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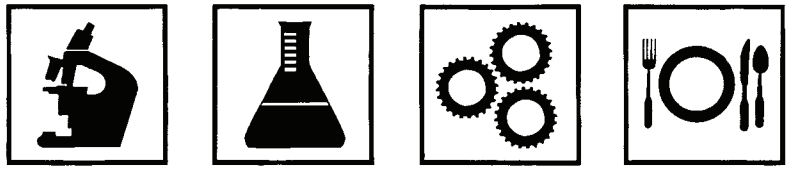

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IAFP 2013 Abstracts

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Scope of the Journal. The *Journal of Food Protection* is intended for publication of research and review articles on all aspects of food protection and safety. Major emphases of *JFP* are placed on studies dealing with (i) causes (microorganisms, chemicals, natural toxicants) and control of all forms of foodborne illness; (ii) contamination (microorganisms, chemicals, insects, rodents) and its control in raw food and in foods during processing, distribution, preparation, and service to consumers; (iii) causes of food spoilage and its control through processing (low or high temperatures, preservatives, drying, fermentation, irradiation, pressure, and other innovative technologies); (iv) food quality and microbiological, chemical, and physical methods to assay food quality; and (v) wastes from the food industry and means to use or treat the wastes.

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Ivan Parkin Lecture

The Future of Food Safety

David W.K. Acheson, M.D., F.R.C.P.
Leavitt Partners LLC
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Food safety generally evolves slowly and methodically over time without a lot of planning interspersed with the occasional growth spurt such as the FSIS Mega Reg for HACCP in the mid '90s, and the current FDA focus on the Food Safety Modernization Act. Both of these events were regulatory, and both were triggered by one or more public health disasters involving loss of life from foodborne illness. In the past, improvements in food safety have largely been driven by regulatory change such as the two mentioned above. Today, the primary drivers for food safety have shifted for many in the food industry with the focus now being primarily on risk management and brand protection not necessarily focusing first, and sometimes only, on regulatory compliance. But despite the capacity for media and consumer buying habits to put brands into bankruptcy, there are some in the food industry that simply don't understand what to do, are complacent about what they are doing, or worse, are occasionally putting profit before safety.

As we look to the future for food safety, what does our crystal ball tell us? Will brand protection be the primary driver in the future? Or will regulatory compliance if the regulators become more powerful and more prescriptive? Maybe technology drives our actions with scientific advances impacting the industry? What will be the impact of the growing global food supply chain? As you can see, these ever-changing dynamics create the possibility for lots of speculation, so let's indulge in just that. What would look right – what would we wish for food safety – what would we do if we were king (or queen) for the day? This platform provides a unique opportunity to speculate on just such topics – to think big picture and to pose some provocative possibilities of where food safety should be in 10, 20 or more years into the future.

To be thinking what the regulatory structure may look like, who is going to pay, what consumers may be demanding are all fun topics for speculation, yet are tangible issues that do need substantive thought and planning if they are going to be successful. But isn't that a novel concept, "substantive thought and planning for food safety?" How do we accomplish that?

Food is a multi-billion dollar business with very high stakes in a commodity that every human and animal needs every day of their lives and thus present both massive opportunity and substantive risk. This presentation will touch on the past and the present and use them as a launching pad to speculate on what the future for food safety should look like if we as an industry plan it, and what it may look like if we don't.

John H. Silliker Lecture

Food Safety Risk Management for a Multinational Company; Things I Wish I Knew before Taking the Job and Things I Have Learned along the Way!

Dane Bernard

Vice President, Food Safety and Quality
Keystone Foods LLC
West Conshohocken, PA

The field of Food Safety is challenging and personally rewarding for many reasons. For those who practice Food Safety Risk Management from an industry view point, accomplishing your food safety management objectives will involve applying deep food safety knowledge as well as other skills needed to navigate the political channels between business units and the corporate office. And as Henry Kissinger once said about becoming Secretary of State, (paraphrasing) "you should know what you need to know before you take the job because you won't have a chance to learn it on the job." This is not to say that there is no learning once you take that Food Safety job for a multinational company but the time will be taken up with the issue of the moment and the multiple issues that arise each day. Be ready, there won't be time to learn the basics on the job!

It has been my experience that food safety roles and responsibilities within a multinational are not transparent. Most companies of significant size will have divisions or business units and depending on corporate philosophy, these units may operate more or less independently. Even if a company culture is tilted toward central control, human nature takes over and rebel camps are inevitable resulting in even less transparency. In some companies all the quality and food safety staff reports to a central authority and in others, they will report within the divisions with a dotted line to corporate. Understand what the landscape is and engage top management in defining what the roles and responsibilities are and how these will be communicated through the business. RACI charts are a good tool to lay out the pattern for who makes decisions under what conditions and who is responsible for subsequent actions.

Make certain to have your foundational documents in place, up to date, and relevant to your business. You can't expect conformance to expectations unless the expectations are clearly laid out and relevant to the current situation. A factory or Business Unit will find ways around policies that are not compatible with their business or products so we must always work to understand the business situation in each geographical area and in the markets to which product is shipped. At the same time, keep "thou shalt" policies to a minimum. Simpler is better and more sustainable.

Encourage management to use incentives for the business units to assist in keeping focus. For example, linking bonus for the business unit to performance on audit scores, microbiological performance, customer complaints, first quality product, and conformance to HACCP plans, are all candidates for incentivizing focus. A system for capturing data that is completely transparent is ideal for this purpose but is not often a reality considering how often things change in terms of company structure for sizable companies.

Running a successful program will require much more than a good understanding of hazards and their controls. Your company's future and the well being of your customers may rest on your ability to address the additional factors needed to implement an effective, global food safety program.

Symposium Abstracts

S1 Fresh Produce: Are Current GAPs Recommendations Sufficient for Food Safety?

REGGIE BROWN: Florida Tomato Committee, Maitland, FL, USA
MICHELLE DANYLUK: University of Florida, Lake Alfred, FL, USA
MANAN SHARMA: U.S. Department of Agriculture-ARS, Beltsville, MD, USA
ELAINE BERRY: U.S. Department of Agriculture-ARS, Clay Center, NE, USA
ELIZABETH BIHN: Cornell University, Geneva, NY, USA
DAVID ORYANG: U.S. Food and Drug Administration, College Park, MD, USA

Good Agricultural Practices (GAPs) for the production of fresh produce have been in use since 1999; however, many of these standards vary across the fresh produce industry and available science does not always offer definitive recommendations. This symposium will delve into GAPs research with respect to manure and compost application, irrigation water quality and buffer zones, while reviewing current GAPs standards to determine if they are sufficient for produce safety with additional presentations addressing real world scenarios based on diversity in farm size, risk modeling and industry perspectives concerning GAPs implementation and challenges.

S2 Foodborne Outbreaks – Domestic and International: What are We Learning?

SHERRI MCGARRY: U.S. Food and Drug Administration, College Park, MD, USA
SCOTT GILLIAM: Indiana State Department of Health, Indianapolis, IN, USA
BRAD JOHNSTON: MultiCorr, Indianapolis, IN, USA
IAN WILLIAMS: Centers for Disease Control and Prevention, Atlanta, GA, USA
FRANK BOELAERT: EFSA, Parma, Italy

This symposium will be the annual foodborne disease outbreak symposium the Control of Communicable Foodborne Disease has sponsored for several years. The specific outbreak updates to be presented will be selected in December or January to make them as current as possible. We are considering including the 2012 *Salmonella* Typhimurium outbreak associated with cantaloupe from Indiana and an outbreak from outside from North America. For the outbreaks outside North America, we are considering the *S.* Stanley outbreak with illnesses in Austria, Belgium, Czech Republic, Germany, Hungary and Poland that is likely linked to turkey.

S3 Getting the Word Out for a Safe Food Supply

CATHERINE JAMAL: Centers for Disease Control and Prevention, Atlanta, GA, USA
BRUCE APPLEGATE: Purdue University, West Lafayette, IN, USA
JANET RILEY: American Meat Institute, Washington, D.C., USA
ARON HALL: Centers for Disease Control & Prevention, Atlanta, GA, USA
WENDY WHITE: Golden State Foods, Conyers, GA, USA
TIMOTHY SELLNOW: University of Kentucky, Lexington, KY, USA

The media landscape has changed dramatically since the dawn of the digital age. The emergence and popularity of social media outlets such as Twitter, Facebook, podcasts, blogs and vlogs have resulted in information being spread faster and more broadly than ever before. This symposium will highlight the influence of these new media outlets on the world of food safety, while providing multiple opportunities to learn how to effectively use social media as an effective communication tool. Topics chosen for this symposium will collectively teach the audience how to effectively use social media outlets to communicate important messages to a broad audience, especially for raising awareness and increasing food safety education. Instances where social media has had a profound influence on public perception will be presented, which will allow the audience to learn how to effectively respond to crisis situations involving these new media outlets. Examples of how social media is being used at the university level, both in a research setting and in a classroom setting, will also be presented. The digital age has had a profound impact on data acquisition, even spawning new fields such as "digital epidemiology". The audience will learn how digital tools, such as internet search queries, are being used to monitor disease trends in an attempt to improve public health. By the end of the symposium, the audience will have a greater appreciation for the major impact that the ever-changing media landscape has had on both food safety and our society as a whole. The various topics chosen for this symposium will educate the audience to better understand how to use social media as an effective communication tool.

S4 Chemical Risk Assessment 101: A Better Understanding of a Complex Subject Made Easier

MARK MOORMAN: Kellogg Company, Battle Creek, MI, USA
JAMES COUGHLIN: Coughlin & Associates, Aliso Viejo, CA, USA
SUZANNE FITZPATRICK: U.S. Food and Drug Administration-CFSAN, College Park, MD, USA

A key component of compliance with the Food Safety Modernization Act (FSMA) is development of a written food safety plan to identify and implement preventive controls to address all potential chemical, biological, physical and radiological hazards. As foods intrinsically are chemicals that are sourced/grown and processed with chemicals, determining the risks of chemicals is a necessary and important component of a food safety plan. This symposium will scope the universe of chemicals hazards that may be in foods, what we know about the presence of these chemicals through existing monitoring programs and explain the process necessary to determine if exposure of these chemical hazards is a risk to human health. This symposium will also describe the drivers that need to be considered when establishing your company's chemical preventive controls program and what the FDA considers important to include in your program.

S5 Persistent and Ongoing Food Allergen Challenges: Labeling, Detection and Control

STEVEN GENDEL: U.S. Food and Drug Administration, College Park, MD, USA
JOSEPH BAUMERT: University of Nebraska-Lincoln, Lincoln, NE, USA
DOUGLAS MARSHALL: Eurofins Scientific, Inc., Fort Collins, CO, USA

Failure to declare the presence of a major food allergen on the label of a food is a leading cause of food recalls. While the U.S. Food and Drug Administration has provided guidance to help industry understand how and when to declare the presence of allergens, the large number of allergen labeling-related recalls is an indication of ongoing problems. An in-depth review of allergen labeling legislation and guidelines in the U.S. will be given to help clarify how such recalls can be avoided.

We know that the allergen world is not static. There is an ongoing evolution of both the number and types of allergens as well as the methodology with which these allergens are detected and measured. An overview of various allergen detection and measurement technologies for both environmental and finished goods testing will be presented.

Understanding that an ounce of prevention is worth a pound of cure, the final presentation will focus on allergen control and contamination prevention. Best practices to control allergens, monitor persistence of allergens in manufacturing areas and effectively document these measures will be discussed.

S6 Linking Pests and Pathogens of Food Safety

JOHN BARCAY: Ecolab Inc., St. Paul, MN, USA
ROY COSTA: Food Industry Consultant, R.S., DeLand, FL, USA
GALE PRINCE: Retired - Corporate Regulatory Affairs, Cincinnati, OH, USA

This short symposium focuses on recent links of pest activity around foods and human illness. The disease-carrying potential of cockroaches, rodents, filth flies and other urban pests will be reviewed along with routes of transmission and related case studies specific to pests in fruits and vegetable operations, dairy operations, food service and food retail.

In these food industry operations, presenters will report on recent foodborne illness incidents and data trends with specifics on identified pathogens, allergens and other contaminants with either direct or indirect linkages to pest infestations.

S7 Culture Independent Diagnostics

MARIO MARCON: The Ohio State University, Columbus, OH, USA
JOHN BESSER: Centers for Disease Control and Prevention, Atlanta, GA, USA
PHILIP BRONSTEIN: U.S. Department of Agriculture-FSIS, Washington, D.C., USA

Most diagnoses of foodborne infections are currently done by culturing specimens from patients. However, methods that do not require isolation of an organism by culture are increasingly being implemented by clinical laboratories for some pathogens, e.g., enzyme immune assays (EIAs) for Shiga toxin-producing *Escherichia coli* (STEC) and *Campylobacter*. Molecular methods that simultaneously detect the presence of the most common bacterial, viral and parasitic diarrheal pathogens without culture have been developed and the first are being considered by FDA for licensing and will be available commercially shortly.

Since all public health laboratory surveillance systems including PulseNet and NARMS use data from the characterization of cultured isolates, the introduction of culture independent diagnostic methods in clinical practice will drastically change our ability to detect, investigate and control outbreaks, follow trends to document the effect of public health interventions to prevent foodborne infections and antimicrobial resistance of foodborne pathogens.

This symposium will present the problem and discuss the implications of this new technology to the food regulatory agencies and the food industry. The symposium will also address possible solutions to the problems including opportunities to strengthen the surveillance system and food safety in general.

S8 From Grapes to the Glass: Food Safety Issues That Affect Wine throughout the Production Chain

RANDY WOROBO: Cornell University, Geneva, NY, USA
BRENT TRELA: Texas Tech University, Lubbock, TX, USA
GLENN O'DELL: Constellation Brands, U.S., Inc., Acampo, CA, USA

Wine is an important and popular part of the global and U.S. food chain. In 2011, worldwide wine production reached about 7 billion gallons, with the top wine producing countries being: 1. France, 2. Italy, 3. Spain, 4. United States, 5. Argentina, 6. China, 7. Australia, 8. Chile, 9. South Africa, 10. Germany. In the U.S., there are wineries operating in all 50 states, with total production reaching some 495 million gallons and $1.39 billion in revenues in 2011.

WinesVinesDATA (2011) identified 7,345 wineries in the United States (more than 100 in North Carolina, IAFP's 2013 host state), with California bottling more than 90 percent of the nation's wine.

From a food safety standpoint, alcohol provides protection from bacterial foodborne pathogens, but chemical hazards such as sulfites, mycotoxins and other possible chemical contaminants are still a concern for wine. Wines are susceptible to microbial and chemical spoilage (oxidation) through their production.

Good winery sanitation practices are essential in preventing contamination with common wine spoilage microorganisms. The use of chlorinated compounds is avoided in wineries due to the formation of trichloroanisole. However, non-chorinated chemical sanitizers commonly used in the food and dairy industries do not target common wine spoilage microorganisms, or have not been validated for target wine spoilage microorganisms. In addition, the wine industry has porous surfaces (barrels) that are not easily cleaned and sanitized. The potential for misuse of chemical sanitizers in wineries may result in a chemical hazard for workers and consumers.

During this symposium respected wine industry professionals will address 1.) winery sanitation and water management, including the range of disinfection methods for winery environments, 2.) international wine labeling regulations for food safety, allergen traceability, ochratoxin, plasticizers, ethyl carbamate, public health and export issues and 3.) and wine storage, bottling, closure and packaging safety and quality issues.

