 log CFU/ml of *S. enterica* was added to 0 and 100 NTU solutions and incubated with 9 log PFU/ml bacteriophage for 0 and 135 min at 37°C.

Results: All incubation studies with bacteriophage and organic load levels yielded no statistical differences ($P < 0.05$). In 100 NTU romaine lettuce solutions with bacteriophage and *S. enterica*, bacteriophage grew 1.84 + 0.12 PFU/ml, the 0 NTU control grew 1.36 + 0.12 log PFU/ml. *S. enterica* was reduced 1.81 + 0.07 log CFU/ml in control solutions and 1.79 + 0.10 log CFU/ml in 100 NTU solutions within 135 min.

Significance: Results show that unlike chlorine, bacteriophage remain viable in wash water with high organic load. Bacteriophage also delivered a reduction in pathogen load that is comparable to those reported for chlorine. Our data suggests that bacteriophage treatments represent a potential alternative to chlorine for on-farm disinfection of produce.

P3-202 Assessing the Effect of Chlorine Concentration on the Transfer of *Salmonella* in the Wash Water for Production of Minimally Processed Lettuce

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❖ ***Developing Scientist Competitor***

Introduction: Washing is a critical step in the production of minimally processed vegetables where a significant reduction in the microbial load may be achieved. However, wash water can be a point of cross-contamination if the water becomes contaminated by pathogens.

Purpose: This study measured the transfer of *Salmonella* from inoculated to non-inoculated portions of iceberg lettuce during washing in water containing chlorine at different concentrations, simulating different production scenarios.

Methods: The experiments were carried out with portions of iceberg lettuce (100 g) inoculated with *Salmonella* at ~6 log CFU/g. The *Salmonella* strain used in the study was modified to express Green Fluorescent Protein to facilitate recovery. Two scenarios were investigated: *i)* washing of one inoculated lettuce portion in non-chlorinated water followed by washing of several non-inoculated portions in the same water and *ii)* washing of one inoculated lettuce portion in chlorinated water (5, 10, 20, 30, 40, 50 and 250 mg/l) followed by washing of several non-inoculated portions in the same water.

Results: After washing the inoculated lettuce portion in non-chlorinated wash water, the population of *Salmonella* in the water was ~4.8±0.1 log CFU/ml. Washing of non-inoculated lettuce portions in this contaminated non-chlorinated wash water resulted in counts of *Salmonella* around 4.1±0.1 log CFU/g. Chlorinated water reduced *Salmonella* concentration by 0.5 to 2.0 log CFU/g and was able to prevent *Salmonella* transfer when at least 10 mg/l of chlorine was used. When the concentration was 5 mg/l, *Salmonella* in non-inoculated lettuce was below quantification (<100 CFU/g), but was detected by enrichment (e.g., at least 1 CFU/25g).

Significance: Our findings show the importance of using chlorine at proper concentrations in the wash water for production of minimally processed lettuce. To prevent cross-contamination with *Salmonella*, the concentration of chlorine should be above 10 mg/l.

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P3-203 Efficacy of Peroxyacetic Acid in Float Tank Water in Reducing Native Microbiota during Commercial Walnut Hulling

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Introduction: During postharvest hulling, the water-filled tanks commonly used to separate inshell walnuts from rocks and soil are potential vehicles for microbial cross-contamination.

Purpose: To evaluate the efficacy of a peroxyacetic acid (PAA)-based sanitizer against native microbiota in float tank water and on walnuts collected during commercial walnut hulling.

Methods: Walnuts were commercially hulled with either water (control) or PAA (25 ppm) in the float tank. Duplicate individual samples of water and walnuts from each of three trials (n = 6) were neutralized with Dey/Engley broth at 0, 5, 10, 15, 20, and 25 min of hulling, and plated onto tryptic soy agar (aerobic plate count [APC]) and onto CHROMagar ECC (coliforms and *E. coli*). Total dried solids, PAA and peroxide concentrations, pH, temperature, and oxidation/reduction potential of the float tank water were determined throughout hulling.

Results: Populations of APC, coliforms, and *E. coli* were 5.6, 5.0, and 2.8 log CFU/ml, respectively, in the unused float tank water and 6.7, 5.5, and 4.3 log CFU/g, respectively, on the pre-hulled inshell walnuts. Populations of coliforms (4.3 - 5.7 log CFU/ml) and *E. coli* (1.8 - 3.1 log CFU/ml) were significantly lower ($P < 0.05$) in the PAA-containing float tank water than in the control water (5.0 - 6.7 log CFU/ml and 3.4–5.0 log CFU/ml, respectively) at all sampling times but PAA did not have a significant impact on the microbiota of the inshell walnuts or the APC of the float tank water. Within minutes, significant increases in total solids and decreases in PAA and hydrogen peroxide concentrations were observed (15, 10, and 25 min after initiation of hulling, respectively).

Significance: Addition of a PAA-based sanitizer reduced some microbial counts in the float tank but not on the corresponding walnuts; addition of PAA to the float tank should be balanced with an assessment of cost and potential negative impact on walnut quality.

P3-204 Evaluating the Use of Indicator Microorganisms in Assessing the Effectiveness of Chlorine Wash in Fresh Produce Processes

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Introduction: Chlorine has been widely used in controlling cross-contamination during fresh produce washing processes. It is important to identify different approaches for monitoring the efficacy of chlorine washing. The suitability of indicator tests for assessing chlorine effectiveness is still unclear.

Purpose: To determine how indicator microorganisms behave during chlorine inactivation and washing when compared with *E. coli* O157:H7.

Methods: Inactivation of various indicator groups [total viable count (TVC), *Enterobacteriaceae*, total coliforms] and *E. coli* in wash water after exposure to chlorine (0 - 30 ppm) with added organic load (0.1% lettuce juice powder) was determined. Cross-contamination of *E.coli* O157:H7 and the indicators to water was evaluated in 50-ml lettuce washing. Indicator microorganisms enriched from lettuce were co-inoculated with *E. coli* O157:H7 (~5 log CFU) on lettuce, which was washed in 30 ml of water or 1 ppm chlorine. Bacterial survival or transfer to wash water was enumerated by plating on appropriate media.

Results: *E. coli* and total coliforms were nearly eliminated by exposure to 10 ppm chlorine, whereas the TVC was only reduced by ~3.5 logs after exposure to 30 ppm chlorine. *Enterobacteriaceae*, total coliforms and *E. coli* decreased similarly as the chlorine concentration increased from 0 to 5 ppm. Results suggest that the indicator groups, except TVC, showed correlation in chlorine inactivation. In lettuce washing, approximately 3 log CFU/ml was transferred into the water for all indicator groups in the absence of chlorine. With 1 ppm chlorine, less than 30 total CFU were detected for *Enterobacteriaceae*, total coliforms and *E. coli* O157:H7; however, higher levels of TVC (~ 1 log CFU/ml) were detected in some replicates.

Significance: Microbial indicator tests might be useful in assessing effectiveness of chlorine washing for fresh produce.

Poster

P3-205 Prevention of *Salmonella* Cross-contamination between Green Round Tomatoes in a Laboratory Flume System

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Introduction: Harvested green round tomatoes are typically transferred from field bin to packing lines utilizing a flume system containing 150 - 200 ppm hypochlorous acid (HOCl). Preliminary studies indicate that the currently required minimum HOCl concentrations in packinghouse flume systems may exceed necessity.

Purpose: This study evaluated the minimum concentration of HOCl required to prevent cross-contamination of uninoculated tomatoes by *Salmonella*-inoculated tomatoes under clean and organic loading conditions.

Methods: Unwashed green round tomatoes inoculated with rifampicin (rif) resistant *Salmonella* (9 log CFU/tomato) were introduced into a recirculating waterbath along with uninoculated tomatoes. Tomatoes in the wash water were exposed to different HOCl (25, 50, 75, 100 ppm) and organic loading (0, 500 ppm Chemical Oxygen Demand [COD]) levels for 0, 30, 60, 120 s. At each time point, three inoculated and uninoculated tomatoes were placed in separate Stomacher® bags containing tryptic soy broth (TSB) and 0.1% $Na_2S_2O_3$ for *Salmonella* enumeration. If levels were below the detection limit (< 2 log CFU/tomato), tomatoes were enriched in TSB (+rif) at 37°C for 48 h. Each experiment was performed in triplicate (n = 9).