S9 Food Safety Education: The Value of Education and Outreach to Advancing the Development of Future Food Safety Leaders

KALMIA KNIEL: University of Delaware, Newark, DE, USA
TRAVIS CHAPIN: Cornell University, Ithaca, NY, USA
ISABEL WALLS: U.S. Department of Agriculture-NIFA, Washington, D.C., USA

Foodborne illnesses continue to pose a substantial public health burden in the United States and around the world. One persistent contributing problem is a relatively poor level of food safety "knowledge" in the population at-large. To address this, there is a clear need to expand existing food safety education and awareness programs, and to establish new ones. However, this cannot be done without a critical mass of food safety professionals. Unfortunately, career and educational opportunities in food safety are largely unrecognized by K-12 and undergraduate students, meaning that many students come to food safety relatively late in their academic training. The purpose of this symposium is to highlight innovative approaches to engaging future food safety professionals by targeting K-12 and undergraduate populations. The symposium will kick off with two presentations on different strategies used recently to reach this important pool of future food safety professionals followed by a presentation describing USDA-NIFA priorities for food safety outreach. Attendees will come away with an understanding of why it is important to include food safety education in K-12 and undergraduate curricula and how engaging these students can help grow the population of food safety professionals around the world.

S10 The USDA-NIFA Food Virology Collaborative (NoroCORE): A Model for an Integrated, Multidisciplinary Approach to Addressing the Leading Cause of Foodborne Disease

ARON HALL: Centers for Disease Control & Prevention, Atlanta, GA, USA
KALMIA KNIEL: University of Delaware, Newark, DE, USA
LEE-ANN JAYKUS: North Carolina State University, Raleigh, NC, USA
BEATRIZ QUINONES: U.S. Department of Agriculture-ARS, Albany, CA, USA
ANGELA FRASER: Clemson University, Clemson, SC, USA
ALVIN LEE: Institute for Food Safety and Health, Bedford Park, IL, USA

Human noroviruses (HuNoV) are the most common cause of foodborne disease and viruses are likely responsible for a large proportion of foodborne diseases of unknown etiology. Recent advancements in molecular biology, bioinformatics, epidemiology and risk analysis have aided the study of these agents, but they remain difficult to control. The USDA-NIFA Food Virology Collaborative (NoroCORE), a 5-year, $25 million project funded by the USDA-National Institute of Food and Agriculture, was established in 2011 to address this important public health issue. Using a multi-disciplinary, integrated approach, our long-term goal is to reduce the burden of foodborne disease associated with viruses through efforts focusing on six core functions: (i) Molecular Virology; (ii) Detection; (iii) Epidemiology and Risk Analysis; (iv) Prevention and Control; (v) Extension and Outreach; and (vi) Education and Capacity Building. Through research, outreach and educational activities, along with stakeholder involvement, the Collaborative builds greater appreciation for the role of viruses to foodborne illness, with the long-term goal of producing a measurable reduction in burden of viral disease burden. The purpose of this session is to present recent findings of NoroCORE investigators and stakeholders, and to describe how these findings are changing how we study and control these important agents of foodborne illness. This session is sponsored by NoroCORE and the Viral and Parasitic Foodborne Disease PDG.

S11 Molecular Methods for Advancing Food Safety

BART WEIMER: University of California-Davis, Davis, CA, USA
SHANNON MANNING: Michigan State University, East Lansing, MI, USA
DAVID PINCUS: bioMérieux, Hazelwood, MO, USA

Methodologies employed in food safety range the gamut from traditional and applied culture-based methods to modern cultureless techniques, based on protein and DNA analysis and sequence recognition. Nevertheless, a large chasm exists between basic research with molecular tools and the applied techniques that can be used in day-to-day food safety testing. The goal of this symposium is to concisely translate information regarding relevant molecular methodology to an audience of food safety personnel, for private testing laboratories, regulatory, industrial or research-based food safety testing and protocols. Experts from the field have been chosen to address topics such as "Genomics in Food Security: 100K Pathogen Genome Project," which itself has a goal of utilizing molecular technologies for the benefit of food safety; proteomic analysis and identification of foodborne pathogens; and molecular typing applications for epidemiological investigations and food safety. This symposium will be of interest to professionals of all knowledge levels and expertise in the food industry, academia, research and food testing laboratories, as well as regulatory agencies.

S12 The Application of Bioinformatic Analyses in Foodborne Pathogen Characterization

ANDREA OTTESEN: U.S. Food and Drug Administration, College Park, MD, USA
WEN ZOU: U.S. Food and Drug Administration, Jefferson, AR, USA
KEITH LAMPEL: U.S. Food and Drug Administration, College Park, MD, USA
MIN LIN: Canadian Food Inspection Agency, Ottawa, ON, Canada
EDUARDO TABOADA: Public Health Agency of Canada, Lethbridge, ON, Canada

Foodborne outbreaks continue to occur at an unacceptable pace. Although the pathogens involved appear to be the same cast of characters, the food matrix varies considerably. Two common themes persist with microbes that cause human illness via the ingestion of contaminated foods. First, the genetic information each pathogen possesses enables these microorganisms to express their virulence potential. Second is their ability to evolve and adapt to their environment—how they can either grow or survive under the intrinsic and extrinsic conditions present. With the advent of whole genome sequencing, the genetic mystery of each pathogen can be revealed by its order of nucleotides. However, this provides information contained at the tip of the iceberg. Bioinformatic analyses and database development allow researchers to analyze large amounts of omics data efficiently, and provide a deeper insight into the genetic potential of each pathogen to answer the two points raised above. For foodborne pathogens, in addition to the virulence factors, data revealing how genetically, a microbe can persist in food matrices or any environment has significant impact on all levels of food safety.

S13 Sanitation Stories: Tall But True

DAVID BLOMQUIST: Ecolab Food & Beverage Division, Eagan, MN, USA
JEFFREY KORNACKI: Kornacki Microbiology Solutions, Inc., Madison, WI, USA
JOSEPH STOUT: Commercial Food Sanitation, LLC, Chicago, IL, USA

Cleaning and sanitation procedures fail for a variety of reasons including, misapplication of cleaning and/or sanitation, maintenance/repair associated issues and equipment or facility design related issues. This Symposim will illustrate real life examples of all the above.

S14 Ecology of *Campylobacter* and *Salmonella* in Pasture Poultry/Mixed Farm and Their Control with Natural Organic Antimicrobials

DEBABRATA BISWAS: University of Maryland-College Park, College Park, MD, USA
WALID ALALI: University of Georgia, Griffin, GA, USA
IRENE HANNING: University of Tennessee-Knoxville, Knoxville, TN, USA

Pasture poultry and mixed farms (grown poultry with other farms animals and vegetables and fruits) are now gradually replacing the conventional and big size farms and making a strong position in the U.S., Canada and Europe. Organic poultry and other farm animals are raised with access to the outdoors for at least one-third of the entire life cycle. The lack of proper biosecurity measures potentially increases the possibility of coming into close contact with sources of foodborne pathogens including birds, pests and other wild animals. The objective of organic livestock production is to produce meat, meat products, eggs and milk in an environmentally friendly manner without the use of medical drugs and chemicals. Consumers believe that organic products are healthier than conventional foods. To avoid the antibiotics and growth hormones, the organic farmer needs some natural products that can replace those components and sustain their business long term. The possible effective natural organic products are needed to be established as alternative source of antimicrobial agents, proteins and nutrients in both conventional and organic livestock production. This symposium will focus on the sustainable management of organic poultry/mixed farms including best practice, improving food safety and consumer confidence.

S15 Where the Wild Things are: Role of Wildlife in the Safety of the Food Supply

DAVID PEARL: University of Guelph, Guelph, ON, Canada
JUDY GREIG: Public Health Agency of Canada, Guelph, ON, Canada
SALAH UDDIN KHAN: University of Florida, Gainesville, FL, USA
MALCOM BENNETT: University of Liverpool, Liverpool, United Kingdom
MICHELE JAY-RUSSELL: University of California-Davis, Davis, CA, USA
ALAN FRANKLIN: U.S. Department of Agriculture-NWRC-WS, Ft. Collins, CO, USA

Although wild game represents only a very small fraction of the food supply in most developed countries, wildlife may be indirectly contributing to microbial food safety hazards in the food chain. Wildlife may serve as a reservoir of a large number of zoonotic pathogens. Approximately 75% of all new and emerging pathogens have putative wildlife reservoirs. This symposium will explore the role that a variety of terrestrial and avian species have in transmitting infections to food animals and contamination of the food supply with zoonotic pathogens and antibiotic resistant organisms. This symposium is sponsored in-part by a competitive grant (2011-51110-31199; LeJeune, PI) awarded by the USDA, National Institute of Food and Agriculture, National Integrated Food Safety Initiative.

S16 Antimicrobial Resistance and Food Safety in the 21st Century

P. MICHAEL DAVIDSON: University of Tennessee-Knoxville, Knoxville, TN, USA
STUART REID: University of Glasgow, Glasgow, United Kingdom
DAVID WHITE: U.S. Food and Drug Administration, Washington, D.C., USA
PAULA FEDORKA-CRAY: U.S. Department of Agriculture-ARS-BEAR, Athens, GA, USA
BILL SHAW: U.S. Department of Agriculture-FSIS, Washington, D.C., USA
BETSY BOOREN: American Meat Institute Foundation, Washington, D.C., USA

Antimicrobial resistance of foodborne pathogens has been an increasing problem in the last two decades. Resistance of bacteria to (1) food processing antimicrobials, (2) therapeutic and prophylactic clinical agents used in animals and humans, as well as, (3) growth-promoting antibiotics for livestock is universally acknowledged. Nevertheless, the underlying reasons for the increase in microbial resistance as well as the best means of controlling and/or preventing future bacterial resistance is not agreed upon among the scientific, medical, veterinary and agricultural communities around the globe. This symposium will provide a timely overview of the current knowledge and opinions pertaining to antimicrobial resistance of foodborne pathogens, as well as potential means of mitigating this problem by experts in academic, governmental research, U.S. regulatory, food industry and the European community. This symposium will be of interest to food industry personnel, as well as researchers, regulators, academicians and students.

S17 Sanitation and Sanitary Design – A Holistic Approach

RONALD SCHMIDT: University of Florida (Retired), Gainvesville, FL, USA
ROLANDO GONZÁLEZ: Bühler, Minneapolis, MN, USA
MARK MORGAN: Purdue University, West Lafayette, IN, USA
JOHN HOLAH: Campden BRI, Gloucestershire, United Kingdom
STEPHEN PERRY: AIOE, Annapolis, MD, USA
STEVE BLACKOWIAK: Bühler Aeroglide, Raleigh, NC, USA
MARK DAVIS: PepsiCo, Chicago, IL, USA

Sanitary equipment and facility design and function have always been of utmost importance in producing excellent quality and safe food. Incidents of food contamination are often avoidable and frequently the result of cross-contamination due to processing and handling food with equipment that does not meet basic sanitary design principles. Multiple groups such as 3-A SSI, EHEDG, USDA, FDA, BISSC, AMI, GMA-SSG and

regional Dairy Equipment Review Committees as well as food manufacturers and equipment manufacturers evaluate food processing equipment and facility design. Equipment that is designed, constructed and installed according to sound sanitary principles is the foundation to easier cleaning and improved sanitation, which in turn mitigates food safety risk by preventing product contamination, satisfying regulatory requirements, and meeting customer demands. In addition to strengthening food safety and sanitation programs, good engineering design lowers operating costs by making sanitation efforts more efficient and more economical, and maintenance and repairs less costly. Of interest to attendees that represent multiple food disciplines, the goal of this symposium is to provide a balanced perspective of sanitary equipment design and the role hygienic design of food manufacturing facilities plays in ensuring food safety. Besides delivering an overview on the topic, this comprehensive discussion will address current issues, risk assessment, industry collaboration, organizational and cultural changes and development of an analytical tool to estimate the overall impact of sanitary design of equipment and facilities. Case studies will be presented to offer insights from both a manufacturer and user perspectives.

S18 Emerging Technologies for Detection and Characterization of Foodborne Pathogens

ARUN BHUNIA: Purdue University, West Lafayette, IN, USA
JOSEPH IRUDAYARAJ: Purdue University, West Lafayette, IN, USA
KURT LAWRENCE: U.S. Department of Agriculture-ARS, Athens, GA, USA
EVANGELYN ALOCILJA: Michigan State University, East Lansing, MI, USA
BYRON BREHM-STECHER: Iowa State University, Ames, IA, USA
CHARLENE MELLO: U.S. Army Natick Soldier Research, Natick, MA, USA

Traditional means for microbial detection can no longer match the pace and reach of today's food processing and distribution networks. Rapid detection of pathogens in foods has never been more important. Emerging sensor and detector techniques may provide timely and actionable information useful in lessening the human and economic burdens levied by foodborne disease. This symposium features presentations on novel optical, spectroscopic and electrochemical technologies for pathogen detection, some of which are amenable to high-throughput, multivalent screening of foods. Benefits and challenges of these new methods and their comparison with existing techniques will be discussed. Label-free approaches and the advantages of novel bioaffinity ligands will be highlighted.

S19 U.S. Interagency Collaboration on Foodborne Illness Source Attribution

KARA MORGAN: U.S. Food and Drug Administration, Washington, D.C., USA
NEAL GOLDEN: U.S. Department of Agriculture-FSIS, Washington, D.C., USA
DANA COLE: Centers for Disease Control and Prevention, Atlanta, GA, USA

This session will present methods and preliminary results from a number of joint federal efforts to estimate the number of foodborne illnesses attributable to different food commodities for four priority foodborne pathogens in the United States. In 2010, the U.S. Centers for Disease Control and Prevention (CDC), Food and Drug Administration (FDA), and USDA Food Safety and Inspection Service (FSIS) formed the Interagency Food Safety Analytics Collaboration (IFSAC) to serve as an analytic hub for source attribution work of high priority to all three agencies.

IFSAC projects are developing novel approaches to move forward the state of the science of source attribution, and results will be used to inform FDA and FSIS regulatory and management activities including priority setting, program evaluation, and targeted risk management. We propose talks focusing on the methods, data, and preliminary results of three ongoing IFSAC projects: (1) development of a multi-agency food classification scheme to improve the quality of data from nationally reported foodborne disease outbreaks that are used to estimate attribution, (2) analysis of foodborne outbreak data as representative of sporadic illness data for purposes of foodborne illness source attribution, and (3) a method of combining source attribution estimates obtained from studies of sporadic illness with data from foodborne outbreaks to estimate the proportion of *Salmonella* Enteritidis illnesses caused by major food commodities.

S20 Best Practices in Recall Management

NICOLE NOLAN: U.S. Food and Drug Administration, College Park, MD, USA
MARK VARE: Inmar, Inc., Winston-Salem, NC, USA
JORGE HERNANDEZ: U.S. Foodservice, Rosemont, IL, USA
JIM BADALATI: Stericycle, Indianapolis, IN, USA

This symposium focuses on recalls further down the supply chain continuum with retail and food service. The FDA will also provide an analysis of recalls and the Reportable Foods Registry. Recalls remain a relevant topic, as grocery retailers handling hundreds of recalls a year. Over the past year, retailers and restaurants have seen ground beef recalled for *Escherichia coli* contamination; cantaloupes recalled due to *Salmonella*; and onions, smoked salmon, mushrooms, trail mix, cheese, more cantaloupes, and packaged salads recalled due to contamination by *Listeria monocytogenes*.

It is essential for retail grocery and food service operators to partner with their suppliers, regulatory agencies, and food safety peers to implement innovative, best practices for proper execution of recalls at retail and food service. Not only will proper recall management improve consumer confidence, but it will also reduce and/or prevent expanded foodborne illness.

S21 Pathogen Environmental Sampling Plans – The Latest on What, How and Why

JENNY SCOTT: U.S. Food and Drug Administration-CFSAN, College Park, MD, USA
RICHARD BROUILLETTE: Mondelez, Bournville, United Kingdom
THEODORA MORILLE-HINDS: Kellogg Company, Battle Creek, MI, USA

At many companies, environmental monitoring programs have been place for 10–15 years, while other companies are just beginning to set up programs. New sampling requirements may be added or changed with new regulations, as there are significant areas awaiting comment in the FSMA Proposed Rules. This session will review learnings from programs that have been successful, from programs that are evolving, and from regulatory and auditing perspectives.

S22 Food Safety in Paradise: Issues and Initiatives in the Caribbean

NEELA BADRIE: University of the West Indies, St. Augustine, Trinidad and Tobago
ANDRÉ GORDON: Technological Solutions, Limited, Kingston, Jamaica
CRISTINA TIRADO: Pan American Health Organization/World Health Organization, Rio de Janiero, Brazil

Alluring for its crystal blue waters, inviting sandy beaches, lush tropical beauty and laid back lifestyle, the Caribbean is a region consisting of the beautiful Caribbean Sea, its islands and the surrounding coasts. Situated largely on the Caribbean Plate, the region comprises more than 7,000 islands, islets, reefs, and cays. These islands generally form island arcs that delineate the eastern and northern edges of the Caribbean Sea. The Caribbean islands are part of the somewhat larger West Indies grouping, which consists of the Greater Antilles on the north, the Lesser Antilles on the south and east (including the Leeward Antilles), and the Bahamas and Turks and Caicos Islands. Some 42 million strong, the people of the islands constitute a myriad indigenous, international heritages and ethnicities. The Caribbean welcomed an estimated 25 million tourists in 2012.

Given the scope, scale and diversity of the Caribbean, its people and cuisines, and the significant tourism component of its economy, it's no surprise there are many challenges to achieving and maintaining a high level of food safety, many of which are unique to the region.

This symposium will showcase these island challenges and how they're being addressed by stakeholders. Expert speakers who are actively engaged with the islands' food chain from farm to fork will cover 1.) food safety issues and collaborative solutions shared by major island food manufacturers, 2.) the impact of the Food Safety Modernization Act on Caribbean inspections, audits, imports and exports and 3.) local public health issues, including those associated with indigenous foods, food service and tourism.

S23 The Pacific Rim: Food Safety Issues and Initiatives

BRIAN BEDARD: World Bank, Washington, D.C., USA
MOSES PRETRICK: Department of Health & Social Affairs, Palikir, Pohnpei, Micronesia
PETER HOEJSKOV: World Health Organization, Suva, Fiji

Welcome to the spectacular Pacific Rim, the global dynamo incorporating all places around the edge of the magnificent Pacific Ocean. This welcome includes the Pacific Basin, specifically the Pacific Rim and islands in the Pacific Ocean. The Pacific Ocean is the largest of the Earth's oceanic divisions. It extends from the Arctic in the north to the Southern Ocean (or, depending on definition, to Antarctica) in the south, bounded by Asia and Australia in the west and the Americas in the east. At 64.1 million square miles (165.2 million square kilometers) in area, this largest division of the World Ocean covers 46% of the Earth's water surface and about one-third of its total surface area, making it larger than all of the Earth's landmass combined. Extending approximately 9,600 miles (15,500 kilometers) from the Bering Sea in the Arctic to the northern extent of the circumpolar Southern Ocean at 60°S, the Pacific Ocean reaches its greatest east-west width at about 5°N latitude, where it stretches approximately 12,300 miles (19,800 kilometers) from Indonesia to the coast of Columbia – halfway across the world, and more than five times the diameter of the Moon.

It's old news that the Pacific Ocean is a continual hotbed of overseas shipping, including food and ingredients bound for myriad of destinations and imports coming in. The 45-some Rim nations are home to no less than 29 of the world's 50 busiest container shipping ports. Given the magnitude of the Pacific Rim and the diversity of its citizens, its food production and commerce, it is fitting to address some of the food safety issues specific to the region, including those that impact its many trading partners. Catering to anyone involved with and/or interested in food safety issues relative to the Pacific Rim, this symposium showcases science-based food safety management initiatives and collaborations among dynamic Pacific Rim supply chain stakeholders.

S24 Consumer Food Safety Behaviors: How to Change Them and How to Know When We've Done It

KAREN HILYARD: University of Georgia, Athens, GA, USA
DEBBIE CLAYTON: Cardiff Metropolitan University, Cardiff, Wales
LINDA ALDOORY: University of Maryland-College Park, College Park, MD, USA
WILLIAM HALLMAN: Rutgers Food Policy Institute, New Brunswick, NJ, USA
MARY BRENNAN: Newcastle University, Newcastle uponTyne, United Kingdom
DAVID DIEHL: University of Florida, Gainesville, FL, USA

Illnesses due to both emerging and familiar foodborne pathogens continue to afflict modern society. Consumers play an important role in preventing foodborne illness by recognizing and avoiding high-risk or adverse food choices and following safe handling practices. Although surveys can assess consumer awareness of food safety risks, knowledge does not necessarily translate into relevant behavior changes, and self-reported behavior may not reflect actual consumer practices. Educators, extension agents, and others who effectively relay food safety information to consumers also need to know how to formulate and deliver messages that achieve desired changes in food selection and handling. This symposium will tap the expertise of communicators, educators, and researchers in behavioral, social, and public/community health sciences, applied psychology and food marketing to: 1) give guidance on how to target audiences and craft effective education strategies, 2) describe responses consumers have to food safety messages, 3) determine behavioral changes after increasing awareness and understanding of high risk or adverse food choices, and 4) explain how to measure outcomes in terms of behavior/attitude changes.

S25 Global Lab Capacity Building for Ensuring Food Safety

CARL SCIACCHITANO: U.S. Food and Drug Administration, Silver Spring, MD, USA
DEON MAHONEY: Food and Agriculture Organization of the United Nations, Farrer, Australia
MICHAEL ROBACH: Cargill, Inc., Minneapolis, MN, USA
BRIAN BEDARD: World Bank, Washington, D.C., USA
MARIA TERESA DESTRO: University of São Paulo, São Paulo, Brazil
DILEK HEPERKAN: Istanbul Technical University, Istanbul, Turkey

Lab capacity building has taken on a life of its own over the past few years. This facet of global food safety has drawn the attention of countries worldwide as well as all the other entities, e.g., international organizations, food industry, NGOs and regulatory agencies to address the need to improve the food supply in the 21st century. Each of the speakers will address how countries or their respective organizations address food safety within the confines of analytical laboratories. Specifically, they will provide insightful models that were successful and identify the challenges lie for the future.

There remains significant obstacles at many levels to ensure global food safety; this symposium will address one issue-what are the challenges within individual countries to build their infrastructure to improve not only their own domestic consumption but also as a partner in the global economy. A number of international organizations, including government, food industry and the private sector have identified critical gaps in lab capacity. Improvement to an individual country's ability to respond to food safety issues need 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, training, and assessment of capabilities, infrastructure, and continuation of training for developing new skills for new technology. Using this information as a springboard to action, efforts by individual countries, NGOs, and WHO/FAO and domestic regulatory agencies have been implemented to move the infrastructure forward, including personnel training to physical improvements within the laboratory. However, there is much more to accomplish with the added challenges of working within this period of economic stress and uncertainty.

S26 *Listeria monocytogenes* in Retail Delis – Prevalence, Transmission and Control Strategies

HALEY OLIVER: Purdue University, West Lafayette, IN, USA
RENEE BOYER: Virginia Tech, Blacksburg, VA, USA
JANELL KAUSE: U.S. Department of Agriculture-FSIS, Manassas, VA, USA
THOMAS FORD: Ecolab Inc., Greensboro, NC, USA
SUSAN HAMMONS: Purdue University, West Lafayette, IN, USA
HILARY THESMAR: Food Marketing Institute, Arlington, VA, USA

Control of *Listeria monocytogenes* represents a particular challenge for the ready-to-eat (RTE) food industry due to the common presence and persistence of *L. monocytogenes* in virtually all environments along the food continuum. The 2003 FDA/FSIS *L. monocytogenes* risk identified RTE deli meats as the food vehicle responsible for most human listeriosis cases. A subsequent FSIS Comparative Risk Assessment suggested that up to 83% of human listeriosis cases linked to RTE deli meats may be attributable to products contaminated at retail, possibly explaining in part why the frequency of human cases has not declined as expected. To better understand the factors that contribute to contamination of RTE products at retail and evaluate which interventions would be effective to further prevent listeriosis, FSIS and FDA conducted the Interagency Retail *Listeria monocytogenes* Risk Assessment. This risk assessment, developed through collaboration among federal partners and academia with input from industry and consumer groups, provides insight into retail food safety strategies. Since the undertaking of this risk assessment, additional retail studies have been done to further augment advances in retail food safety. The purpose of this session is to update stakeholders on recent studies conducted to investigate *L. monocytogenes* prevalence, persistence and transmission in retail deli systems and to highlight efforts to control this pathogen. Specifically this session will highlight very recent studies aimed to characterize *L. monocytogenes* in retail delis as well as complimentary efforts to control and/or eliminate it from food and non-food contact surfaces (e.g., deep cleans). Further, perspectives from the lead trade association representing the retail food industry will be presented to highlight efforts taken to respond to recent findings. Finally, regulators will provide an update on Interagency Retail *L. monocytogenes* Risk Assessment given the availability of new data and increased efforts to control this pathogen in retail food systems.

S27 Global Practices That Form a Multiple Hurdle Approach to *Salmonella* and *Campylobacter* Reductions in Poultry

SCOTT STILLWELL: Tyson Foods, Inc., Springdale, AR, USA
ALICE JOHNSON: Butterball, LLC, Mt. Olive, NC, USA
JEFFREY FARBER: Health Canada, Ottawa, ON, Canada
MARIA TERESA DESTRO: University of São Paulo, São Paulo, Brazil
LESLEY LARKIN: Animal Health and Veterinary Laboratories Agency, London, United Kingdom
ROY BIGGS: Tegel Foods Ltd, Auckland, New Zealand
SHARON WAGENER: Ministry for Primary Industries, Wellington, New Zealand

Accepted management practices vary across poultry industry segments as well as various regions of the world. This symposium will look at both on-farm and processing plant practices that attempt to integrate a multiple hurdle approach to *Salmonella* and *Campylobacter* reductions. The U.S. broiler and turkey industries have both different and similar practices throughout their respective processes. We will hear from both industries in the U.S. on the progress made in the last decade. The chicken industries in the United Kingdom, Australia/New Zealand and Brazil also operate differently from the U.S. and we will have representatives to address differences in their goals and systems. Shell eggs and poultry are important issues in Canada and initiatives on the control of *Salmonella* Enteritidis in these products will also be discussed.

S28 Discussing Food Safety Risks, Controls and Challenges Associated with Farmers' Markets

VELMA LAKINS: U.S. Department of Agriculture-AMS, Riverdale, MD, USA
CLAUDIA COLES: Washington Department of Agriculture, Olympia, WA, USA
JUDY HARRISON: University of Georgia, Athens, GA, USA
MARISA BUNNING: Colorado State University, Fort Collins, CO, USA
SARAH SMATHERS: North Carolina State University, Raleigh, NC, USA
ERIN JOBE: Carrboro Farmers' Market, Carrboro, NC, USA

With consumers increasingly looking to have a greater connection to food, farmers' markets are growing in popularity, not just in the U.S., but worldwide. In 2012, USDA documented almost 8,000 markets nationally (up from only 1,700 in 1994). Coupled with world-wide economic downturns in the past decade, niche producers of fresh produce and small-scale processed foods have found farmers markets to be a viable place to market products. Farmers' markets allow for development of local food communities and are venues where patrons concerned about the quality of their food can meet with like patrons and those who grow and/or process their food.

Vendors at farmers' markets sell all food commodities and a common misconception revealed post-national outbreaks is that is that locally grown or produced foods from a small-scale farm are automatically safe as compared to foods transported long distances from a large-industrial sized farm. Considering the popularity of farmers' markets and the increasing number of outbreaks and recalls related to fresh produce, reheatable foods and dairy products a focus on safety of food products sold at farmers' markets can protect farmers, patrons and local economies.

This session will focus on presenting an overview of the growth of farmers markets as a sector, challenges associated with regulating risk reduction at these sites and applied research and extension activities currently addressing this sector. A unique view from a farmers' market manager will also be shared. It is anticipated that this symposium will reveal opportunities for further work and resource development with the farmers' market community.

S29 Assessing the Safety of Water Used in the Production of Fresh and Minimally Processed Produce

MIEKE UYTTENDAELE: Ghent University, Ghent, Belgium
LISE KORSTEN: University of Pretoria, Pretoria, South Africa
ANA ALLENDE: CEBAS-CSIC, Murcia, Spain
MABEL GIL: CEBAS-CSIC, Murcia, Spain
LIESBETH JACXSENS: Ghent University, Ghent, Belgium

Accessibility to abundant sources of high quality water is integral to the production of safe and wholesome fresh produce. This is becoming increasingly difficult in many parts of the world, increasing the risk of setting to the market products contaminated with pathogenic bacteria, viruses or parasites. The International Life Sciences Institute (ILSI)-Europe Emerging Microbiological Issues Task Force in collaboration with EU FP7 Veg-i-Trade initiated a study to assess the scientific evidence regarding the safety of water used in the production of fresh and minimally processed produce. An international group of experts identified the microbial food safety concerns of significance to fresh produce in different regions of the world. As such, they explored the relationship and interaction between water microbiology and food microbiology. Issues discussed in the ILSI report and within the EU FP7 Veg-i-Trade project include the impact of crop production and irrigation practices on the safety of fresh produce, including microbial ecology; the variation in microbial standards and recommendations for 'clean' water to be used in fresh produce production; issues associated with the use of alternative sources of water (including wastewater) in fresh produce production. Specific practices (risk factors) impacting the microbial quality of water used were identified and when possible, best practice guidelines were provided. Within the framework of quantitative microbial risk assessment, the experts also identified data gaps and needs for further risk-based efforts related to managing the safety of water used in fresh produce production. The white paper produced by this effort will be used for capacity-building and training at all stages of the supply chain and in all areas of the world. It will also provide guidance to critical national and international organizations whose mission is to improve human health by assuring adequate and equitable access to clean and abundant food and water.

S30 The Next Risk Analysis Challenge: Linking HACCP and Risk Assessments

ALEJANDRO AMEZQUITA: Unilever, Sharnbrook, United Kingdom
LAURENT GUILLIER: ANSES, Paris, France
JOHN HELFERICH: MIT, Rockport, MA, USA
ROBERT BUCHANAN: University of Maryland-College Park, College Park, MD, USA
ELIZABETH WILLIAMS: University of Maryland-College Park, College Park, MD, USA
LEE-ANN JAYKUS: North Carolina State University, Raleigh, NC, USA

Chemical and microbial risk assessment techniques are being increasingly used to provide sound food safety advice and inform food safety decisions. However, their benefits have not been fully realized due to the lack of integration into the internationally accepted food safety risk management system, Hazard Analysis Critical Control Point (HACCP). The ability to link the stringency of HACCP programs to food safety public health outcomes is crucial to ultimately developing risk-based food safety systems at the facility level. The symposium will explore how the techniques of microbial risk management metrics, risk assessment, risk ranking, systems thinking, and statistical process control can be used to enhance the ability of facility specific HACCP plans to better consider the highly complex supply chains that are becoming typical of today's global food markets.