Results: In the absence of HOCl, *Salmonella* was recovered in both the wash water (≥ 3.5 log CFU/ml) and uninoculated tomatoes (≥ 4.5 log CFU/tomato). Presence of HOCl in the wash water reduced *Salmonella* levels on uninoculated tomatoes to below the detection limit (< 2 log CFU/tomato). Enrichment revealed the presence of *Salmonella* on uninoculated tomatoes exposed to 25 and 50 ppm HOCl. However, the use of 75 or 100 ppm HOCl without organic loading, prevented *Salmonella* cross- contamination of uninoculated tomatoes, regardless of the contact time.

Significance: Results suggest that 75 ppm HOCl is sufficient to control *Salmonella* cross-contamination in a model flume system, in the absence of organic loading. The ability of packers to use less sanitizer could reduce associated chemical and disposal costs.

P3-206 Use of Aqueous Ozone in Sanitizing Fresh-cut, Organic Romaine Lettuce (*Lactuca sativa* L.)

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Introduction: In consideration of the present restrictions or standards presented by organic farming, an alternative sanitizer to chlorine would address the demand for consumer safety. It is then imperative that a cheap and environment-friendly sanitizer be investigated and evaluated.

Purpose: The use of aqueous ozone at 125 ppm concentration for sanitizing fresh-cut, organic Romaine lettuce was evaluated against chlorine at 200 ppm concentration and tap water at different time exposures (2, 5, 10 min) and at two incubation temperatures, 10°C and 30°C.

Methods: Log reduction in heterotrophic plate count and *Escherichia coli* count was computed, over-all visual quality rating was evaluated during storage, and microbiological analysis for natural microflora and *Escherichia coli* of incubated samples was conducted. Kruskall-Wallis and Tukey-Kramer tests were used to determine the significant differences between the treatments.

Results: Samples washed with tap water had the least HPC and *E. coli* log reduction which was significantly lower than the log reductions in samples washed with ozone (125 ppm) and chlorine (200 ppm) at any time exposure ($P < 0.05$). Chlorination and ozonation showed similar HPC log reduction at 2 and 10 min time exposure ($P > 0.05$) but all samples washed with chlorine produced better *E. coli* log reduction ($P < 0.05$). All samples stored at 10°C did not exceed the limit of acceptability during the whole storage period. Even so, by the end of the recommended use-by-date, the HPCs of all treatments were already unsatisfactory. On the other hand, no sample stored at 30°C reached the suggested use-by date of 6 days with good OVQR. All samples, at 30°C, regardless of the treatment, exceeded the limit of acceptability by day 2. Similar trend was observed in *E. coli*-inoculated samples.

Significance: These data suggest that aqueous ozone is a very good candidate as a sanitizer for use by organic farmers in the Philippines.

P3-207 Impact of Wash Water Flow Rate on the Extent of *E. coli* O157:H7 Cross-contamination during Postharvest Washing of Cut Lettuce

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❖ ***Developing Scientist Competitor***

Introduction: Maintaining the antimicrobial efficacy of produce washing systems can be challenging due to the dynamic nature of the process. An in-depth understanding of factors influencing performance of produce wash will allow better wash water management.

Purpose: Investigate how water flow rate may affect the extent of *E. coli* O157:H7 cross-contamination during washing of inoculated lettuce in chlorinated water.

Methods: Cut romaine lettuce (8 g) inoculated with 7 log CFU/g of *E. coli* O157:H7 were added into 40 liters of water together with uninoculated lettuce (800 g) and washed for 2 min. Washing trials were performed at 3°C with various levels of chlorine (0, 5, and 10 ppm) and wash water flow rates (fast, medium, or slow by setting the valve pressure at 3.5, 5, or 7 psi, respectively). The degree of cross-contamination was determined by measuring the presence of *E. coli* O157:H7 in wash water and uninoculated lettuce.

Results: In the absence of chlorine, the spread of *E. coli* O157:H7 occurred and resulted in the contamination of wash water and uninoculated lettuce at levels of 3.0 ± 0.0 log CFU/ml and 3.1 ± 0.1 log CFU/g, respectively. At 10 ppm of chlorine, regardless of water flow rate, no *E. coli* O157:H7 was found in wash water or uninoculated lettuce. At 5 ppm of chlorine, while no *E. coli* O157:H7 was found in wash water or uninoculated lettuce samples (0/6) collected during washing at a slow flow rate (valve pressure at 7 psi), cross-contamination of *E. coli* O157:H7 was observed in washing runs operated at higher flow rates, with the pathogen detected in 2/6 wash water and 1/6 uninoculated lettuce samples collected during washing at the fast flow rate (3.5 psi).

Significance: Under certain conditions, wash water flow rate may have an impact on the extent of *E. coli* O157:H7 cross-contamination during lettuce washing.

P3-208 Inactivation of *Bacillus cereus* Spores on Red Chili Peppers by Combined Treatments of Aqueous Chlorine or Chlorine Dioxide and Heat Drying

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Introduction: Chili pepper or chili powder is easily contaminated with various microorganisms from postharvest to distribution and *Bacillus* spp. has been frequently isolated from them.

Purpose: We determined effect of combined treatments of aqueous chlorine or chlorine dioxide and heat drying on inactivation of *B. cereus* spores on red chili peppers.

Methods: Ten samples [half of pepper (*Capsicum annuum L.*)] washed and dried were inoculated with 4 - 5 drops of *B. cereus* spore suspension). Each samples were washed with sterile distilled water, aqueous chlorine (0, 50, 100, 200 ppm) or chlorine dioxide (50, 100 or 200 ppm) for 1 min and air-dried (25 ± 1°C, 47 ± 1% RH). The samples were then heat-dried at 55°C for up to 48 h. During the drying, populations of spores on samples were enumerated and a_w values and color of the samples were also measured every 12 h.

Results: The results showed that initial spore numbers (6.2 log CFU/sample) decreased to 4.4, 4.8, 3.8, 2.9, and 2.7 log CFU/sample immediately after washing with sterile water and 0, 50, 100, and 200 ppm of aqueous chlorine, respectively. After 12 h of drying, the populations of spores on samples washed with 100 or 200 ppm of aqueous chlorine reduced to < 2.0 log CFU/sample. Initial spore numbers (6.4 log CFU/sample) decreased to 4.9, 5.4, 4.3, and 4.1 log CFU/sample immediately after washing with sterile water and 50, 100, and 200 ppm of chlorine dioxide, respectively. Treatments of red chili peppers with aqueous chlorine or chlorine dioxide prior to drying did not cause changes in a_w values and colors of dried chili peppers.

Significance: More rapid reduction rates in population of spores occurred on pepper samples washed with higher concentration of aqueous chlorine, whereas concentrations of aqueous chlorine dioxide did not cause different reduction rates on spore numbers on samples.

P3-209 Bromine-based Biocides for the Control of *Salmonella* on Fruits and Vegetables

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Introduction: *Salmonella* is a pathogen of major concern in the fruits and vegetables industry. Chlorine-based antimicrobial interventions are common in the fruits and vegetables industry but they have several limitations. Bromine-based antimicrobials have been widely used in recreational and industrial water treatment for many years and have recently been approved for use in some food safety applications. Some advantages of bromine-base biocides are: their effectiveness at a wider range of pH values than chlorine, low odor and minimal effect on organoleptic properties.

Purpose: To evaluate DBDMH (1,3-Dibromo-5,5-Dimethylhydantoin), a bromine-based biocide as means to control *Salmonella* on cantaloupes and tomatoes.

Methods: *Salmonella enterica* was grown overnight at 37°C in Luria-Bertani broth. Cantaloupes and Roma tomatoes purchased from local stores were inoculated with 8 log CFU/cm^2 of *Salmonella* and allowed to attach at room temperature for 2 and 3 h, respectively. Simulating a wash tank, cantaloupes or tomatoes were submerged in a biocide solution at room temperature for 5 or 3 min, respectively; the treatments included DBDMH at 300 or 400 ppm (as bromine), chlorine at 300 or 400 ppm (as bromine) and peroxyacetic acid (PAA) at 100 ppm. After treatment, cantaloupes were drained and allowed to dry for 1 h, and 3.14 cm^2 of the surface were taken and homogenized. Tomatoes were washed with distilled water for 1 min and rinsed for microbiological analysis. The solutions were decimally diluted with 1% peptone water, and analyzed with APC 3M™ Petrifilm™ incubated at 37°C for 24 h.

Results: DBDMH on inoculated tomatoes showed bacterial reductions between 2.8 and 4.2 log CFU/cm^2. DBDMH treatment at 300 ppm on inoculated cantaloupes showed a *Salmonella* reduction of 1.7 log CFU/cm^2 while PAA and chlorine had a reduction of 1.6 and 1.5 log CFU/cm^2, respectively.

Significance: Bromine-based antimicrobials are an effective intervention to reduce pathogens in the fruits and vegetables industry.

P3-210 Utilization of Emulsified Clove Bud Oil and Thyme Oil to Inactivate *Salmonella* on Cherry Tomatoes and *Escherichia coli* O157:H7 on Baby Spinach

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❖ ***Developing Scientist Competitor***

Introduction: In the produce industry, post-harvest rinses to remove debris are frequently used. This step has also been shown to be a primary point of contamination if antimicrobials are not utilized. While chlorine and peroxyacetic acid are approved and effective, many organic producers prefer to use alternatives. The effectiveness of essential oils, including clove bud (CBO) and thyme oils (TO), have been recognized, but their hydrophobic nature makes implementation in aqueous systems difficult.

Purpose: Evaluate the efficacy of CBO emulsified with whey protein and TO emulsified with gum arabic against *Salmonella* on cherry tomatoes and *E. coli* O157:H7 on baby spinach.

Methods: Tomatoes and spinach were inoculated with *Salmonella* or *E. coli* O157:H7, respectively, and rinsed in: 1) water, 2) 200 ppm free chlorine, 3) emulsion with 0.5% CBO, and 4) emulsion with 0.5% TO. Tests were conducted in clean water and water with 1% organic load. Post-treatment, surviving populations on produce were enumerated on TSA with ammonium ferric citrate and sodium thiosulfate (*Salmonella*) or CT-SMAC (*E. coli* O157:H7). Two samples were analyzed for each treatment and all experiments were replicated three times (n = 6). Analysis of variance was conducted to determine significant differences.

Results: A 2-log reduction of *Salmonella* was observed with CBO emulsion and was more effective than chlorine in the clean water system ($P < 0.05$). TO emulsion also lowered the population of *Salmonella* by nearly 1 log CFU/g and performed similarly to chlorine. Both treatments performed better than chlorine when water carried an organic load. *E. coli* O157:H7 populations significantly decreased in essential oil emulsions with and without organic load. TO emulsion performed better than chlorine in the systems with organic load with a more than 2-log reduction ($P < 0.05$).

Significance: These data demonstrate potential for organic oil emulsions to be used as alternative antimicrobials for produce wash water systems.

Poster

P3-211 Inactivation of *Toxoplasma gondii* on Blueberries Using Low-dose Irradiation

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Introduction: *Toxoplasma gondii* is the most common parasite that contaminates produce. However as more cases of *T. gondii* contamination are being linked to produce, current washing steps in produce processing may not be effective or suitable for some varieties of produce.

Purpose: The objective of this study was to evaluate the effect of low-dose irradiation on inactivating *T. gondii* oocysts on blueberries.

Methods: Blueberries (10 g) inoculated with *T. gondii* (5 log oocysts/g) were exposed to an absorbed dose of 0 (control), 0.2, 0.4 or 0.6 kGy at 4°C. A self-contained Lockheed Corporation ^{137}CS gamma radiation source at a dose rate of 0.075 kGy/min was utilized. After treatment, oocysts were recovered from berries. Human foreskin fibroblast (HFF) cells were maintained as monolayers in a 96-well microplate, exposed to excysted *T.* gondii recovered from berries, and incubated for 7 days. The viability of HFF cells was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) assay. In addition, blueberries were analyzed for compression firmness, surface color, and total anthocyanins, immediately after each treatment.

Results: HFF cells inoculated with oocysts recovered from the 0.6 kGy treated berries retained viability at 93% compared to the control, indicating that treated oocysts were less infectious to HFF cells. The result showed that gamma radiation significantly ($P < 0.05$) inactivated *T. gondii* oocysts on blueberries. Quality analysis showed that there was no significant change in texture, anthocyanins, or color in berries after the irradiation treatment

Significance: Findings of this study indicated that low-dose irradiation is a potential intervention measure for controlling *T. gondii* contamination on blueberries without affecting product quality.

P3-212 Effectiveness of a Spontaneously Emulsified Carvacrol Nanoemulsion Acidified with Organic Acids against a *Salmonella* Species Cocktail on Contaminated Mung Beans

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❖ ***Developing Scientist Competitor***

Introduction: Outbreaks of foodborne illness from the consumption of sprouts has been linked to contaminated seeds prior to germination. Currently, the recommended treatment involves soaking seeds in 20,000 ppm hypochlorite for 15 – 25 min prior to germination. In this study, an alternative treatment involving soaking *Salmonella* spp. coated mung beans in an acidified carvacrol nanoemulsion was tested.

Purpose: The purpose of this study was to evaluate the effectiveness of a novel, food-grade, and label friendly treatment on contaminated sprouting seeds.

Methods: Mung beans were inoculated to levels of approximately 8, 5, 4, or 3 log CFU/g of seed and treated by soaking inoculated seed batches in acidified (acetic or levulinic acid) nanoemulsion (4,000 or 8,000 ppm) for 30 or 60 min. Numbers of surviving cells were determined after treatment by suspending seeds in broth and performing plate counts and/or Most Probable Number enumeration. Treated seeds were sprouted and tested for the presence of the pathogen.

Results: In the mung bean system, levels of *Salmonella* spp. were reduced by 5 log, regardless of concentration and/or treatment time when compared to the control or organic acids alone. When seeds were inoculated at either 4 or 3 log CFU/g seed, pathogens were not detected in mung bean sprouts grown from acidified carvacrol nanoemulsion treated seeds, regardless of treatment concentration or time.

Significance: These results show that the use of the acidified carvacrol nanoemulsion may be an alternative, food-grade, and label friendly antimicrobial treatment for mung beans.

P3-213 Estimating the Burden of Foodborne Illness for *Campylobacter*, *Salmonella* and *Vibrio parahaemolyticus* in Japan, 2006–2012

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Introduction: In Japan, the numbers of food poisoning and cases are reported mandatory; however, these do not exactly reflect the real burden of foodborne illnesses due to the passive surveillance nature. We have been estimating the real burden of foodborne diseases for *Campylobacter*, *Salmonella* and *Vibrio parahaemolyticus* in Japan.

Purpose: The purpose of this study was to estimate the burden of foodborne illnesses associated with three pathogens in Japan, by making use of the laboratory confirmed numbers of infections.

Methods: Data on laboratory-confirmed infections of three pathogens were collected from clinical laboratories that test stool samples submitted from all over Japan or from Miyagi Prefecture, from January 2006 to December 2012. The physician consultation rate and the stool submission rate were estimated from telephone population surveys conducted for whole of Japan and for Miyagi prefecture. Each estimate was introduced into the Monte-Carlo simulation model as a probability distribution, which was run for 10,000 iterations.

Results: The estimated mean numbers per year of foodborne illnesses for *Campylobacter*, *Salmonella* and *V. parahaemolyticus* in whole of Japan were 2.2 - 5.3 million, 0.47 - 1.1 million, and 44 - 170 thousand during 2006 - 2012, respectively. Those estimated for whole of Japan from data on Miyagi prefecture were 0.75 - 1.6 million, 78 - 190 thousand, and 7 - 63 thousand during 2006 - 2012, respectively. The numbers of reported foodborne illnesses per year in Japan during 2006 - 2012, for *Campylobacter*, *Salmonella* and *V. parahaemolyticus*, were 1,800 - 3,100, 670 - 3,600 and 90 - 1,300, respectively.

Significance: These data reveal a significant difference in numbers and trends between our estimates of burden of foodborne illnesses and the reported foodborne disease cases associated with three pathogens. Need for continuing active surveillance system to complement the present passive surveillance is strongly suggested, in order to identify and prioritize food safety issues more precisely and to monitor the effectiveness of risk management options.

P3-214 Analysis of Restaurant-related Foodborne Illness Outbreaks and Restaurant Inspections Data from Local Health Departments: Are Sanitarians Having to Inspect Too Many Restaurants?

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❖ ***Developing Scientist Competitor***

Introduction: Ensuring food safety among restaurants is an important feature of local health department efforts to protect public health. Restaurant inspections are performed to remedy food safety violations and prevent foodborne illness among patrons.

Purpose: The purpose of this study is to investigate the hypothesis that restaurant-associated foodborne outbreaks are associated with the frequency of restaurant inspections.

Methods: We invited all local US health departments to complete a web-based survey to collect jurisdictional demographics, frequency of foodborne outbreaks and frequency of restaurant inspections. One hundred sixty-seven jurisdictions from 36 states participated in the survey. To examine sanitarian workload, we calculated the mean restaurant to sanitarian ratio (the average number of restaurants assigned to each sanitarian) and the mean inspection to sanitarian ratio (the average number of inspections performed by each sanitarian). Pearson correlation coefficients were calculated to describe the relationship between the outbreak rates and these continuous variables.