S31 Farm to Fork Cantaloupe Risks and Interventions

TREVOR SUSLOW: University of California-Davis, Davis, CA, USA
STACY DRAPER: Sun-Rich Fresh Foods, Corona, CA, USA
DAVE PODESTA: Sysco Corporation, Chandler, AZ, USA

The consumer demand for convenient and healthy fresh-cut RTE fruit continues to increase which poses special foodborne illness risks to the retail and food service industry. Processing melons such as cantaloupes into fresh-cut fruit increases the risk of pathogen contamination.

There have been 17 outbreaks linked to cantaloupes since 1985 and most recently, this past summer, two deaths and over 141 illnesses were linked to cantaloupes. Last summer, cantaloupes were the cause of the most deadly foodborne illness outbreak in 25 years. The CDC reported 30 people died and over 133 people were sickened. Satisfying the demand for convenient and wholesome fresh-cut cantaloupe to customers continues to be a critical competitive strategy for many retail and food service companies. The purpose of this session is to take a deep dive into controlling pathogen contamination on cantaloupes from the farm to the fork. Specifically, expert leaders from academia and the fresh-cut fruit manufacturing and distribution industry will present best agricultural practices for preventing contamination during farm production, harvesting, storage, processing and transportation. Best practices relating to supplier approval, building and equipment design, sanitation, processing interventions, personal hygiene, temperature controls, traceability and training will be presented.

S32 Food Safety Sampling, Risk Assessment and Regulatory Standards: Arbitrary or Science-Based?

MARCEL ZWIETERING: Wageningen University, Wageningen, Netherlands
SEBASTIAN HIELM: Finnish Ministry of Agriculture and Forestry, Helsinki, Finland
ARIE HAVELAAR: Dutch National Institute for Public Health and the Environment, Bilthoven, Netherlands

Effective control of foodborne illnesses requires 1) disease incidence and severity assessments, 2) food safety management practices or governmental policy based on the most accurate scientific information, and 3) knowledge of food contamination levels and sampling accuracy. Inaccurate assessments of these criteria, however, occur due to A) underreporting of illnesses, B) transmission of pathogens by means other than the foodborne route, and C) errors in sampling plans, in part, based on non-uniform pathogen distribution. These uncertainties necessitate the use of estimations, predictions and modeling in determining acceptable risk and establishing microbiological standards by governmental authorities. This symposium will address these issues with leading experts in the field, discussing bacterial distributions and sampling plans, the utility of predictive modeling in risk assessment and setting policy, setting food standards in the U.S. and the FAO, as well as a discussion on the applicability of the ALOP and FSO concepts in food safety and specific case studies.

S33 Food Defense Reprised: What Do We Need to Know about Fraud, Counterfeiting and Tampering?

JOHN SPINK: Michigan State University, Okemos, MI, USA
BOB FAHY: Kraft Foods, Inc., Chicago, IL, USA
GEORGE HUGHES: U.S. Food and Drug Administration, Rockville, MD, USA

At the past two IAFP annual meetings, the food defense symposia focused on a combination of innovative tools and programs available for industry and regulatory agencies worldwide to evaluate our systems and improve our readiness for a possible intentional agroterrorism attack. Now is the time to expand our focus to include a dialogue on other intentional attacks on our food systems related to fraud, counterfeiting and tampering that can also have significant public health outcomes along with the economic risk.

The global food supply chain is becoming increasingly complex while the economic and political fragility of the world marketplace in increasing. Food fraud is growing in attention as an emerging risk, and is occurring across multiple commodities and supply chain pathways. This symposium will showcase some current activities related to food fraud tracking, insight from regulators on issues of diverted products, hijacked trucks and other intentional activities and perspectives and experiences from the processing and retail industries related to food fraud. Please join with us to raise your awareness of intentional food fraud, the possible impact to your organization, and to understand where you can get more information on this timely topic.

S34 Enhancing the Value of Restaurant Inspections to Drive Food Safety Improvements

RUTH PETRAN: Ecolab Inc., Eagan, MN, USA
BENJAMIN CHAPMAN: North Carolina State University, Raleigh, NC, USA
ANN MARIE MCNAMARA: Jack in the Box, Inc., San Diego, CA, USA

Are you using the results of your restaurant's health department reports to drive food safety improvements in your restaurants? Or are you filing health reports with passing scores in a drawer? This symposium will discuss the practical benefits of inspections, how to glean important data from the report to drive improvements in your restaurant's food safety programs and practices, discuss how to compare the approaches and differences of sets of data from different regulatory jurisdictions and how to communicate food safety risks and hazards to food inspectors and retail personnel. In addition, the Conference for Food Protection workgroup on electronic reporting of health inspection reports will be discussed. Practical information on how to use health inspection reports to drive improvements should benefit retail personnel, educators, and federal and state regulators.

S35 Prevention and Control of *Listeria monocytogenes* Contamination of Cheese

BENJAMIN SILK: Centers for Disease Control and Prevention, Atlanta, GA, USA
KATHERINE HEIMAN: Centers for Disease Control and Prevention, Atlanta, GA, USA
LORALYN LEDENBACH: Kraft Foods, Inc., Glenview, IL, USA
MICHAEL ROBERSON: Publix Super Markets, Inc., Lakeland, FL, USA
OBIANUJU NSOFOR: U.S. Food and Drug Administration-CFSAN, College Park, MD, USA

Listeria monocytogenes contamination of cheese has been a notorious threat to food safety for decades. In 1985, a landmark outbreak of listeriosis linked to Mexican-style cheese caused at least 142 illnesses and 48 deaths/fetal losses in Los Angeles County. Numerous microbiological studies subsequently characterized the growth of *L. monocytogenes* in various cheese types; a considerable amount of data have been published on specific interactions among *Listeria* spp. and various cheese properties, including the effects of moisture and other parameters, the roles of aging and ripening, and the importance of production with pasteurized milk. Nevertheless, *L. monocytogenes* contamination of cheese persists, along with costly recalls and deadly listeriosis outbreaks. Human illness may continue because of the increasing popularity of a diverse array of artisanal cheeses. Recent investigation of a large outbreak in the United States suggests that cross-contaminated cheese maybe an important source of illness. Cross-contamination seems to occur in distribution and retail settings during cutting and repackaging of cheeses, which may be another challenge for control of *L. monocytogenes*.

The symposium begins with an epidemiologist's historical overview of U.S. outbreaks of listeriosis associated with cheese, and a detailed presentation on the epidemiological, microbiological and product traceback investigations that implicated ricotta salata as a source of illness in the United States. Subsequent presentations by industry representatives and federal officials will share their perspectives on interventions to control *L. monocytogenes* in cheese. The session's purpose is to increase stakeholders' appreciations for the relationships between controlling *L. monocytogenes* contamination of cheese and overall listeriosis prevention.

S36 Food Safety for Large Events: Lessons Learned from the Olympics and Conventions

DONNA WANUCHA: U.S. Food and Drug Administration, Charlotte, NC, USA
JULIE CASANI: North Carolina Department of Health and Human Services, Raleigh, NC, USA
DOMENIC LOSITO: Retired from Vancouver Coastal Health, Vancouver, BC, Canada
LARRY MICHAEL: North Carolina Department of Health and Human Services, Raleigh, NC, USA

Managing food safety and defense for large events such as international sporting competitions and political conventions provides challenges for organizers, vendors, suppliers and regulatory agencies that oversee them. Large events are seen as economic drivers to communities and local food businesses often wish to promote their wares to a wider audience. The high-profile nature of these events draw extra attention from spectators and the media and increase the chance of intentional contamination incidents. The vast number of food handlers are volunteers with no formal training which also increases complexity for food safety risk-reduction as individuals are not well versed in prerequisite programs and HACCP-based principles. The aim of this symposium is to expose attendees to the complexities surrounding sourcing, preparing and inspecting food handling during these events. Individuals who were instrumental in organizing and participating in large events will talk about risks, how they were managed and lessons learned from their experiences. Presenters will provide case studies of the Democratic National Convention and recent Olympic games as models.

S37 What is Dry Sanitation? What is Dry Cleaning?

DON ZINK: U.S. Food and Drug Administration-CFSAN, College Park, MD, USA
STEPHEN GROVE: Institute for Food Safety and Health, Bedford Park, IL, USA
SCOTT BURNETT: MOM Brands, Lakeville, MN, USA
DEANN AKINS-LEWENTHAL: ConAgra Foods, Omaha, NE, USA

Pathogen contamination of dry ingredients has increased the focus on dry sanitation, dry cleaning and what it means to maintain a dry facility. There are many types of products considered to be dry products and the way in which they are handled can be different for each product. Peanut butter and chocolate are handled very differently than flour and spices. Additionally, recent recalls have involved multiple product types that were produced 1 or 2 years earlier but implicated from being made on the same processing equipment as the contaminated product. This raises questions of how we define microbiological lots in a dry facility and how we validate dry cleaning cycles to prove 'clean' and create lot separation.

There are many questions the industry has. What is dry sanitation? What is the difference between dry cleaning and dry sanitation? Does dry sanitation apply only to facilities where no water is introduced for cleaning? Or can it apply to a facility that wet cleans a couple times a year? How do we validate a dry clean sanitation cycle? A microbiological lot has traditionally been considered as wet clean to wet clean. How do we define a lot for dry cleaning? Does push through of product work? What kinds of dry cleaning techniques have been tested or validated? While we can't answer all of these questions in one symposium, it's important for the industry to start discussing this topic. This symposium will cover the topic of dry sanitation, dry cleaning, validation strategies, dry cleaning approaches and how we might start to define microbiological lots.

S38 Validation of Sanitation – Expectations and Approaches

JEFFREY KORNACKI: Kornacki Microbiology Solutions, Inc., McFarland, WI, USA
JOSEPH BAUMERT: University of Nebraska-Lincoln, Lincoln, NE, USA
ZHINONG YAN: Intralox, LLC, Okemos, MI, USA
MARK DRAKE: Kraft Foods, Inc., Glenview, IL, USA

Sanitation is an essential component of food safety systems at food manufacturing facilities. Both wet and dry sanitation procedures are preventive controls to reduce the likelihood of microbiological or allergen contamination from equipment or facilities that could cause a food safety hazard. Validation of sanitation is important to assure procedures are effective for preventing contamination hazards. Sampling program and the methods used to test the surfaces are also important considerations. This symposium includes information on hazard thresholds and expectations for validation of procedures to control both microorganisms and allergens to assure sanitary food manufacturing equipment and facilities. Various current, and novel or proposed validation methods will be described for both wet sanitation and dry sanitation.

S39 From Cocoa Beans to Baking Chips, Candy Bars and Bunnies: Food Safety Issues That Affect Chocolate throughout the Global Production Chain

DAVID KUHN: U.S Department of Agriculture-ARS, Miami, FL, USA
LAURIE POST: Mars Global Chocolate, Hackettstown, NJ, USA
STERLING THOMPSON: Hershey Company, Inc., Hershey, PA, USA
TIM JACKSON: Nestle USA, Inc., Glendale, CA, USA

Arguably the world's favorite and most economically significant confection, chocolate is a real big deal. Globally, chocolate is an $83 billion a year business. In the United States alone, retail sales of chocolate confectionery in all channels in 2011 reached $18 billion. 2010 U.S. chocolate consumption was 3.616 billion pounds (12.6 pounds per person), just over 20% of world consumption. Europe accounts for nearly half of all chocolate consumption.

In 2012, chocolate sales in China were expected to rise 19% to $1.2 billion. India expects to see a 7% jump to $633 million and Indonesia is projected to reach nearly $2 billion by 2015. Asian markets are expected to hold a 20% share of the global market by 2016. Europe has a net world cocoa import of nearly 60%, while the United States posts some 21% in this category. In 2010, the U.S. exported over $1 billion worth of bulk and retail chocolate to markets around the world.

Long-term, sequencing the cocoa genome should improve yield and other traits of cocoa that will certainly improve food safety. For example, by improving yields through more disease resistant cultivars, cocoa will not have to be grown in marginal areas, allowing proper harvest and drying of the beans, which will reduce post-harvest contamination issues.

Salmonella can be a problem in chocolate. Therefore, constant efforts must be made to eliminate or minimize the risks of contamination with *Salmonella* during chocolate production by introducing preventive measures and by adherence to good manufacturing practices.

Expert speakers will address the impact of unlocking the cocoa bean's genome on pre-harvest and post-harvest food safety, manufacturing contamination issues associated with cocoa, especially the potential for *Salmonella* contamination, solutions to optimize food safety throughout production, including implementing a HACCP plan in chocolate manufacturing and validation systems for roasting cocoa beans.

S40 Making Traceability Work across the Entire Supply Chain

JENNIFER MCENTIRE: Leavitt Partners, Frederick, MD, USA
TEJAS BHATT: Institute of Food Technologists, Washington, D.C., USA
PAUL LOTHIAN: Tyson, Fayetteville, AR, USA
SHERRI MCGARRY: U.S. Food and Drug Administration, College Park, MD, USA

The Food Safety Modernization Act mandated that the United States Food and Drug Administration (FDA) conduct two product tracing pilots, one including a produce item and another including a processed food and its ingredients. The Institute of Food Technologists (IFT), along with many stakeholders from industry, academia, state and federal regulatory agencies, and technology solution providers, led the efforts on these pilots, which were completed in the summer of 2012. The purpose of this session is to discuss the pilots, gain an appreciation for the scope of stakeholder input that was received, describe the methodology of the pilot process and the cost/benefit study conducted. In addition, the session will highlight the results and key findings of the pilots and IFT's recommendations to FDA for improving product tracing, which will help inform any additional FDA regulatory recordkeeping requirements for high risk foods for product tracing. The cost and benefits discussion will span from social benefits realized by reduced public health impact due to better product tracing to the factors that influence costs in the private sector when adopting and implementing the recommendations from the pilots. Additional perspectives from technology solutions, industry, and regulatory agencies will also be presented on what the implications of the pilot recommendations mean to enhancing food safety and traceability.

S41 Using the Food Emergency Response Network to Improve National Food Defense through Integration of Federal, State and Local Laboratories

RANDY LAYTON: U.S. Department of Agriculture-FSIS-FERN, Athens, GA, USA
JAMES RUDRIK: Michigan Department of Community Health Bureau of Laboratories, Lansing, MI, USA
BLAINE RHODES: Washington State Department of Health, Shoreline, WA, USA
DON BURR: U.S. Food and Drug Administration-USPHS-CFSAN, Bedford Park, IL, USA

FERN is a joint effort between the USDA's Food Safety and Inspection Service and the DHHS' Food and Drug Administration. Partnering Federal, State, and Local food testing laboratories, FERN has the capacity and capability to respond to food contamination events, both intentional and unintentional. FERN laboratories have testing capabilities that encompass the microbiology, chemistry and radiochemistry disciplines. Through the use of cooperative agreements and joint activities, the FERN has been instrumental in the development or verification of methods for the detection of threat agents in foods, responding to national and international events affecting food safety and food defense, as well as providing testing capability during unique situations. Speakers in this symposia will include Federal and State personnel relating the functions of FERN as well as specific examples of FERN activities including response to the Fukushima Daiichi Reactor meltdown, activities related to the National Political Conventions, and a laboratories experience with testing for unknowns in a food sample.