Results: During 2012, 59 jurisdictions reported at least one foodborne outbreak (total 187 outbreaks, range 1 - 27). On average, there were 0.55 restaurant-associated outbreaks per jurisdiction per 100,000 population (range 0 - 6.98/100,000). Sanitarians visited an average of 159 restaurants (range 12 - 546) and performed 296 inspections (range 17 - 1012). The mean number of annual inspections per restaurant did not correlate with outbreak rate (mean = 2.02, range 0.65 - 4.9, p = 0.8). However, both the mean restaurant to sanitarian ratio (mean = 159, range 12 - 546) and mean inspection to sanitarian ratio (mean = 295, range 17 - 1012) were significantly associated with higher outbreak rates (P = 0.029 and P = 0.036, respectively).

Significance: These data reveal that the number of restaurant inspections performed per sanitarian varies considerably throughout the country and that a greater inspection workload is associated with higher foodborne illness outbreak rates. Future studies are needed to determine if there may be differences in the quality of inspections performed by inspectors with higher workload.

P3-215 Systematic Review of the Microbiological Safety Status of Foods at Retail in Costa Rica

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Introduction: Most of the available information on the burden of foodborne pathogens is limited to industrialized countries and little is known about their prevalence in developing regions.

Purpose: To systematically summarize and critically analyze the current knowledge on the microbiological safety of foods at retail in Costa Rica

Methods: To determine the current food safety information available about Costa Rica, the European Food Safety Authority guidelines for conducting systematic reviews in food and feed safety were followed. An online search in English and Spanish was performed on four databases – FSTA, Pubmed, SciELO, and Web of Science – every three months during two years. Any study reporting the "presence," "prevalence," "occurrence," "incidence," "concentration" or "levels" of any microbial pathogen in any kind of retail food sold in Costa Rica was considered.

Results: Forty four primary research reports were identified. Reports dealing with microbial contamination in institutional settings, primary food production, or street foods were excluded Most of the evidence reports described contamination of dairy products and produce. Other commodities included meat and poultry, fish and seafood, sugar, spices, and eggs. Very limited information about most commodities has been published. In what is published, extremely low samples sizes were used. Furthermore, no emergent pathogens were tested for on any commodity, except for one study of *Clostridium difficile* in retail meats. No national surveys were conducted for any product or pathogen, except for one study reporting the presence of *Salmonella* and Shiga toxigenic *Escherichia coli* in retail beef products in urban and rural areas. Finally, studies reporting seasonality or presence of antimicrobial resistant bacteria were scarce. Most studies pointed out the presence of microbial pathogens at unacceptable levels.

Significance: This study identifies knowledge gaps and research opportunities to characterize and improve the safety of retail foods in Costa Rica.

P3-216 Zoonotic Species of the Genus *Arcobacter* in Poultry from Different Regions of Costa Rica: Frequency of Isolation and Comparison of Two Sampling Methods

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Poster

Introduction: In recent years, interest in emerging pathogens has received special attention due to their consequences for public health, and the genus *Arcobacter* is one of these, because of its potential as foodborne zoonotic agents.

Purpose: These bacteria have been isolated in Costa Rica from commercial poultry samples, so the aim of this research was to determine its isolation frequency from laying hens, broilers, ducks and geese, and to compare two sampling methods, namely cloacal swabs and stool collection.

Methods: Two hundred samples were randomly taken from 25 different farms in the country. De Boer and Houf broths were used as enrichment media; isolation was done using Arcobacter selective medium and membrane filtration using blood agar. Typical colonies were identified using a genus-specific PCR reaction and species identification was made using the multiplex polymerase chain reaction (*m*-PCR) proposed by Douida *et al*.

Results: *Arcobacter* was isolated from 22 samples of the 200 samples examined (11%). Of the strains isolated, 15 (55%), 8 (30%) and 4 (15%) were identified as *A. butzleri, A. cryareophilus* and *Arcobacter* spp., respectively. Also, there is a statistically significant difference between the isolation frequencies of *Arcobacter* for the sampling methods evaluated, yielding more isolates when stool collection was performed than when cloacal swabs were used.

Significance: The presence of this emerging pathogen in poultry samples from Costa Rica represents a health risk for human beings as well as for animals, efforts shall be focused in reducing it by introduction and application of good agricultural and manufacturing practices through food chain.

P3-217 Prevalence of *Salmonella* Species in Ground Beef in Guadalajara, México

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Introduction: *Salmonella* in raw meat is considered a significant public health issue in countries around the world, including México. Traditional methods for detection of *Salmonella* are labor-intensive with a typical time to result of 4 - 5 days.

Purpose: To determine the prevalence of *Salmonella* spp. in ground beef obtained from butcher shops in Guadalajara, México, using the 3M™ Molecular Detection Assay (MDA) for *Salmonella* in comparison to the USDA/FSIS MLG 4.08 reference method.

Methods: Ninety-three ground beef samples were obtained for the study. The Guadalajara city was divided in seven geographical zones for data analysis according to the number of butcher shops in each zone. Each sample (25 g) was homogenized in 225 ml of BPW-ISO and incubated for 24 h, followed by a 75-min analysis with the 3M MDA for *Salmonella.* All positive and negative results by MDA were confirmed following the USDA/FSIS MLG 4.08 method.

Results: Seventy-four out the 93 ground beef samples tested positive for *Salmonella* (79%). The percentage of positive samples for the Minerva, Olímpica, Oblatos, Industrial, Tetlán, Huentitán and Centro zones was 57%, 61%, 77%, 78%, 84%, 93% and 100%, respectively. All positive samples by 3M MDA were biochemically and serologically confirmed, and there were no false negative results observed, reflecting a 100% accuracy, sensitivity and specificity in comparison to the reference method.

Significance: Results suggest that there is significant prevalence of *Salmonella* in ground beef obtained from butcher shops in Guadalajara, México. The 3M MDA proved to be a reliable method for detection of *Salmonella* spp. in ground beef, delivering fast and accurate results.

P3-218 Prevalence and Dynamics of *Campylobacter jejuni* in Dairy Cattle Farms in Korea

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Introduction: *Campylobacter jejuni* is the leading cause of bacterial foodborne disease worldwide. As large outbreaks have been associated with consumption of unpasteurized milk, the significance of *Campylobacter* in dairy cattle farms should be concerned. Furthermore, relatively is known about the dynamics and transmission of *Campylobacter* genotypes in dairy cattle farms.

Purpose: The objectives of this study was to investigate the prevalence of *Campylobacter jejuni* in dairy cattle farms and its association with farm management variables and to determine the genetic relatedness of the isolates between farms, with various sources and within a farm.

Methods: A total of 501 cattle samples were collected from 19 dairy cattle farms from August 2012 to Dec 2014 to identify the presence of *Campylobacter jejuni* using a PCR-based culture method. Questionnaires were distributed and farm management factors were evaluated. *Campylobacter jejuni* isolates were then subtyped using *fla*A typing and PFGE method. Forty-six *Campylobacter* isolates from different host and year of isolation were included in the subtyping. The digested band profiles were then analyzed by UPGMA method with 1% optimization using Bionumerics software version 6.6.

Results: The herd prevalence for *Campylobacter jejuni* was 7/19 (36.8%) and the individual prevalence was 113/501 samples (22.6%). Farm management factors such as sanitary conditions, biosecurity measures, and feeding management of 15 farms were analyzed. The discriminatory index of *fla*A typing and PFGE were 0.89 and 0.86, respectively. Genotype heterogeneity was observed in all farms except farm 2. Part of the isolates from different farms, sources (cattle and human, chicken and human), and different time period within a farm were indistinguishable. Combined *fla*A-PFGE showed that certain pattern was in peak before dominated by another pattern within a farm.

Significance: These data suggests that dairy cattle farms may act as a reservoir for human campylobacteriosis and by understanding the dynamics of *Campylobacter*, new intervention strategies can be exploited.

P3-219 Prevalence and Comparison of Detection Methods of Enterohemorrhagic *Escherichia coli* in Culled Dairy Cows

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❖ ***Developing Scientist Competitor***

Introduction: Enterohemorrhagic *E. coli* (EHEC) serogroups O26, O45, O103, O111, O121, O145 and O157 (EHEC-7) account for the majority of EHEC cases in the U.S. and are adulterants in non-intact, raw beef according to the USDA-FSIS.

Purpose: The objectives of this study were to: 1) determine the prevalence of EHEC-7 in fecal, hide, and pre-intervention carcass samples from culled dairy cows at harvest, and 2) evaluate detection methods for EHEC-7 in these matrices.

Methods: One hundred culled dairy cows were sampled at harvest (June to July 2014). Matched fecal, hide and carcass samples were enriched in *E. coli* (EC) broth and by immunomagnetic separation (IMS). Enriched samples were plated on CHROMagar™ STEC and recovered IMS beads were plated on STEC heart infusion washed blood agar with mitomycin-C, CHROMagar™ O157 and USMARC STEC agar. Colonies were tested for Shiga Toxin (*stx*), EHEC-7 O-groups (*wzx*, *wbq*, or *rfbE*), and intimin (*eae*) by PCR. Samples with EHEC-7 recovered by any agar were considered culture positive.

Enriched samples were tested by NeoSEEK™ (Neogen® Corp.), and the Atlas® EG2 Combo assay (Roka®Bioscience). Agreement between methods was determined by Cohen's kappa coefficient and McNemar's Chi-square test.

Results: By culture, EHEC-7 were recovered from 7.0%, 16.0% and 1.0% for feces, hides and carcasses, respectively. By NeoSEEK™, the prevalence of EHEC-7 was 26.0%, 65.0%, and 7.0% for feces, hides and carcasses, respectively. By Atlas® EG2, 29.0%, 46.0%, and 28.0% were non-O157 EHEC positive and 29.0%, 51.0%, and 3.0% were O157:H7 positive for feces, hides, and carcasses, respectively. Moderate agreement was observed between culture and NeoSEEK™ for detection of EHEC-O26 (Kappa = 0.5773, $P < 0.05$), EHEC-O121 (Kappa = 0.4975, $P < 0.05$) and EHEC-O157 (Kappa = 0.4012, $P < 0.05$). No agreement was observed between other methods.

Significance: Detection of EHEC-7 on carcasses indicates that effective interventions are critical to ensure food safety.

P3-220 Prevalence, and Pheno- and Genotypic Characteristics of *Campylobacter jejuni/coli* Isolates from Chicken and Duck at Wet Markets in Korea

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Introduction: *Campylobacter jejuni* and *Campylobacter coli* are common bacterial cause of human gastrointestinal infection, and poultry carcasses are common sources. Intake of undercooked poultry and cross-contamination from the carcasses may cause campylobacteriosis.

Purpose: The purpose of this study was to analyze prevalence, and pheno- and genotypic characteristics of *C. jejuni/coli* isolated from chicken and duck carcasses at wet markets in Korea.

Methods: One hundred sixty samples of poultry carcasses (chicken and duck) were analyzed by PCR and mCCDA medium to evaluate the prevalence of *C. jejuni/coli* during summer from June to August. Isolated colonies on the medium were further analyzed for antibiotic resistances to ciprofloxacin, nalidixic acid, tetracycline, erythromycin, enrofloxacin, amikacin and chloramphenicol, using agar dilution method and for presence of cytolethal distending toxin genes (*cdtA*, *cdtB*, and *cdtC*) with PCR. The isolated colonies were also used for serotyping, and genotyping to evaluate genetic correlations among isolates by pulsed-field gel electrophoresis (PFGE).

Results: *C. jejuni/coli* were isolated from 22 samples [7 chicken samples (8.8%), 15 duck samples (18.8%)], and 19 isolates of 22 isolates were *C. jejuni,* and three isolates were *C. coli*. The antibiotic resistances of *Campylobacter* were observed to enrofloxacin, nalidixic acid and ciprofloxacin, and most isolates had all of *cdt A*, *B*, and *C* genes. As a result of genotyping by PFGE, only 2 isolates (*C. jejuni*) of 22 strains showed high genetic correlation (96.8%).

Significance: These results indicate that poultry carcasses in wet markets were contaminated with *C. jejuni/coli* at relatively high prevalence rate, and the isolates had antibiotic resistance and cytolethal distending toxin genes. Thus, food safety management to control *C. jejuni/coli* should be improved.

P3-221 Prevalence of *Listeria monocytogenes* from Chicken and Duck, and Genetic Analysis for the Isolates Using Molecular Typing Methods

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Introduction: *Listeria monocytogenes* has been recognized as a high-risk foodborne pathogen. The pathogen can contaminate fresh meat and poultry, and especially, the prevalence of *L. monocytogenes* in poultry could be higher in wet markets than in grocery stores.

Purpose: The objective of this study was to evaluate the prevalence of *L. monocytogenes* in poultry carcasses in wet markets, and to analyze pheno- and genotypic characteristics of the isolates.

Methods: During summer from June to August, 160 poultry carcasses (chicken: 80; duck: 80) were purchased from 17 wet markets in Korea. One poultry carcass was washed by 400 ml of 0.1% buffered peptone water, and 0.25 ml aliquots of washings were plated on Palcam agar, followed by incubation at 30°C for 48 h. Presumptive colony on *L. monocytogenes* on Palcam agar was further analyzed for identification by 16s rRNA sequencing. The presence of *actA*, *inlA*, *inlB*, *plcB*, and *hlyA*were confirmed by PCR, and serotypes were also determined by multiplex-PCR and agglutination test. Genetic correlations among isolates were also evaluated by PFGE patterns formed by Asc I.

Results: Of 160 poultry samples, *L. monocytogenes* were isolated from 10 samples (6.25%) of poultry carcasses [two chicken samples (2.5%), and 8 duck samples (10%)]. Fifteen *L. monocytogenes* isolates were from wet markets in Gimhae, Gwangju, Jeonju and Wonju. Most isolates had virulence genes such as *actA*, *inlA*, *inlB*, *plcB* and *hlyA*, and the presence of high prevalence serotypes were confirmed. Genetic correlations among *L. monocytogenes* isolates mostly ranged 70% to 90%.

Significance: This result indicates that food safety to control *L. monocytogenes* on poultry needs to be improved.

P3-222 Molecular Subtyping of Human and Food-environmental *Listeria monocytogenes* Isolates by PFGE in Northern Italy (2012–2014)

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Introduction: Human invasive Listeriosis is a relatively rare but life threatening foodborne disease, with high hospitalization and fatality rates in vulnerable populations. The main route of transmission to humans is through the consumption of contaminated food, but the heterogeneity of food products, the extended shelf life of many marketed products and the prolonged incubation periods prejudice the identification of common source outbreaks and food vehicles.

Purpose: The aim of this study was to evaluate the molecular correlation between human and food-environmental *Listeria monocytogenes* isolates in the Lombardy region during the period 2012-2014, in order to improve the network of integrated surveillance and support epidemiological investigations.

Methods: Seventy-three human isolates were collected by the regional reference laboratory of the Lombardy region at University of Milan. Simultaneously, 301 food-environmental isolates were detected from the food chain by the Department of Food Microbiology (IZSLER) in the same period and area under study. All *L. monocytogenes* isolates were subtyped by PFGE according to the PulseNet protocol with *AscI* enzyme and statistical analysis was performed using the Chi-square test ($P \leq 0.05$).

Results: Among the 73 human isolates were recognized 30 pulsotypes and 8 clusters, four of which were reported in previous years (*Mammina C.* et al., 2013). While 54 pulsotypes and 9 clusters were identified among the 301 food-environment isolates, the number of clusters observed in food-environment isolates was significantly lower than those observed in human isolates ($P < 0.01$). The correlation between human and

food-environmental isolates showed 65 pulsotypes and 15 clusters, in particular *L. monocytogenes* strains were isolated from meat products and preparations (43.2%, n = 73), fishery products (24.9%, n = 42), other Ready-to-Eat products (26.6%, n = 45) and cheeses (5.3%, n = 9).

Significance: This study identified the main pulsotypes that spread from the food supply chain to humans as well as the ecological niches or foods potentially involved in Listeriosis cases in Lombardy.

P3-223 Pheno- and Genotypic Characteristics of *Listeria monocytogenes* Isolates from Various Foods

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❖ ***Developing Scientist Competitor***

Introduction: *Listeria monocytogenes* exists widely in the nature, and the pathogen can cross-contaminate fresh produce, Ready-to-Eat (RTE) meat and poultry, and fish. *L. monocytogenes* can cause 30% of mortality by intake of the contaminated foods.

Purpose: The objective of this study was to analyze phenotypic and genotypic characteristics of *L. monocytogenes* isolated from different food groups.

Methods: Chromosomal DNAs were extracted from 14 *L. monocytogenes* isolates from meat (7), RTE food (4), and fish samples (3). The presences of virulence genes (*actA*, *inlA*, *inlB*, *plcB*, and *hlyA*) were identified by PCR. Genetic correlations among the isolates were also evaluated by the PFGE (pulsed-field gel electrophoresis) patterns formed by Asc I.