S42 Preserve This! Novel Preservatives and Applications in Acid and Acidified Foods

P. MICHAEL DAVIDSON: University of Tennessee-Knoxville, Knoxville, TN, USA
TONY JIN: U.S. Department of Agriculture-ARS, Wyndmoor, PA, USA
LARRY STEENSON: DuPont Nutrition and Health, New Century, KS, USA
BILL KING: Food Safety Consultant, Walnut Creek, CA, USA
LESLIE KRASNY: Keller and Heckman LLP, San Francisco, CA, USA

Driven by consumer demand for natural products, and the increasingly complex food safety regulatory landscape, the food industry has been looking for alternative preservatives or new applications of the more traditional preservatives to enhance value as well as assure safety. In this symposium recent trends in the use of traditional and novel preservatives will be explored, with emphasis on acid and acidified foods. Topics will include the more recent use of preservatives to enhance food safety; technological innovations to deliver antimicrobials such as package coatings; natural alternatives such as compounds extracted from plants or other natural sources, protective cultures, fermentates and purified fermentation-based antimicrobials. The symposium will wrap up with a discussion on labeling of these new and often novel compounds.

Roundtable Abstracts

RT1 Food Safety Links in Cross Border Health Initiatives between the U.S., Canada and Mexico

EILISH CLEARY, Chief Medical Officer of Health, Fredericton, NB, Canada
CARRIE RIGDON, Minnesota Department of Agriculture, St. Paul, MN, USA
WAYNE TURNBERG, Washington State Department of Health, Shoreline, WA, USA
ALLISON BANICKI, Texas Department of State Health Services, Austin, TX, USA

Health and Human Services and CDC allocated funds to the northern and southern states bordering Canada and Mexico for the U.S. Border State Early Warning Infectious Disease Surveillance (EWIDS) Project. The purpose of EWIDS is establishing a database of laboratories, drafting MOUs to share data, personnel and equipment between jurisdictions during an infectious disease public health emergency; maintaining laboratory surveillance through PulseNet and FoodNet, working with tribes whose land is on the border and crosses into Mexico and Canada, expanding the Laboratory Response Network (LRN) into Canada and Mexico; conducting tabletop workshops to discuss bi-national communication strategies, developing bi-national communication strategy including bi-national crisis and emergency health alert communication, case reporting and notification protocols and expanding sentinel and active surveillance for infectious diseases. More recently, there have been subcommittees or groups set up to consider food safety and defense issues. Four cross-border regions are focused on during this roundtable, the Northeast, Great Lakes, Northwest and California and Texas with Mexico. In the Eastern Health Initiative with seven states and provinces, Dr. Cleary is the lead for the Food Protection Programs for New Brunswick. In the south, Carrie Rigdon, Minnesota Rapid Response Team, heads up the FoodSHIELD program and maintains the sharing of food safety information with those in the Great Lakes Border Health Initiative, Food Protection and Defense Subcommittee. Wayne Turnberg is co-chair of the Epidemiology Workgroup of the Pacific Northwest Border Health Alliance. Co-chair Shaun Kennedy is the former Director of the National Center for Food Protection and Defense. The panel is expected to raise issues dealing with both food safety and potential threats under food defense where immediate reaction by members is required, but also to learn from each regional group's activities.

RT2 China Food Safety: Needs, Challenges and Approaches

YANBIN LI, University of Arkansas, Fayetteville, AR, USA
PAMELA WILGER, Cargill, Inc., Wayzata, MN, USA
XIUMEI LIU, Ministry of Health, Beijing, China
LESLIE BOURQUIN, Michigan State University, East Lansing, MI, USA
JASON WAN, Institute for Food Safety and Health, Bedford Park, IL, USA

With the rapid growth of food trade in China and recent food safety scares, China food safety has become a global concern. Although China's food safety has made great improvement, it is still not thoroughly understood and good food safety practices have not been well implemented due to system, language and cultural barriers. During the symposium and roundtable sessions on China food safety at the 2012 IAFP Annual Meeting, the challenges which multinational enterprises and education groups encountered in China provoked active discussions among the participants requiring further more communications. Hence, this roundtable is designed to focus on the differences between U.S. and China food safety systems, the challenges food safety professionals are facing and possible solutions to improve China food safety following the short symposium described in a separate proposal. The panelist from government, education and multinational companies will introduce the food safety systems, programs and experiences/difficulties they have when working in China. Approaches for solutions to overcome these issues and improve food safety levels together with Chinese shareholders will also be discussed. The objective of this roundtable is to enhance the understanding of China food safety issues and provide suggestions/solutions for the food safety professionals working in China food safety area.

RT3 Current Controversies in Food Safey

FRANCISCO DIEZ, University of Minnesota, St. Paul, MN, USA
DOUGLAS POWELL, Kansas State University, Manhattan, KS, USA
MICHAEL ROBACH, Cargill, Inc., Minneapolis, MN, USA
JOSEPH MEYER, Covance Laboratories, Inc., Battle Creek, MI, USA
DAVID ACHESON, Leavitt Partners, Glenelg, MD, USA
LINDA HARRIS, University of California-Davis, Davis, CA, USA

This interactive roundtable is intended to engender lively discussion of important topics in food safety. The session will cover three topics: "Is there scientific evidence that organic produce is as microbiologically safe as conventional produce?"; "The food industry has historically not used food safety in a competitive manner. Should food safety become a marketing tool?"; and "USDA FSIS /FDA: *Listeria* Mulligan – To Have or Not to Have?" Each topic will include a 9 minute presentation in support of (PRO) followed by a 9 minute presentation in opposition to (CON) for the proposed question. Each speaker will have 3 minutes for extemporaneous rebuttals. A 6 minute question/answer session will then follow to allow for audience participation. The session is intended to be informative, lively and humorous. Electronic gizmos will be provided that the audience can use to vote Yes/No/Undecided; one takes a vote at the beginning, and again at the end to see whether people's views have changed.

RT4 Careers in Food Safety: Traveling the Path of Those before Us

KENDRA NIGHTINGALE, Texas Tech University, Lubbock, TX, USA
DAVID GOMBAS, United Fresh Produce Association, Washington, D.C., USA
DAVID WHITE, U.S. Food and Drug Administration, Washington, D.C., USA

The focal point of this roundtable will be career experience and advice from experts in food safety. The participants in the roundtable include three speakers, one from government, industry and academia. The members of the Student PDG, Developing Food Safety Professional PDG and others striving to make the most of their careers in food safety will gain an expounding level of information through hearing the career advice from those individuals well established in the realm of food safety. The inclusion of a roundtable portion will allow for interaction between the audience and speakers. Invaluable information portrayed from those speaking will include where they have been in their careers, where they see their careers going and what they would have done differently. The hindsight provided from those speaking will provide proper foresight to those up-and-coming individuals in food safety. Key learnings from the proposed discussions will lead to audience members thinking "What can I do next with my career and education?" and "I never thought of going down that path, but I would love to take myself there." The selected speakers provide a variety of individuals who have been consistently growing in their careers, of which range from early to seasoned careers. A spread of newer professionals as well as veteran experts will allow for a diversity of experience to be portrayed.

RT5 Codex Alimentarius at 50: Accomplishments and Challenges

KAREN HULEBAK, Resolutions Strategy, LLC, Warren, VA, USA
SAMUEL GODEFROY, Health Canada, Ottawa, ON, Canada
JEROEN FRIEDERICY, European Commission, Brussels, Netherlands
JENNY SCOTT, U.S. Food and Drug Administration-CFSAN, College Park, MD, USA

Fifty years ago, two United Nations organizations, the World Health Organization and the Food and Agriculture Organization, formed the Codex Alimentarius Commission with the objective of incorporating the food safety work of FAO, WHO and other international bodies into a unified program. The first session of the Commission was attended by 120 participants from 30 countries and 16 international organizations. Today Codex convenes food safety regulatory officials from 184 member countries, together with industry and consumer observer organizations, to develop standards, guidelines and Codes of Practice ("Codex text") designed to ensure that food that is on world markets is safe for everyone everywhere. It covers a huge range of topics from good hygiene practices and HACCP implementation to residue limits for chemicals in food to the use of testing and risk assessment in food safety control programs. Once adopted, Codex standards are used both by governments and industries around the world. Over the past 50 years Codex has evolved in an increasingly transparent and inclusive way to meet ongoing and emerging challenges in protecting consumer health and ensuring fair practices in food trade. Codex standards are referenced in the World Trade Organization's Agreement on Sanitary and Phytosanitary measures (SPS Agreement) as the benchmark standards for food in international trade and they serve as a basis for national legislation in many countries.

Past and present members of the Executive body of the Codex Alimentarius Commission, and representatives of member countries or organizations will reflect on its achievements and challenges and consider the way forward to ensure that Codex continues to provide valuable advice to governments in improving their food safety standards.

RT6 Benefits of Food Safety Beyond Saving Lives

C. HAROLD KING, Chick-fil-A, Inc., Atlanta, GA, USA
WILL DANIELS, Earthbound Farm, San Juan Bautista, CA, USA
BENJAMIN WARREN, Land-O-Lakes, Arden Hills, MN, USA
GILLIAN KELLEHER, Wegmans Food Markets, Inc., Rochester, NY, USA

For those of us whose professions are centered on food safety, one of the strangest questions we hear is "Why is food safety important?" This question seems odd to us because the answer is so self-evident to us that it can be difficult to articulate and answer. This problem can be compounded when we are speaking with our own management for whom the priority is managing a successful business and who see food safety programs as simply a form of insurance. The view that food safety is insurance is, to a point, correct. Food safety programs do protect lives, brands and businesses. However, well implemented food safety programs can do more than just supply insurance and protect a business. A food safety program that is effective and thorough can provide benefits beyond protecting consumers and businesses. The extra benefits that one gets from an effective food safety program can include:

1) The same practices that are needed for growing, processing, distribution, selling, preparing, storing and consuming safe food also lead to higher quality food.
2) Safe food has a longer shelf-life, therefore reducing shrink and increasing sale potential
3) Employees that have been trained in food safety are more committed and easier to retain and an employee that is trained in food safety is a better representative of the company if they have a consumer facing role resulting in less turn over and more investment in their jobs.
4) A retail or food service environment that helps to ensure safe food is a more pleasant environment for consumers helping to retain customers and allowing greater margins on goods sold, leading to better brand perception and consumer trust.
5) Safe food practices, e.g., proper temperature control during distribution, clean and sanitary equipment, results in energy and cost savings and other potential benefits

Safe food and the processes and practices that encourage the production, processing and preparation of safe food do more than just protect lives. They add to a business' bottom line. They do this not just by saving money and protecting brands, they also strengthen brands and sales. This roundtable will help attendees understand what other food safety professionals have found to be effective ways to communicate the full value of food safety programs within their own companies.

RT7 Bridging Gaps between Scientific Assessment of Risk and Public Perception

GARY ACUFF, Texas A&M University, College Station, TX, USA
CAROLINE SMITH DEWAAL, Center for Science in the Public Interest, Washington, D.C., USA
LINDA LEAKE, Food Safety Consultant, Wilmington, NC, USA
DONALD SCHAFFNER, Rutgers University, New Brunswick, NJ, USA
DAVID GOMBAS, United Fresh Produce Association, Washington, D.C., USA
DOUGLAS POWELL, Kansas State University, Manhattan, KS, USA

Public perception of risk and the scientific assessment of risk are often not congruent. The lack of consensus between peer-reviewed evidence, epidemiology and the consumer view can often impact the image of the food industry and its food safety efforts. While data may show that more public health attention should be paid to microbial risks, lean finely textured beef, mercury in seafood, BPA in packaging and the stigma around pesticides in fresh produce have all affected how food safety is defined within these food systems. To confound this issue, consumers are presented with confusing messages surrounding organic, sustainable, local and other product differentiators. The goal of this roundtable session is to discuss differences between scientific assessment of risk and public perceptions, where challenges lie and how industry, regulators and academia can engage the public to discuss risks. The aim is to produce a road map of where risks/benefit discussions have gone awry in the past and provide a framework on how to address them in the future.

Panel members will be provided with a set of perceived risks and data-supported risks prior to the session and moderators will generate dialogue among audience members and the panel about each of the case studies.

RT8 Validation of Process Control in a HACCP System: Practical Application

MARGARET HARDIN, IEH Laboratories and Consulting Group, Lake Forest Park, WA, USA
GARY ACUFF, Texas A&M University, College Station, TX, USA
JAMES DICKSON, Iowa State University, Des Moines, IA, USA
HARSHAVARDHAN THIPPAREDDI, University of Nebraska-Lincoln, Lincoln, NE, USA

Validation is universally acknowledged as an essential part of an effective HACCP plan, however, uncertainty exists regarding implementation and value to food safety. Our proposed roundtable will address the IAFP program priority areas Safety and Microbial Quality of Foods (Dairy, Meat and Poultry, Seafood, Produce, Water) and General-applied Food Safety Microbiology (advances in sanitation, laboratory methods, quality assurance, food safety systems). This roundtable is proposed as a result of our pre-meeting workshop that was conducted at IAFP 2012 and discussions will focus on the latest changes in guidelines and regulations on validation and verification programs.

According to the United States Department of Agriculture's Food Safety and Inspection Service (FSIS), inadequate validation of process control has resulted in HACCP programs that may be ineffective at controlling the hazards they are designed to address. It is clear that the Food and Drug Administration (FDA), through implementation of the Food Safety Modernization Act (FSMA), and the FSIS will require all food processors to provide evidence of HACCP plan validation. Proper validation of HACCP controls may be difficult for all food processing operations to implement, however, there is concern that small to medium processors will find the task to be particularly burdensome. Therefore, this roundtable will provide an atmosphere for practical discussion of validation, covering experimental design, implementation and application, including appropriate microbiological testing, analysis and reporting. Convened by the Consortium of Food Process Validation Experts (CFPVE), a group of experienced scientists who support the promotion and application of scientifically sound approaches and protocols for food process validation, this roundtable will provide a platform to facilitate a dialog among researchers, processors, regulatory personnel and technology providers on proper validation implementation. As part of the roundtable, specific discussions will be directed towards small-to medium-sized meat and poultry processors to provide viable food safety options.

RT9 Microbial and Chemical Hazards in Veal: Identification of Contributing Factors, Data Gaps and Solutions

PETER EVANS, U.S. Department of Agriculture-FSIS, Baltimore, MD, USA
ADNAN AYDIN, American Veal Association, Gladstone, MO, USA
JOSEPH BOSILEVAC, U.S. Department of Agriculture-ARS, Clay Center, NE, USA
GARY ACUFF, Texas A&M University, College Station, TX, USA
PHILIP BRONSTEIN, U.S. Department of Agriculture-FSIS, Washington, D.C., USA
JOHN LUCHANSKY, U.S. Department of Agriculture-ARS-ERRC, Wyndmoor, PA, USA

Prevalences of Shiga toxin producing *Escherichia coli* (STECs) and/or chemical residues in various veal products may exceed those of other beef products*. There is a perceived need for improvement in veal processing to effectively control these hazards**. A roundtable discussion by subject matter experts would help producers, regulatory agencies and the scientific community better understand factors that contribute to the prevalence of microbial and chemical hazards in various classes of veal, leading to science based farm to fork practices for the production of safer veal products.

Target outcomes of the roundtable discussion include: 1) suggestions for defining classes of veal, 2) insights (biology, husbandry, processing) into factors which increase the prevalence/magnitude of hazards (microbial and chemical), 3) identification of farm to fork practices to minimize the occurrence of hazards, 4) identification of potential critical control points, and 5) identification of critical data gaps and research needs.

To increase the value of this discussion, an article summarizing the discussion will be prepared and submitted for publication in a food safety and/or meat industry publication.

*U.S. National Residue Program for Meat, Poultry, and Egg Products, 2010 Residue Sample Results, USDA, FSIS, OPHS, June 2012

**http://www.meatingplace.com/Industry/Blogs/Details/35266

RT10 Changes in Academic Food Safety Microbiology Teaching Laboratories: Are We Throwing the Baby Out with the Bathwater?

RUTH GYURE, Western Connecticut State College, Danbury, CT, USA
ROBERT NOBLES, Texas A&M University, College Station, TX, USA
FRANCISCO DIEZ-GONZALEZ, University of Minnesota, Saint Paul, MN, USA
FRANCISCO DIEZ-GONZALEZ, University of Minnesota, St. Paul, MN, USA
KELLY STEVENS, General Mills, Golden Valley, MN, USA
RONALD SMILEY, U.S. Food and Drug Administration-ORA, Jefferson, AR, USA

Because of recent cases of laboratory-acquired infection and universities' desires to minimize student and institutional risk, the academic sector is facing more stringent university-wide biosafety regulations in research laboratories. This has had a trickle-down effect on undergraduate teaching laboratories. An adhoc survey of undergraduate food microbiology instructors illustrates the types of changes underway, from required biosafety training of undergraduates to mandated containment of microbial pathogens (biosafety hoods) for all BSL-2 agents, to the complete prohibition of working with BSL-2 agents in the teaching laboratory environment. These changes have resulted in undergraduate students receiving less hands-on training with important foodborne pathogens such as *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus*. Such changes impact our ability to adequately deliver our curricula and train future food safety professionals in methods that will be critical to their careers. The proposed roundtable provides a venue for discussion of this issue amongst academic, industry and government stakeholders, as well as our students. Key questions to be explored include the following: To what extent is there uniformity in biosafety requirements for undergraduate teaching laboratories across U.S. campuses? What is the appropriate usage of non-pathogenic surrogates to replace microbial pathogens for the sake of student training? What is the best way to approach BSL-2 training at the undergraduate level? What would be the training implications if university Environmental Health and Safety divisions ban the use of pathogens in undergraduate teaching labs? For example, how do future employers feel about hiring young food safety professionals who do not have hands-on training for work with BSL-2 agents? How should investigators approach university biosafety officers in order to promote rigorous and applicable student training while minimizing risk? The IAFP roundtable format is ideal for promoting dialogue on this important topic. The intention is to create a white-paper for publication in Food Protection Trends.

RT11 Call to Action – Let's Put Water on a HACCP Plan

DON ZINK, U.S. Food and Drug Administration-CFSAN, College Park, MD, USA
DEAN DAVIDSON, Consultant/ILSI, Arlington, VA, USA
KAARIN GOODBURN, Chilled Food Association UK, Kettering, United Kingdom
GORDON HAYBURN, QMI-SAI Global, Toronto, ON, Canada
TREVOR SUSLOW, University of California-Davis, Davis, CA, USA
JOSEPH ODUMERU, Ministry of the Environment, Etobicoke, ON, Canada
JOSEPH COTRUVO, JosephCotruvo & Associates LLC, Washington, D.C., USA

The roundtable will be a forum to debate what the minimal water standards and testing regimen should be in different sectors of our global food production system. Waterborne illness, contaminated foods and foods rejected for export and denied entry as imports often trace back to polluted water used in irrigation, unsafe water used in manufacturing and processing, as well as breakdowns in the regulatory infrastructure. Four speakers will describe in brief vignettes (10 minutes maximum) a water quality situation and what water standard and testing protocol he/she believes is acceptable for a given food product or process. Vignettes will cover: irrigation water in FSMA, UK's law treating water as food and the implications, ILSI's new beverage processing standards, and Canadian water standards for food. Two additional panel members and a moderator will join the speakers (with audience participation) to discuss and debate when water should be treated as a Critical Control Point (CCP) in a HACCP plan, be in a pre-requisite program or stay within sanitation Standard Operating Procedures (SOP). Since water quality is so critical to the manufacturing of safe food it must be managed from sourcing/receipt through production and packaging. Effective water programs need to be in place to control microbiological, chemical and physical attributes. Handouts of various water standards – U.S. Environmental Protection Agency Safe Drinking Water Standard, World Health Organization standards, USDA irrigation water standards, Canadian Environmental Protection Act, European Drinking Water Directive and the U.S. Model Food Code will be provided to attendees. The goal of this roundtable is to develop a white paper on what ideal risk-based water standards and testing programs need to be in place to ensure sufficient controls for water quality and food safety attributable to water.

International Association for Food Protection®

Symposium Series on Food Microbiology

Sponsored by the

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

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

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

RT3 Current Controversies in Food Safety

This interactive roundtable is intended to engender lively discussion of important topics in food safety. The session will cover three topics: "Is there scientific evidence that organic produce is as microbiologically safe as conventional produce?"; "The food industry has historically not used food safety in a competitive manner. Should food safety become a marketing tool?" and "USDA FSIS /FDA: Listeria Mulligan – To Have or Not to Have?" Each topic will include a 9 minute presentation in support of (PRO) followed by a 9 minute presentation in opposition to (CON) for the proposed question. Each speaker will have 3 minutes for extemporaneous rebuttals. A 6 minute question/answer session will then follow to allow for audience participation. The session is intended to be informative, lively and humorous. Electronic gizmos will be provided that the audience can use to vote Yes/No/Undecided; one takes a vote at the beginning, and again at the end to see whether people's views have changed.

Is There Scientific Evidence that Organic Produce is as Microbiologically Safe as Conventional Produce?

FRANCISCO DIEZ-GONZALEZ, Professor, Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN, USA

The question of the microbiological safety of organic produce has been examined for more than a decade. From the moment that organic food products became popular in the late 1990's, the concern for increased risks of contamination with foodborne pathogens has been a polarizing factor among scientists, consumer groups, government and industry. The alleged increased risk of organic produce stems from the fact that organic practices limit the kind of fertilizers, and animal manure is the most widely available soil amendment. Animal manure can be a vector for transmitting pathogenic microorganisms such as Salmonella, Escherichia coli O157, Campylobacter and Cryptosporidium, but the food and human prevalence data available do not support the hypothesis that organic fruits and vegetables are less safe than conventional produce. Given that evidence, a null hypothesis that declares similar levels of risks between the two types of produce systems is the most viable alternative. In this participation, the epidemiological evidence of outbreaks caused by organic produce as well as the incidence of organic produce recalls in comparison to similar parameters for conventionally grown produce will be deliberated. A short discussion of the publicly available research studies that have compared the presence of indicator and pathogenic microorganisms in organic and conventional fresh produce will also be used as argument in support of lack of microbial safety differences. Based on the evidence discussed here, the conclusion will be that organic produce is as microbiological safe as conventional fresh fruits and vegetables.

Should Food Safety Become a Marketing Tool?

DOUGLAS POWELL, Kansas State University, Manhattan, KS, USA

A culture of food safety is built on a set of shared values that operators and their staff follow to produce and provide food in the safest manner. Maintaining a food safety culture means that operators and staff know the risks associated with the products or meals they produce, know why managing the risks is important, and effectively manage those risks in a demonstrable way.

Marketing microbial food safety at retail provides consumer choice, and is an incentive to embed a food safety culture throughout a production system, from farm-to-fork. Restaurant inspection disclosure is one form of food safety marketing; the concept should be extended to all forms of retail so consumers can choose based on safety rather than faith.

DAVID ACHESON, Leavitt Partners, Glenlg, MD, USA
LINDA HARRIS, University of California-Davis, Davis, CA, USA
JOSEPH MEYER, Covance Laboratories, Inc., Battle Creek, MI, USA
MICHAEL ROBACH, Cargill, Minneapolis, MN, USA

S7 Culture Independent Diagnostics

Most diagnoses of foodborne infections are currently done by culturing specimens from patients. However, methods that do not require isolation of an organism by culture are increasingly being implemented by clinical laboratories for some pathogens, e.g., enzyme immune assays (EIAs) for Shiga toxin-producing Escherichia coli (STEC) and Campylobacter. Molecular methods that simultaneously detect the presence of the most common bacterial, viral and parasitic diarrheal pathogens without culture have been developed and the first are being considered by FDA for licensing and will be available commercially shortly. Since all public health laboratory surveillance systems including PulseNet and NARMS use data from the characterization of cultured isolates, the introduction of culture independent diagnostic methods in clinical practice will drastically change our ability to detect, investigate and control outbreaks, follow trends to document the effect of public health interventions to prevent foodborne infections and antimicrobial resistance of foodborne pathogens. This symposium will present the problem and discuss the implications of this new technology to the food regulatory agencies and the food industry. The symposium will also address possible solutions to the problems including opportunities to strengthen the surveillance system and food safety in general.

Culture Independent Diagnostic Tests, An Overview

MARIO MARCON, Former Director, Clinical/Molecular Microbiology and Immunoserology Labs (retired), Department of Laboratory Medicine, Nationwide Children's Hospital, Clinical Professor of Pathology and Pediatrics, Ohio State University College of Medicine, USA

Culture independent assays designed for direct detection in stool of infectious agents of gastroenteritis can be categorized as either immunologic assays for detection of specific protein antigens or nucleic acid amplification tests (NAAT) for detection of unique DNA or RNA sequences. Immunoassays including microwell plate enzyme immunoassays and lateral flow immunoassays for detection of Campylobacter spp. and Shiga toxin producing Escherichia coli (STEC) have been available as FDA-cleared tests for clinical diagnostic use for several years. Very recently, two NAAT assays utilizing PCR for detection of multiple bacterial agents or multiple bacterial, viral, and parasitic agents of gastroenteritis have been FDA-cleared and others are in development. The primary advantages of culture-independent assays are that they are capable of providing results more quickly than culture, do not require a viable organism for detection, and eliminate the inherent variability in culture-based methods. The primary disadvantage in culture-independent assays is that they do not provide an isolate of the infecting organism for epidemiologic studies and antibiotic susceptibility testing when appropriate. Because of literature reports of falsely-positive immunoassay results, there is debate as to whether immunoassays for Campylobacter spp. should be used alone or whether culture should be performed on all or questionable positive immunoassay results. Some have questioned the need for performing both immunoassay for STEC and culture for E. coli serogroup O157 on all stool specimens submitted on patients with suspected bacterial gastroenteritis. There are currently no published peer-reviewed articles relating to performance of the FDA-cleared PCR assays. Based on the available data presented in the instructions for use brochure of these two assays, one can conclude that they are highly sensitive assays compared to culture or other diagnostic methods used for their evaluation on targeted pathogens in human stool specimens. It is premature to recommend use of these PCR assays as replacements for culture and/or immunoassays. Independent clinical studies published in peer-reviewed journals are needed to confirm the performance characteristics of these assays in both high and low prevalence populations. In addition, there are a number of challenges associated

with implementation of NAAT for detection of GI pathogens including (1) cost, medical necessity, and reimbursement; (2) technical expertise needed to perform the assays; and (3) impact of the lack of a culture isolate of the infecting organism on public health issues including case definition, test of cure, outbreak surveillance and changes in antimicrobic susceptibility patterns. All of these challenges will need to be addressed with practical solutions as laboratories work toward implementing NAAT for detection of GI pathogens.

Culture Independent Diagnostic Testing – The End of Surveillance of Foodborne Infections?

JOHN BESSER, Deputy Chief, Enteric Diseases Laboratory Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA

Foodborne disease surveillance is an increasingly critical component of our food safety system. Through outbreak detection and monitoring of disease trends, surveillance makes it possible to detect safety gaps that would otherwise be unrecognized, and to measure the effectiveness of control efforts. PulseNet and NARMS have been particularly effective in these respects, stimulating improvements to a wide range of food commodities by way of industry action and regulatory oversight. Unfortunately, these programs are completely dependent on a reliable flow of bacterial isolates from clinical diagnostic laboratories. The likely shift from culture to culture-independent diagnostic technology poses both threats and opportunities for these programs and for the safety of our food supply. If efforts are not taken to preserve isolates and develop new technology for public health, we could see the premature demise of PulseNet, NARMS, and other isolate-dependent surveillance programs. On the other hand, if we are successful at developing new technology for disease tracking our ability to monitor trends, detect outbreaks, and solve them quickly and precisely will likely be revolutionized. This presentation will describe the complex implications of the shift to culture-independent diagnostics, actions that are being taken to address the issue, and the promise of new technology for preserving and strengthening our disease surveillance systems.

A World without Cultures – Food Regulatory Implications

PHILIP BRONSTEIN, U.S. Department of Agriculture-FSIS, Washington, DC, USA

S37 What is Dry Sanitation? What is Dry Cleaning?

Pathogen contamination of dry ingredients has increased the focus on dry sanitation, dry cleaning and what it means to maintain a dry facility. There are many types of products considered to be dry products and the way in which they are handled can be different for each product. Peanut butter and chocolate are handled very differently than flour and spices. Additionally, recent recalls have involved multiple product types that were produced 1 or 2 years earlier but implicated from being made on the same processing equipment as the contaminated product. This raises questions of how we define microbiological lots in a dry facility and how we validate dry cleaning cycles to prove 'clean' and create lot separation. There are many questions the industry has. What is dry sanitation? What is the difference between dry cleaning and dry sanitation? Does dry sanitation apply only to facilities where no water is introduced for cleaning? Or can it apply to a facility that wet cleans a couple times a year? How do we validate a dry clean sanitation cycle? A microbiological lot has traditionally been considered as wet clean to wet clean. How do we define a lot for dry cleaning? Does push through of product work? What kinds of dry cleaning techniques have been tested or validated? While we can't answer all of these questions in one symposium, it's important for the industry to start discussing this topic. This symposium will cover the topic of dry sanitation, dry cleaning, validation strategies, dry cleaning approaches and how we might start to define microbiological lots.

What is Dry Cleaning vs. Dry Sanitation? What Defines a "Dry Plant"?

DON ZINK, U.S. Food and Drug Administration-CFSAN, College Park, MD, USA

Dry Cleaning and Dry Sanitation Techniques – Best Practices for Cleaning and Sanitizing Nut Butter Processing Equipment

STEPHEN GROVE, Institute for Food Safety and Health, Bedford Park, IL, USA

Environmental Hygiene Control in a Dry Environment

SCOTT BURNETT, Manager, Corporate Quality and Food Safety, Internal Programs, MOM Brands, Lakeville, MN, USA

Traditionally in several low moisture food manufacturing categories, approaches to sanitation have been aqueous based. Outbreaks of salmonellosis and associated recalls of implicated low moisture food products have spurred a paradigm shift in best practices for sanitation upon recognition that use of aqueous-based methods can increase microbiological risk. Putting into practice a successful and sustainable transition from wet to dry sanitation practices in plants is a considerable challenge requiring a sound scientific process to support new methods, a creative mindset in overcoming barriers to success, and a thoughtful approach in engaging sanitation personnel. This presentation will detail a cereal manufacturer's journey in 'drying out' their facilities in an effort to enhance the microbiological safety on the processing environment. It will provide perspective on the hurdles, successes, and process which evolved which became the company's 'War On Water', a sustainable set of sanitation practices which govern how equipment and its environment are maintained in hygienic condition.

How Do You Validate Dry Cleaning?