Results: Of 14 isolates, most *L. monocytogenes* isolates possessed *actA*, *inlA*, *inlB*, *plcB*, and *hlyA*, and no differences in gene expression patterns of the virulence genes among sample groups (meat, RTE food, and fish) were observed. In general genetic correlations among the *L. monocytogenes* isolates mostly ranged 70% to 90%, and genetic categories among the sample groups were not detected.

Significance: This result indicates that *L. monocytogenes* strains isolated from various foods possess virulence genes, and genetic correlations among isolates were categorized by sample groups.

P3-224 Molecular Characterization of *Listeria monocytogenes* Strains Isolated from Italian Blue Cheese Production Plants and Comparison with Human Clinical Strains from the Same Geographical Area

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Introduction: *Listeria monocytogenes* causes invasive syndromes with mortality rates as high as 30% in specific population groups such as elders, immuno-compromised and newborn or fetuses. Fresh and unpasteurized cheeses have been frequently implicated in outbreaks and sporadic cases both in Europe and USA. Italian blue cheese is a raw milk soft cheese mainly produced in Western Northern Italy, with a high export rate (Europe, USA, Canada, Far East and Australia) and a yearly retail turnover exceeding 500 million Euros.

Purpose: Given the above, it is important to better understand *L. monocytogenes* isolates obtained from blue cheese. Therefore, the aims of this study were to characterize strains of *L. monocytogenes* isolated in blue cheese production plants and compare them to isolates from human cases diagnosed in the same geographical area.

Methods: Forty-one *L. monocytogenes* strains from cheese and environmental samples obtained from 22 production plants (2004-2007) were subtyped with Multi Virulence Locus Sequence Typing (MVLST) and compared to 60 *L. monocytogenes* strains from sporadic human cases (2005-2014) and previously typed strains. New allelic sequences were assigned arbitrary virulence type (VT).

Results: Five VTs were identified among food/environmental samples: 83% (n = 34) showed two new allelic profiles, 12% (n = 5) were VT14 (milk chocolate outbreak, 1994, USA), 0.02% (n = 1) was VT80 (ricotta salata outbreak, 2012, USA) and 0.2% (n = 1) was VT46. Twenty-six VTs were observed among human strains, with 62% (n = 37) of samples showing VTs previously observed in outbreaks. VT14 and VT80 were also identified in 3 and 4 of the clinical samples, respectively.

Significance: The production environment of blue cheese can allow growth and persistence of *L. monocytogenes*, and while this cheese shares many characteristics with other cheeses considered at risk for Listeriosis, the overall strain population appears to be homogeneous and relatively distinct from human cases strains from the same region.

P3-225 Characterization and Relatedness of *Clostridium difficile* Strains Isolated from Animals, Meat and Humans in Belgium

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Introduction: *Clostridium difficile* is an anaerobic spore-forming bacterium recognized as a major cause of nosocomial colitis and antibiotic associated diarrhea. Over the past few years, several studies have focused on the possible role of animals and food as contamination routes for human *C. difficile* infections.

Purpose: The aim of this study was to isolate and compare the *C. difficile* strains circulating in animals, food and humans in Belgium.

Methods: Fecal samples of newborn pigs and calves were collected from breeding farms. Intestinal contents and carcass samples were collected from cattle and pigs at slaughterhouse. Raw meat was obtained from the retail trade. Horse fecal samples were collected from hospitalized animals. Human *C. difficile* isolates were obtained from care home residents and hospitalized patients. *C. difficile* strains were compared with respect to the toxin gene profile, PCR-ribotyping, antimicrobial activity, multilocus sequence typing (MLST) and multiple-locus variable number tandem repeat analysis (MLVA). A neighbor-joining phylogenetic tree was constructed in order to determine the correlation between human, animal and food isolates.

Results: A total of 127 isolates belonging to 32 different PCR-ribotypes were collected. The PCR-ribotypes most prevalent in terms of number of isolates were 078, 014 and 027. For a given PCR-ribotype, strains presented a similar susceptibility to the antimicrobials tested, irrespective of the isolation source. Phylogenetic analysis showed that human, meat and animal isolates with the same PCR-ribotype cluster in the same lineage.

Significance: The overlap between strains from animal, food and human origins suggest a potential risk of interspecies and foodborne transmission.

P3-226 Introduction of Hygiena SIB (*Salmonella* Incubation Broth) Device as Rapid Method for Surface Surveillance for Stressed *Salmonella*

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Introduction: The contamination of surfaces with *Salmonella* species is one of the major routes for cross-contamination. InSITE *Salmonella* is a simple-to-use, rapid device for screening surfaces for *Salmonella* species.

Purpose: The InSITE *Salmonella* device is designed to swab a large area for possible *Salmonellae* contamination, pre-enrich and then selectively enrich in the same device giving a presumptive positive in 24 h from sample collection. This allows better and more rapid surveillance of high risk surfaces.

Methods: *Salmonella* Typhimurium ATCC 14028 and *Salmonella* arizonae ATCC 13314 were grown and diluted into diluent. Sterile stainless steel squares were inoculated with 100 µl from each dilution -1 through to -9 and air dried overnight under asepsis. Each square was swabbed with one InSITE *Salmonella* device and incubated at 6 h in the pre-enrichment phase, a further 18 h in the selective phase. Devices are positive if a vivid yellow color appears in the viewing window. All devices used in the study were analyzed for confirmation of positivity using the BAM *Salmonella* confirmation steps with a final identification by biochemistry. Directly inoculated devices without a drying step were performed as controls.

Results: The drying of the *Salmonella* on the stainless steel surface rendered the bacteria stressed. Dilutions -1, -2, -3 and -4 were all positive for both *Salmonella* Typhimurium and for *Salmonella* arizonae. The -5 dilution dried was also detected using the InSITE *Salmonella* device. All dilutions from the directly inoculated devices were positive. All devices were then confirmed for negativity and positivity using a confirmation protocol from BAM, the confirmation was 100% for both positives for *Salmonella* but also negative for *Salmonella* from those swabs that indicated a presumptive negative at 18 h.

Significance: An easy to use enclosed device that will reliably indicate presumptive positive and negative *Salmonella* from surfaces has advantages to many food producers and manufacturers.

P3-227 GenomeTrakr: A Pathogen Database to Build a Global Genomic Network for Pathogen Traceback and Outbreak Detection

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Introduction: The use of Next Generation Sequencing (NGS) in outbreak investigations is being adopted by public health agencies, and a state and federal pathogen detection network is being implemented: GenomeTrakr. In this network, public health agencies will share genomic data publicly to build a transparent reference database.

Purpose: This pilot study demonstrates how desktop Whole Genome Sequencing (WGS) data can be used, in a combined analysis, for source tracking of foodborne pathogens. Additionally, we describe the components of the NGS pathogen network that integrates state public health laboratories (AK, AZ, FL, HI, MD, MN, NM, NY, NY_Ag, TX, VA, and WA) as well as federal laboratories.

Methods: Multiple data analysis pipelines (Examples) were tested to combine draft bacterial genomes for phylogenetic analyses to provide leads in outbreak investigations. Results of over 12,000 draft bacterial genomes plus the phylogenetic trees of these results are available from the GenomeTrakr bioprojects at the NCBI.

Results: The hardware and software implemented allowed us to compare and cluster complete genomes of thousands of taxa at a time, and the software outputs daily phylogenetic trees for source tracking of food, clinical and environmental isolates. We report enhanced molecular epidemiological insights gained by comparative analysis of *Salmonella* and *Listeria* genomes previously deemed indistinguishable by conventional subtyping methodologies. Details will be provided, for two outbreak case studies with roughly 50 isolates for each case one in nut butter and one in Latin style cheeses The nut butter case documents the ability to link a few clinical cases to an environmental swab from a food facility. The *Listeria* case was the first example of FDA using WGS for regulatory action.

Significance: These results demonstrate the role of WGS tools within a regulatory environment while highlighting the novel additional insights provided to epidemiological investigations through comparison to a reference database.

P3-228 Dynamics of Shiga Toxin-producing *Escherichia coli* (STEC) Shedding in Beef Cattle

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❖ ***Developing Scientist Competitor***

Introduction: Even with advancements in pre- and post-harvest food safety, Shiga Toxin-producing *Escherichia coli* (STEC) still present challenges to human health. Since cattle are the primary reservoir for STEC, lowering the prevalence of this pathogen in farm animals may reduce STEC outbreaks in humans. However, many factors that modulate the colonization and persistence of STEC in cattle remain unknown.

Purpose: This study reveals the role of animal age and environmental factors influencing the shedding of Shiga Toxin-producing *E. coli* (STEC) in beef cattle.

Methods: Several animal factors like breed, age, sex were investigated to determine their influence on STEC shedding. A cohort of 300 beef calves from a multi-breed beef calf population derived from Brahman and Angus cattle was sampled four times every three months after birth. This study utilized a combination of culture-based and nucleic acid-based methods for the detection and enumeration of Shiga Toxin-producing *Escherichia coli* (STEC) from the fecal samples. Data were analyzed by regression methods and McNemar's test for matched pairs using STATA software.

Results: The herd prevalence of STEC in March was 59.8%, which was significantly higher compared to any of the other sampling times (39.5% in June, 20.3% in August and 20.7% in December). The *stx2* genotype was predominant in the herd, whereas *stx1/stx2* was the lowest in all samplings. There was no significant association between breed group, sex of the calf and average weight gain with the STEC shedding. However, we observed STEC shedding was significantly affected by animal age. Furthermore, seasonal variation of STEC shedding was observed.

Significance: This study provides insight that animal age is a significant factor that influences the prevalence of STEC; therefore this finding has implications for the development of on-farm mitigation strategies.

P3-229 Survival of Generic *E. coli* on Gala and Golden Delicious Apples Near Harvest

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Introduction: Application of overhead, evaporative cooling water to apples could introduce contamination near harvest.

Purpose: Reduction of inoculated generic *E. coli* levels was observed on Gala and Golden Delicious apples with and without overhead cooling water application for up to three weeks.

Methods: A cocktail of rifampicin-resistant, generic *E. coli* was sprayed onto individual Gala and Golden Delicious apples in an orchard using a backpack sprayer after sunset. Galas (18) and Golden Delicious (at least 27) were harvested at each time point after inoculation (0, 8, 24, 32, 56, 176 h); Golden Delicious were also harvested at (344 and 512 h). Samples were enumerated on Chromagar ECC-rifampicin with and without filtration; pre-enrichment in TSB was performed as microbial counts declined. Uninoculated control apples were tested for indicator organisms on Chromagar ECC, as well as for pathogenic *E. coli* and *Salmonella*.

Results: Only one of forty uninoculated control apples was positive for generic *E. coli* (2.5 log CFU) and no pathogens were detected. Average generic *E. coli* levels were reduced 2.5 log CFU/apple within 8 h after inoculation; this time period reflects the minimum anticipated time between a final application of agricultural water and harvest. Average generic *E. coli* levels continued to decline at slightly more than a 0.5 log per day up to 3 days. Although average generic *E. coli* levels at the last sampling point for each variety were low (1.2 - 1.5 log CFU/apple), 3 of 15 Gala apples (20%) were above > 3 log CFU/apple after 1 week, and after 3 weeks, 14 of 57 Golden Delicious apples (25%) were > 3 log CFU/apple.

Significance: Preliminary data indicated that average generic *E. coli* levels were reduced at rates greater than or equivalent to the proposed 0.5 log per day reduction proposed by FDA for mitigation of water that exceeds the proposed standards.

P3-230 *E. coli* O157:H7 Fecal Shedding in Backgrounder Steers Fed with Different Dietary Crude Protein Levels

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✦ ***Undergraduate Student Award Competitor***

Introduction: Shiga Toxin-producing *Escherichia coli* comprise a diverse group of bacteria capable of causing severe gastrointestinal disease in humans. Prior research has suggested that *E. coli* O157:H7 prevalence varies according to the type of diet (fiber vs. starch) fed to cattle. In addition, the protein content in forage diets may also play an important role in O157:H7 fecal shedding.

Purpose: Evaluate the effect of different dietary crude protein concentrations on *E. coli* O157:H7 fecal shedding in beef cattle.

Methods: A total of 192 fecal samples were collected from beef steers fed with forage diets, plus the addition of barley straw, canola meal, and corn silage, to adjust dietary crude protein (CP) levels (CP; T1 = 6.9%, T2 = 10.3%, T3 = 12.1%, and T4 = 13.6%). Similar energy and fiber contents were maintained among the treatments (NDF: T1 = 56, T2 = 54.7%, T3 = 56.9 and T4 = 57.3). *E. coli* O157:H7 detection and confirmation was carried out using conventional microbiology techniques, immunomagnetic separation, latex agglutination and multiplex PCR (*rfbE, flicH7, stx1* and *stx2* genes).

Results: Overall 21.9% (42/191) of the animals tested positive for *E. coli* O157. A total of 42 strains were positive for O157 latex agglutination and were further confirmed with PCR (*rfb*E positives). All isolates were positive for sorbitol and β-glucuronidase, and therefore considered atypical. Results indicated that 15 of these strains were O157:H12 and negative for $stx_{1\text{-}2}$ genes. Chi-square analysis indicated that the distribution differed among dietary CP levels ($P < 0.05$); where T2 showed the highest percentage of *E. coli* O157, 36.2% (17/47), followed by T1: 22.9 (11/48), T3: 22.9 % (11/48) and T4 with the lowest prevalence 6.3% (3/48).

Significance: Results suggest that protein content in the diet may influence *E. coli* O157 fecal shedding in cattle as cattle fed a diet containing 13.6% CP had lower prevalence than cattle fed diets containing 6.9, 10.3 and 12.1% CP.

P3-231 Characterization of *Campylobacter* Species from Flies Sourced from Conventional Turkey Farms

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❖ ***Developing Scientist Competitor***

Introduction: *Campylobacter* is a leading agent of foodborne disease with poultry as major vehicle for human infections. Risk factors for colonization of poultry flocks with *Campylobacter* remain poorly characterized but include poor biosecurity and insect vectors, primarily flies and darkling beetles. Flies may play a vital role in introduction and dissemination of *Campylobacter* through a flock. However, limited information is available on the characteristics of *Campylobacter* from flies in poultry farms.

Purpose: The purpose of this study was to determine the prevalence, species, and antibiotic susceptibility profiles of *Campylobacter* isolated from turkey feces and flies from turkey farms. Additionally, the diversity and correlations between susceptibility profiles of fly and fecal isolates were investigated.

Methods: Visits were made to 31 conventional turkey flocks. At each visit, 12 fecal samples and 10 flies were collected. *Campylobacter* was isolated and enumerated on selective media (mCCDA), speciated (*C. jejuni* vs. *C. coli*) by multiplex PCR and characterized for susceptibility to a panel of antibiotics.

Results: The majority (92.6 %) of flocks were positive for *Campylobacter* both in fecal and fly samples. Most antimicrobial susceptibility profiles of isolates from feces were represented among those from flies. However, isolates from flies tended to exhibit greater diversity of antimicrobial susceptibility profiles than those from feces – 2.45 different profiles/flock in flies vs. 1.92 in feces.

Significance: The prevalence and diversity of *Campylobacter spp.* from turkeys and putative insect vectors, especially flies, remain poorly characterized. The findings suggest that flies from turkey farms are commonly *Campylobacter*-positive and may exhibit a greater diversity of *Campylobacter* than suggested by analysis of isolates from feces. The data will add to the body of literature on this subject and expand into the untapped area of the role of the fly in the spread of not just *Campylobacter*, but associated antibiotic resistances as well.

P3-232 Effect of Management Practices on the Prevalence of Shigatoxigenic *Escherichia coli* on Small-scale Cow/Calf Operations in Oklahoma and Louisiana

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Introduction: *Escherichia coli* O157:H7 is considered an adulterant in meat along with the six non-O157 serogroups (O26, O45, O103, O111, O121, and O145). Cattle coming into feedlots and slaughter facilities are common reservoirs of these shigatoxigenic *E. coli* (STEC), resulting in post-harvest contamination of beef products. On-farm management practices may affect pathogen-loads on cattle entering slaughter facilities. However, little is known about the impact of practices employed at cow/calf operations on the prevalence of STEC.

Purpose: Determine the effect of management practices employed by small-scale cow/calf operations on the prevalence of STEC.

Methods: Fecal, water, sediments, and equipment-swab samples were collected from several cow-calf operations in Oklahoma and Louisiana during summer and fall, over a 2-year period. Each farm was visited twice each year. Presence of *E. coli* O157:H7 and non-O157 (O26, O45, O103, O111, O121, 145) was determined using immuno-magnetic-separation and RT-PCR. A questionnaire on feed-type, water-source, cattle-breed, herd-density and farm-cleanliness was obtained to assess management practices. Data was analyzed using ANCOVA, followed by Tukey-test for water-source, feed, and breed-types and regression analysis for correlation between herd-density and STEC prevalence.