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Sanitation has become an important topic for many dry processing facilities in the food industry due to recent recalls involving low-water activity ingredients and products. One question that food companies are facing is how to validate dry sanitation practices so that the information can not only be used to assess the efficacy of the sanitation but also provide a scientific basis for microbiological lot separation. There are many sanitation strategies and techniques that can potentially be validated for different food ingredients and products. This presentation will discuss one company's journey towards validating dry sanitation practices. This discussion will cover the principles and science behind the development of dry sanitation methods or sanitation methods involving minimal water application for different ingredients and products. Dry sanitation techniques to be discussed will include brushing, product flushing, sweeping, scraping and wiping. Topics will also include information on how hygienic design of the equipment can play a part in the success of the validation as well as understanding the microbial reduction based on the developed sanitation methods. Lastly, the discussion will cover the importance of running multiple trials to demonstrate repeatability and ongoing verification activities such as assurance of sanitation effectiveness program.

Technical Abstracts

T1-01 Diversity among *Campylobacter* spp., Using *flaA* Typing, through a Commercial Poultry Production and Processing Chain

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Introduction: In Australia, *Campylobacter* is the leading cause of foodborne bacterial gastroenteritis, with chicken meat considered the primary vehicle of transmission. As chicken is the most consumed meat, this association is a major public health concern. Thus, understanding the ecology of *Campylobacter* in poultry production is critical to the development of effective management strategies.

Purpose: To determine the genetic diversity of *Campylobacter* spp. through primary production and processing in a commercial poultry operation, using *flaA* typing.

Methods: *Campylobacter* spp. (*C. jejuni* and *C. coli*) were isolated from a range of sampling sites through primary production (grow-out sheds) and subsequent processing of the same flocks. DNA was extracted and the *flaA* gene amplified using PCR. The amplicon from each isolate was digested with one of the restriction enzymes *Hin*fI or *Dd*eI. Fragments were resolved on 2% TBE agarose gels and patterns compared using computer software.

Results: Using *Hin*fI or *Dd*eI, 9 and 25 subtypes were produced, respectively, with 38 subtypes produced when the restriction patterns were used in combination. There was some correlation between *Hin*fI and *Dd*eI subtypes. There was evidence of sequential colonization and dominance of subtypes in primary production. Not only common types persisted through the production chain; a subtype found on only one farm was found on carcasses originating from other farms at the end of processing (post-chill). At the same time, some subtypes found commonly in primary production were not recovered post-chill.

Significance: The discriminatory power of *flaA* typing, based on restriction profiles generated using patterns from both *Hin*fI and *Dd*eI digestions, allows traceability of movement of strains of *Campylobacter* spp. through poultry primary production and processing.

T1-02 Comparison of the Microbiological Status of Commercial Broiler Carcasses after 24 and 48 Hour Continuous Production

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Introduction: In Australia, increased consumer demand for chicken meat has driven greater production and processing. At the same time, companies face pressures in processing, particularly with respect to environmental sustainability. The desire to extend processing operations while avoiding an increase in water use has led to concerns that the microbiological quality and safety of the product will be compromised

Purpose: To determine any difference in the microbiological status of whole chicken carcasses after 24 and 48 h of commercial production

Methods: Over three weeks, whole carcasses were collected hourly, through 24 h and 48 h of production runs, and rinsates prepared. The rinsates were analyzed quantitatively for total viable count (TVC), *Escherichia coli* and *Campylobacter* spp. using the Tempo, and qualitatively for *Salmonella* using the VIDAS UP *Salmonella* assay, all with validated modifications. All microbiological data were analyzed statistically. Throughout the processing runs, the spin chiller water was monitored for temperature, pH, oxidative redox potential (ORP), and hypochlorous acid (HOCl) concentration.

Results: There was no statistically significant difference ($P > 0.05$) in bacterial populations from broiler carcass rinsates between 24 h (mean average; log CFU TVC, 2.2; *E coli*, 0.6; *Campylobacter*, 0.8; *Salmonella* prevalence 37%) and 48 h (mean average; log CFU TVC, 2.3; *E. coli*, 0.8; *Campylobacter*, 0.8; *Salmonella* prevalence 31%) of processing. Additionally, the spin-chiller parameters were not statistically different ($P > 0.05$) between the 24 h (mean average; pH 6.3, ORP 841 mV, HOCl 5.1 ppm; carcass core temperature, 2.4°C) and 48 h (mean average; pH, 6.4; ORP, 821 mV; HOCl, 5.3 ppm; carcass core temperature, 2.4°C) continuous processing.

Significance: Based on the analytical data obtained, commercial production of whole chicken carcasses, in the processing plant under study, can be continued for 48 rather than 24 h with no significant negative impact upon microbiological quality and safety.

T1-03 The Safety and Quality of Recycled Scald Tank Water from Commercial Poultry Processing, Treated Using a Ceramic Membrane System

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Introduction: Poultry processing plants use large quantities of water and energy. With rapidly increasing consumption of chicken meat, processors are under greater strain to increase production in order to meet demand. Site-specific limitations on water consumption and discharge exist within current plants. This, in conjunction with the introduction of a carbon tax in Australia, has driven investigation of recycling water and energy within the plant to maximize processing efficiency.

Purpose: To determine if scald tank wastewater can be treated, using membranes, in a manner that maintains quality and safety, and recycled, reducing water and energy consumption.

Methods: A pilot plant, utilizing a 0.2 µm α-Al_2O_3 ceramic membrane, was used over nine months to treat screened scald tank wastewater via membrane filtration. Trials (4-16 h) were conducted in which concentrate and filtrate samples were collected and subjected to water quality analysis. The concentrate and filtrate were analyzed quantitatively for total viable count (TVC), total coliforms (TC), and *Escherichia coli* using the Tempo, and qualitatively for *Salmonella*. Total suspended solids (TSS) were analyzed using Australian Standard 3550.4:1990, turbidity was measuring using a turbidity meter and pH was measuring using a handheld pH probe.

Results: Treatment of scald tank wastewater reduced populations of *E. coli* from 5×10^5 to <1 MPN/ml. *Salmonella* was not detected in any of the concentrate or filtrate samples. TVC was reduced by 2-4 logs. All water quality indicators (TSS, pH and turbidity) met Australian and major international water recycling guidelines.

Significance: Scald tank wastewater can be treated to allow recycling, reducing the environmental impact of chicken meat processing.

T1-04 *Salmonella* in Broiler Carcass Bone Marrow and Neck Skin: Potential Sources for Ground Chicken Contamination

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Introduction: According to 2011 USDA-FSIS data, the prevalence of *Salmonella* in ground chicken was 30.9%, which is approximately four times higher than the prevalence on broiler carcasses (6.5%). Parts of the broiler neck skin with breast meat and drumsticks are used in ground chicken production. It is unknown whether the presence of *Salmonella* spp. in neck skin and in the drumstick bone contributes to ground product contamination.

Purpose: The objective of this study was to assess the prevalence of *Salmonella* spp. in broiler drumstick bone marrow and neck skin collected from a poultry processing plant in Georgia.

Methods: One week prior to slaughter, broiler flocks were tested for the presence of *Salmonella* using the bootsock sampling method. Positive flocks were followed through processing where drumsticks and neck skin samples were collected. Neck skins were rinsed and stomached and then tested for *Salmonella* by selective enrichment following USDA-FSIS methods. Bones were extracted from drumsticks, sterilized from outside, crushed aseptically to release the bone marrow, and tested for *Salmonella*.

Results: Two hundred and forty-four bones and 245 neck skin samples were collected and tested. Four percent of the rinsed skin samples and 20% of the stomached skin samples were *Salmonella*-positive. Two bone marrow samples (0.8%) were *Salmonella*-positive.

Significance: *Salmonella* entrapped in neck skin and internalized into bone marrow may pose risk toward ground chicken contamination. The outcomes of this study will be helpful to identify intervention opportunities to reduce *Salmonella* prevalence in ground chicken products.

T1-05 Effectiveness of Several Anti-microbials Used in Parts Decontamination Tank to Kill *Salmonella* and *Campylobacter* on Chicken Parts

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Developing Scientist Competitor

Introduction: Cut-up poultry parts are the top selling item in fresh meat retail. *Salmonella* and *Campylobacter* are two pathogens commonly associated with poultry and intervention strategies. Reducing these pathogens is extremely important to public health. This research focused on new technologies and chemical interventions to treat poultry parts.

Purpose: The objectives of this study were to determine the optimal contact time and effectiveness of antimicrobials added to a parts decontamination tank to reduce *Salmonella* and *Campylobacter* on chicken parts.

Methods: Skin-on drumsticks (contact time; n = 160) and cut-up poultry parts (pilot plant study; n = 80) were inoculated with *Salmonella* Typhimurium (10^8 CFU/ml) and *Campylobacter jejuni* (10^8 CFU/ml) with a 30-min attachment period. Antimicrobials (0.003% chlorine, 0.07%, 0.1% peracetic acid; PAA, or 0.35%, 0.60% Cetylpyridinium Chlorine; CPC) were evaluated for effectiveness of contact time (10, 20, 30 s) and used in a decontamination tank (23 s). Drumsticks and parts were then rinsed with 25 or 200 ml of buffered peptone water, respectively. Serial dilutions (0.10 ml) were plated on differential media for enumeration of *Salmonella* and *Campylobacter*. Non-inoculated chicken breast meat and wings from each treatment were used for sensory analysis.

Results: Contact time of antimicrobials had no differences in reducing *Salmonella* and *Campylobacter.* In the pilot plant, treatment with 0.35% or 0.60% CPC was found to be most effective in decreasing *Salmonella* and *Campylobacter* followed by 0.07%, 0.1% PAA. For CPC, the higher concentration was more effective, however, for PAA there were no difference between concentrations. Chlorine at 0.003% was least effective. The only sensory attribute that was affected was juiciness which was perceived as lower for 0.60% CPC and 0.1% PAA.

Significance: Lower concentrations of CPC and PAA combined with a parts decontamination tank are effective for reducing *Salmonella* and *Campylobacter* on chicken parts while maintaining product quality.

T1-06 The Effect of Post-chill Antimicrobials on *Salmonella*, *Campylobacter*, Shelf Life and Quality Attributes of Ground Chicken

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Developing Scientist Competitor

Introduction: Ground chicken is more likely to have higher microbiological loads than whole carcasses and parts. Therefore, it is necessary to identify antimicrobials that reduce pathogens and overall microbial loads without negatively impacting meat quality.

Purpose: The objectives of this research were to evaluate the effect of various post-chill antimicrobials on reducing *Salmonella* and *Campylobacter* and to determine the impact of these treatments on shelf life and quality attributes of ground chicken.

Methods: Five treatments (0.003% Chlorine, 0.07%, 0.10% Peracetic acid; PAA, 0.35%, 0.60% Cetylpyridinuium Chloride; CPC) were evaluated. Samples (1.81 kg; n = 120) of skin-on chicken breast and thigh meat were inoculated with *Salmonella* Typhimurium (10^8 CFU/ml) and *Campylobacter jejuni* (10^8 CFU/ml). Following a 30 min attachment time, parts were rinsed with either chlorine, PAA or CPC in a decontamination tank for 23 s. Parts were then ground, and then samples (25 g) were plated and reduction of *S.* Typhimurium and *C. jejuni* were determined. Non-inoculated ground breast and thigh meat were used for sensory and shelf life determination. Samples (n = 200) for shelf life determination were collected on days 1, 4, 7 and 10 to estimate spoilage microflora of ground chicken stored at 4°C. Additionally, sensory panels were used to evaluate the quality characteristics of cooked ground patties at days 1, 4 and 7.

Results: Ground chicken treated with 0.07% and 0.10% PAA had the greatest reductions in *Salmonella* and *Campylobacter*, followed by 0.35% and 0.60% CPC. Chlorine (0.003%) was the least effective treatment. Treatments with 0.07% and 0.10% PAA also extended the shelf life of ground chicken. None of the treatments affected sensory attributes of cooked ground chicken patties.

Significance: These findings are useful for industry to select suitable post-chill antimicrobials for improving food safety and maintaining quality of ground chicken product.

T1-07 *Salmonella* Concentration, Serotypes, and Antimicrobial Resistance on Raw Poultry in Emerging Market Countries (China, Colombia, Guatemala, Russia, and Vietnam)

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Introduction: Data collection for *Salmonella* on raw poultry will contribute to the global knowledge on protecting the food supply and facilitating U.S. and international trade, as well as providing quantitative risk assessment data on *Salmonella* at the local-country-level for food safety surveillance and pathogen phenotypic characterization

Purpose: To determine the concentrations, serotypes, and antimicrobial resistance profiles of *Salmonella* on raw poultry in China, Colombia, Guatemala, Russia and Vietnam to increase the knowledge on how to protect the global food supply and enhance food safety data collection and risk assessment at the local level

Methods: Whole chicken carcasses (n = 300/country) were collected from retail establishments (large, small, and wet markets), over a wide geographical range in these countries. *Salmonella* concentration was assessed using MPN method (according to USDA-FSIS). *Salmonella* serotypes were determined using the Kauffmann-White scheme, and disk diffusion or broth microdilution methods were used for antimicrobial susceptibility testing (up to 15 antimicrobials).

Results: The prevalence and concentration (mean log MPN/carcass) of *Salmonella* in China, Colombia, Guatemala, Russia and Vietnam were (43%, 0.74), (37%, 0.78), (45%, 1.1), (51%, 1.2), and (49%, 0.98), respectively. Most frequently detected serotypes were *S.* Enteritidis (18.7%) in China, *S.* Paratyphi B (44.7%) in Colombia, and *S.* Infantis (72%) in Russia. Multi-drug resistant (≥ 2 drugs) *Salmonella* were detected in 89% (n = 671) of isolates in China, 79% (n = 378) in Colombia, 74% (n = 73) in Guatemala, and 94% (n = 153) in Russia. Data collection is still ongoing in Guatemala, Russia, and Vietnam.

Significance: Although *Salmonella* concentrations do not appear to be high compared to USDA-FSIS 2008 data (mean log MPN = 1.8), the *Salmonella* prevalence in these countries was high. These data will be helpful to identify common and unique *Salmonella* serotypes on raw chicken meat in relation to public health. In addition, multi-drug resistant isolates may pose a significant public health risk.

T1-08 Comparison of Nitrite from Purified and Natural Sources on Inhibition of *Clostridium perfringens* Outgrowth during Cooling of Cured Turkey Breast According to FSIS Appendix B

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Developing Scientist Competitor

Introduction: USDA, FSIS Appendix B is widely used as a guideline for cooling meat and poultry products and allows extended cooling for products containing an ingoing minimum of 100 ppm $NaNO_2$. Currently, however, products cured with nitrite from natural sources do not qualify for the same extended cooling. The antimicrobial equivalency of nitrite from both purified and natural sources at comparable concentrations has not been evaluated during extended cooling following Appendix B.

Purpose: Compare the outgrowth of *Clostridium perfringens* during a 15 h cooling curve in turkey breast cured with nitrite from purified and natural sources.

Methods: Five treatments of deli-style turkey breast (74% moisture, pH 6.2-6.4, 1.4% NaCl) were prepared: control (0 $NaNO_2$), 100 ppm $NaNO_2$ from both purified nitrite and pre-converted celery juice powder, 100 ppm purified nitrite + 547 ppm ascorbate, and 100 ppm pre-converted nitrite + 547 ppm ascorbate from cherry powder. Treatments were inoculated with *C. perfringens* spores (three-strain mixture) to yield 3 log CFU/g. Individual 50-g portions were vacuum-packaged, cooked to 71.1°C and cooled from 54.4°C to 26.7°C in 5 hours and 26.7°C to 7.2°C in 10 additional hours. Triplicate samples were assayed for growth of *C. perfringens* at 0, 2.5, 5, 7.5, 10, 12.5, and 15 h by plating on tryptose-sulfite-cycloserine agar; experiments were replicated three times.