Results: A 5% and 22% of *E. coli* O157:H7 and 53% and 49% of non-O157 prevalence was observed in Oklahoma and Louisiana, respectively. Serogroups O26, O45, and O103 were most prevalent in both states. In Louisiana, use of municipal-water over well-water increased non-O157 prevalence whereas a combination of water-sources (streams and runoff) in Oklahoma increased prevalence of all STECs ($P < 0.05$). Hay-feeding increased prevalence of *E. coli* O157:H7 (14.5%) and O26 (31.4%) in Oklahoma and Louisiana, respectively. Commercial-feed increased prevalence of O145 (30%) in Louisiana. Positive correlation ($P < 0.05$) between herd-density and prevalence of O45 and O103 was observed in Oklahoma.

Significance: Results can be used to establish guidelines for best management practices and for development of risk management strategies.

P3-233 Emergence of Listeriosis Outbreaks Associated with Produce in the United States, 1983–2014

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Introduction: Listeriosis is caused by *Listeria monocytogenes,* a Gram-positive bacillus transmitted primarily through food. Commonly recognized foods causing outbreaks and illnesses include delicatessen meat and dairy products such as milk and cheese.

Purpose: We describe an increase in the number of US Listeriosis outbreaks associated with produce.

Methods: We queried CDC's Foodborne Disease Outbreak Surveillance System (FDOSS), reviewed published reports, and unpublished CDC data for all Listeriosis outbreaks associated with produce during 1973–2014. We analyzed the number of outbreaks, outcomes (hospitalizations and deaths), and implicated produce items. We considered cases in pregnant women or infants ≤ 28 days old to be pregnancy-associated. Factors contributing to these outbreaks were reviewed.

Results: During 1973–2014, six Listeriosis outbreaks associated with produce were detected in the United States. All six outbreaks occurred after 2008 and involved domestically grown produce. These outbreaks included 216 illnesses (range 2–147, median 15 per outbreak), 207 hospitalizations, 47 deaths, and two fetal losses. Twenty-one illnesses (10%) were pregnancy-associated. These comprised 21% of Listeriosis outbreaks and 59% of Listeriosis outbreak-associated illnesses after 2008. Two outbreaks were associated with sprouts and one each with cantaloupe, caramel apples, celery, and stone fruit. Environmental assessments indicated that structural defects, inadequate produce handling and cleaning techniques, and inadequate facility and equipment design were possible sources of contamination.

Significance: No US Listeriosis outbreaks were linked to produce in the first 25 years of US foodborne Listeriosis outbreak surveillance compared with six outbreaks after 2008. Both vegetables and fruit were implicated. Reasons for the emergence of produce-associated Listeriosis outbreaks are not known. However, improvements in the detection and investigation of outbreaks might have contributed to the identification of previously unrecognized sources. Manufacturers, growers, and packers should implement measures to minimize contamination.

P3-234 Enhanced Enumeration of Heat-injured *Salmonella* Typhimurium in Synthetic and Food Models by Combining Thin Agar Layer Method and Sodium Pyruvate Supplement

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Introduction: *Salmonella* is a leading cause of foodborne illness throughout the world. Previous research indicates thin agar layer (TAL) method can effectively recover pathogenic bacteria sublethally injured under common food processing conditions. Moreover, supplementation of peroxide decomposer chemicals to media improve the recovery of injured bacterial cells.

Purpose: This study aimed to determine the combined effect of these two approaches for recovering heat-injured *Salmonella* Typhimurium from synthetic and food models.

Methods: The TAL procedure was developed by overlaying one or two layers of non-selective plate count agar (PCA) onto pre-poured and solidified *Salmonella* selective medium, xylose lysine deoxycholate (XLD) agar. The recovery effect of these agar media with various peroxide decomposers in PCA was evaluated with healthy and heat-injured *Salmonella* Typhimurium cells inoculated in peptone water, canned chicken broth, and fresh lettuce washing solution.

Results: Supplementation of PCA with sodium pyruvate (SP) significantly enhanced the recovery of heat-injured *Salmonella* Typhimurium in peptone water; the count increased by 0.76 and 2.67 logs compared with PCA and XLD, respectively. Adding SP or $FeSO_4$ into XLD directly did not show any significant effect on the recovery. Heat-injured *Salmonella* Typhimurium in peptone water was counted 2.51 and 3.62 log CFU/ml on one and two layers of SP-supplemented PCA overlaid onto XLD, respectively. The count was only 0.20 and 0.85 log CFU/ml on one and two layers of PCA overlaid onto XLD, respectively. Supplementation of two layers of PCA in TAL with SP increased the count of heat-injured *Salmonella* Typhimurium in chicken broth and lettuce washing solution by 1.48 and 2.78 logs, respectively. This method did not influence the selectiveness of XLD in TAL on background flora in lettuce washing solution.

Significance: The combination of TAL method and SP supplement could potentially be used for rapid and accurate enumeration of injured pathogenic bacteria in food samples.

P3-235 *Salmonella* Serotype Determination Based on High-throughput Genome Sequencing Data

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❖ ***Developing Scientist Competitor***

Introduction: *Salmonella* is the most prevalent foodborne pathogen in the United States, causing more than 1 million cases of illness annually and the largest economic burden among all bacterial pathogens. The U.S. National *Salmonella* Surveillance System has been built upon serotyping, a subtyping method traditionally performed through the agglutination of *Salmonella* cells with specific antisera that detects lipopolysaccharide O and flagellar H antigens. Specific combinations of O and H antigenic types represent serotypes. More than 2,500 *Salmonella* serotypes have been described in the White-Kauffmann-Le Minor scheme.

Purpose: To develop a bioinformatics tool for the determination of *Salmonella* serotypes using high-throughput genome sequencing data.

Methods: Databases for *Salmonella* serotype determinants and bioinformatics pipelines were built to allow *in silico* determination of antigenic profiles from raw sequencing reads and genome assemblies. A web application, SeqSero, was developed to allow public access to this tool.

Results: SeqSero was validated by testing: 1) raw reads from genomes of 308 *Salmonella* isolates of known serotype; 2) raw reads from genomes of 3,306 *Salmonella* isolates sequenced and made publicly available by GenomeTrakr, a U.S. national surveillance network; and 3) 354 publicly available draft/complete *Salmonella* genomes. It achieved accuracy rates of 98.7%, 92.6% and 91.5%, respectively, for the three datasets. Together, SeqSero successfully determined a total of 200 serotypes and was predicted to perform near full spectrum (more than 2,300) *Salmonella* serotype determination. We also demonstrated *Salmonella* serotype determination from raw sequencing reads of fecal metagenomes from mice orally infected with this pathogen.

Significance: Public health microbiology is being transformed by whole genome sequencing (WGS) which opens the door to serotype determination using WGS data. SeqSero is a fast and robust serotype prediction tool that helps to maintain the well-established utility of *Salmonella* serotyping by integrating it into the platform of WGS-based pathogen subtyping and characterization.

P3-236 An Investigation of Food Allergy Knowledge and Training among Restaurant Employees in the U.S

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Introduction: Americans spend half of their food expenditures eating out. With 15 million Americans having food allergies, restaurant employees should be trained and gain better understanding about this health condition.

Purpose: This study investigated restaurant employees' food allergy (FA) knowledge and identified previous FA training, preferred characteristics of a future FA training and reasons for not interested in attending FA training.

Methods: The questionnaire was developed based on interviews with four restaurant employees and previous literature. Research panels consisted of restaurant employees in the U.S. who were recruited by a market research company (Qualtrics Inc.).

Results: A total of 229 participants completed this survey. Approximately 82% (n = 187) participants indicated that they worked for an "allergy-friendly" restaurant. Common strategies to accommodate clients with food allergies included modifying recipes upon request (n = 175) and stating allergen on the menus (n = 97). Of maximum 28 points possible, the mean food allergy knowledge score was 20.76 ± 3.44 (range = 6 to 28). Most participants could identify symptoms of a food allergic reaction (n = 196) and respond to it (n = 174). Only 27% (n = 84) participants indicated that they have been trained about food allergies. Most of the training was conducted when an employee was newly hired (n = 46), lasted for less than 2 hours (n = 62), and in a group setting (n = 46). Eighty percent (n = 183) participants showed interest in attending a future food allergy training. They preferred a training that is self-paced, incorporates examples that mimic the real-world situations, and uses simple language. Forty-six participants were not interested in attending training because they perceived a training to be "*not needed,*" "*time consuming,*" "*not beneficial*" and "*boring.*"

Significance: Employees have some knowledge about food allergy. Employee food allergy training was uncommon but majority participants were interested to be trained. Future training should incorporate characteristics that are most appealing to the restaurant employees.

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