Results: Control, purified nitrite, and pre-converted nitrite treatments showed > 1 log increase after 5 h and 5.3±0.2, 4.2±0.6, and 4.4±0.2 log increases at 15 h, respectively, revealing no difference in growth inhibition between the two nitrite sources and only slight inhibition by 100 ppm $NaNO_2$ relative to control. In contrast, < 1 log growth was observed through 15 h in treatments containing 100 ppm nitrite and 547 ppm ascorbate.

Significance: This study confirmed that equivalent concentrations of nitrite, regardless of the source, provide similar inhibition of *C. perfringens* during cooling and suggests greater inhibition exists when combined with a cure accelerator.

T1-09 Prevalence of Rotavirus, Bovine Enteric Calicivirus and F-RNA Coliphages on Commercial Vacuum Packaged Beef

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Introduction: There are concerns about the potential zoonotic transmission of animal strains of norovirus (bovine enteric calicivirus, BEC), and rotavirus (RV). F-RNA coliphages, part of the gut flora and likely to be deposited on meat along with other enteric organisms during carcass dressing and processing, may be regarded as an indicator and/or surrogate for potential zoonotic enteric viruses. In addition, F-RNA coliphages can be genotyped to differentiate human (genogroups II and III) from animal sources (genogroups I and IV).

Purpose: The objective was to determine the numbers of F-RNA coliphages, BEC and RV on commercial vacuum packaged beef in Canada during the summer and winter months and perform molecular characterization of the isolates.

Methods: The entire surface of each sub-primal, purchased from retail stores, was swabbed with a sponge and viruses were dislodged from the sponges with a stomacher. Infectious F-RNA coliphages were detected by plaque assay. RNA was extracted from clarified and concentrated (ultrafiltration) supernatant and F-RNA coliphage, BEC and RV RNA were detected by real time RT-qPCR.

Results: Infectious F-RNA coliphages were recovered from 46/140 cuts at levels ranging from 0.26-80 plaque forming units/100 cm^2 where 24 samples were positive for F-RNA coliphages of human origin(1 GII and 23 GIII) and 24 were positive for F-RNA coliphages of animal origin (24 GI and 1GIV) while 4 samples contained genotypes of both human and animal origin. RV was detected in 6 samples by molecular detection while BEC was not detected. F-RNA GI was detected in only 1 sample by qRT-PCR

Significance: The low levels of infectious F-RNA coliphages are below the limit of detection by qRT-PCR in most instances. About 4% of samples were positive for RV. In addition, contamination by food handlers may be a concern as 50% of the positive samples were associated with F-RNA coliphages of human origin.

T1-10 Prevalence of Shiga Toxin-producing *Escherichia coli* (STEC) in Beef Cattle and Cattle Farms in Arkansas Delta Region

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) is a major concern for food industry and public health due to its deleterious effects. Since cattle are at the top of integrated beef production, developing pre-harvest intervention strategies would provide the most cost-effective control in beef safety.

Purpose: The objective of this study was to evaluate the occurrence of STEC in small-sized cattle farms in Arkansas Delta Region.

Methods: Rectal Swabs and environmental samples including soil, feed and water were collected from 21 farms in five counties in Northeast Arkansas over 12 months and analyzed for the presence of STEC. Each farm was visited twice (warm spring and colder fall-winter season). Presence of STEC was analyzed by selective culture method using rainbow agar and multiplex PCR for *stx* 1/2 genes and *rfb* gene for O157 serotype.

Results: The overall STEC prevalence in beef cattle farms was 59.1%; however, the STEC prevalence rate greatly varied in each farm from 0% to 76.9%. Non-O157 STEC was more prevalent (49.9%) than O157 STEC (21.2%) in rectal swabs from beef cattle but O157 STEC was more prevalent (47%) in environmental sample than non-O157 STEC (7.6%). No significant correlation was found between STEC prevalence and season or the farm location. In addition, the prevalence of STEC in beef cattle was not correlated to any of the environmental factors. The results from this study indicate a high occurrence of non-O157 STEC in small-sized beef cattle farms and it might have different transmission mechanism than *E. coli* O157:H7. The results also suggest more research needs to be done to better understand the potential reservoirs of non-O157 STEC and develop pre-harvest risk management strategies.

Significance: This was the first study focusing on prevalence of STEC in small-sized cattle farms, and knowledge obtained from this research will ultimately improve food safety by significantly reducing beef-associated human STEC infections.

T1-11 Detection and Characterization of *Salmonella* from Butchery Utensils

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Developing Scientist Competitor

Introduction: *Salmonella* is a foodborne pathogen highly associated with beef consumption. Cross-contamination during beef manipulation is a common source of *Salmonella* to end cuts, and the butcher shops utensils that keep contact with beef play an important role in this context.

Purpose: The present research aimed the identification of *Salmonella* spp. in key utensils currently used in butcher shops, and characterization of the obtained isolates by genetic markers in order to track their origin and persistence in this environment.

Methods: Four butcher shops located in Viçosa city (Minas Gerais state, Brazil) were selected, and each one was visited eight times for sampling of the following utensils (100 cm² per sample): butcher hands, chopping boards, knives, refrigerated room floor, grinders, and tenderizers. Thirty-two samples from each utensil were obtained and subjected to *Salmonella* spp. detection according to ISO 6579, being the suspect isolates subjected to PCR to confirm the identification (gene *omp*C). *Salmonella* confirmed isolates were subjected to *Xbal* macrorestriction and PFGE, and PCR reactions to detect virulence genes (*inv*A, *sef*A, and *spv*C).

Results: *Salmonella* spp. was detected in five chopping board samples, obtained from three distinct butcher shops: one butcher shop presented *Salmonella* positive results in chopping boards in three consecutive visits. A total of 15 suspect isolates were obtained from positive samples, being all confirmed as *Salmonella* by PCR (*omp*C). PFGE showed 10 isolates obtained from two distinct butcher shops with 100% of identity; isolates obtained from the butcher shop with *Salmonella* recurrent results presented 80% of similarity. Thirteen isolates presented positive results for *inv*A, eight for *sef*A, and none for *spv*C.

Significance: The obtained results indicated the relevance of chopping boards as *Salmonella* spp. source in butcher shops, and also a common source of this foodborne pathogen for distinct butcher shops. The presence of virulence genes highlights the relevance of *Salmonella* as a potential hazard associated to beef. Acknowledgments: CAPES, CNPq, FAPEMIG.

T1-12 Prevalence of *Salmonella enterica*, *Escherichia coli* O157:H7 and Non-O157 Shiga Toxin-producing *Escherichia coli* in Beef Cuts Sold at Retail Markets in Costa Rica

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Developing Scientist Competitor

Introduction: In Latin America, approximately 70% of diarrheal cases are due to food- and waterborne illnesses. In Costa Rica, data on the presence of pathogenic bacteria exist; however, these are limited, particularly regarding the occurrence of emerging foodborne pathogens in raw meat products.

Purpose: This study was undertaken to determine the presence of *Salmonella enterica* and seven different Shiga toxin-producing *Escherichia coli*serogroups (STEC) in retail meat samples collected in urban and rural areas of Costa Rica.

Methods: Seventy-seven retail meat establishments (57 urban, 20 rural) were visited. Three non-visceral beef cuts were purchased at every store and each piece was swabbed individually with a sterile sponge. An FSIS-approved PCR protocol was used to assess the presence of *S. enterica*and STEC serogroups O26, O45, O103, O111, O121, O145, and O157 using the DuPont Qualicon BAX® System.

Results: *S. enterica* prevalence was 1.7% (4/231), of which three samples were collected in urban settings and one in rural areas, whereas STEC were deemed positive in 4.8% of the samples (11/231), six in urban stores and five in rural ones. Among the 11 STEC positive samples, 37 serogroup-specific markers were detected and included markers for O45, O121, and O103 were the most prevalent, with frequencies of 11/37 (29.7%), 8/37 (21.6%), and 7/37 (18.9%), respectively. Both O26 and O145 serogroups were present at 16.2% (6/37), while no O111 or O157 serogroups were detected.

Significance: The results of this study help fill the knowledge void regarding occurrence of pathogenic microorganisms in meat products in Costa Rica. These data can help prioritize food safety policies, interventions, and control measures in Costa Rica.

T2-01 High School Students as the Target of Food Safety Education: Successful Results from a Pilot Study

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Developing Scientist Competitor

Introduction: High school students represent an important population for food safety knowledge intervention because many are or will soon be responsible for food preparation for themselves and others.

Purpose: The objectives of this study were to perform and assess a food safety and hand hygiene curriculum in high school students that included an educational comic book.

Methods: From a convenience sample of 231 students at a predominantly minority Chicago high school, baseline and follow-up cross-sectional surveys were conducted with 195 and 159 students, respectively. The 34-question survey instrument was self-administered to obtain information about knowledge, behaviors, and personal hygiene of the students. Frequencies of correct answers to each knowledge question and self-reported behavioral changes were examined to determine post-intervention change. Student comments to behavioral change questions were examined to determine overall themes.

Results: Compared to the baseline score, the intervention produced a knowledge score increase of 7.3 questions (from 37% to 62%; $P < 0.05$). Students were significantly more likely to identify as false that it is safe to thaw a frozen chicken breast by putting it on the counter at room temperature (increase from 32% to 70%; $P < 0.05$). Knowledge that reaching a high enough temperature as measured on a metal stem thermometer is the only way to determine that a frozen chicken breast is safe to eat increased from 12% to 55% ($P < 0.05$). Among the self-reported behaviors and comments, commonly reported areas of behavioral change included washing hands for a longer period of time, sanitizing surfaces after contact with potentially contaminated food, making sure to cook meat to the correct temperatures, thawing meat appropriately, and avoiding cross-contamination.

Significance: These data demonstrate substantial knowledge increases and behavioral change in a predominantly minority high school student population following an educational intervention. Further research enhancing the curriculum based on lessons learned and determining efficacy in other populations is needed.

T2-02 Implementing Good Agricultural Practices (GAPs) in School and Community Gardens

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Introduction: The interest in school and community gardens has increased over the past decade as a method to connect students and communities with food production. Although fresh produce is a common source of pathogens, a regulatory authority or guidance does not exist for gardens.

Purpose: The purpose of this study was to create a set of evidence-based garden-specific best practices for gardens, place into an outreach intervention, and evaluate its effectiveness. The best practices suite was based on the U.S. FDA's Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables.

Methods: The project was structured as a summative evaluation with a pre-intervention and post-intervention visit to gardens. Gardens (n = 20) were selected through a convenience sample and information was collected on practices through semi-structured interviews as well as observations. Interview response data were coded and gardens were awarded a risk score based the USDA Good Agricultural Practices/Good Handling Practices Audit Verification Checklist.

Results: Baseline data demonstrated differences within the sample with respect to fencing (n = 13), handwashing policies (n = 4), and compost bins placement (n = 14). Post-intervention, five more gardens required pre-harvest handwashing ($P < 0.01$) and four gardens reduced risk by switching to municipal water. The mean risk score on the first visit was 54.74% but it was 65.14% by the second visit, an increase of 10.39% ($P < 0.001$). Sixteen gardens improved their overall scores, three gardens lessened their scores, and one garden stayed the same.

Significance: With readily-available information, garden managers can take steps towards a safer garden. Food safety was not a topic on which they concentrate, but they were able to make critical changes with the curriculum. This intervention is a suitable starting point for employing GAPs in school and community garden settings.

T2-03 Food Safety Knowledge and Behavior among Low Socioeconomic African-Americans in Chicago: Results of Focus Groups

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Developing Scientist Competitor

Introduction: Foodborne illness disproportionally affects the African-American community due to a large percentage of this population living below the poverty level and holiday food preferences that may include pork chitterlings. There is limited information of food safety knowledge regarding food handling and meat preparation among this population.

Purpose: To inform the design of a targeted educational intervention, this study determined food safety knowledge gaps related to food handling and meat preparation and food safety in general.

Methods: Five focus groups were conducted including a total of 34 African-American City of Chicago Department of Public Health Clinic clients. Questions on food safety knowledge gaps and behaviors were discussed and a knowledge survey was administered. Focus groups were audio-taped and the information was transcribed and analyzed allowing for the determination of key food safety knowledge gaps and behaviors.

Results: Twenty-six percent (26) of participants were female. Food safety knowledge gaps among focus group discussion participants indicated participants were less concerned with foodborne illness risk at home compared to getting ill from food prepared outside the home. Usage of a meat thermometer to ensure doneness of meat instead of utilizing visual cues of doneness only became a concern when preparing food for others outside the home. Only 6% (2) owned a meat thermometer. Twenty-six percent (9) reported it was acceptable to defrost frozen meat on the kitchen counter. Hand washing was emphasized before cooking rather than as a cross contamination preventative measure.

Significance: These results revealed important issues to emphasize in educational material targeted to low socioeconomic African-Americans. A photonovella incorporating these issues has been created and is currently undergoing testing in this population for efficacy at educating and changing related food safety behavior.

T2-04 Substantial Efficacy of a Food Safety Educational Intervention for Persons Living with AIDS Using a Comic Book Format

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Introduction: Persons living with AIDS who have CD4 T-lymphocyte counts below 200 cells/ml are highly vulnerable to foodborne enteric infections with the potential for substantial morbidity and mortality. Educational materials intended for this immunocompromised population have not been assessed for their efficacy in improving knowledge or encouraging behavior change.

Purpose: To determine the efficacy of a food safety educational intervention for persons living with AIDS using a comic book format.

Methods: AIDS patients in four healthcare facilities in Chicago, New Orleans, and Puerto Rico were interviewed to determine food safety knowledge gaps and risky behaviors. A food safety educational comic book that targeted knowledge gaps was created, piloted, and provided to these patients who were instructed to read it and return at least 2 weeks later for a follow-up interview. The overall food safety score was determined by the number of the 29 knowledge/belief/behavior questions from the survey answered correctly.

Results: Among 150 patients who participated in both baseline and follow-up questionnaires, the intervention resulted in a substantial increase in the food safety score (baseline 59%, post-intervention 81%, $P < 0.001$). The intervention produced a significant increase in all the food safety knowledge, belief, and behavior items that comprised the food safety score. Many of these increases were from baseline knowledge below 80 percent to well above 90% such as concerning the potential danger of eating eggs that are not fully cooked, store bought hot dogs that have not been cooked, and food that is past its expiration date even if it does not smell or look bad. Most (85%) of the patients stated they made a change to their behavior since receiving the educational booklet.

Significance: This comic book format intervention to educate persons living with AIDS was highly effective. Future studies should examine to what extent long-term behavioral changes result.

T2-05 Principles of Effective Produce Safety Training and Program Delivery for Fresh Fruit and Vegetable Farmers

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Introduction: The Produce Safety Alliance (PSA) is developing a science-based, on-farm food safety education and training program for fresh fruit and vegetable farmers and regulatory personnel. This curriculum will help prepare farmers to meet the first ever fresh produce regulation promulgated by the FDA as part of the Food Safety Modernization Act and will aid in providing a consistent food safety message for both growers and regulatory personnel.

Purpose: The purpose of the focus groups was to gather data directly from farmers to understand their educational needs and expectations from training programs, including why and where farmers prefer to attend trainings. Understanding their learning preferences will ensure the curriculum content, design and delivery will effectively meet their needs.

Methods: Eight focus groups were conducted with eighty-nine fruit and vegetable farmers across the country representing different geographical locations, farm sizes, cultures, commodities and production practices. The focus groups were tape recorded and qualitative data was transcribed and analyzed using constant comparative analysis.

Results: Several common themes emerged, including the need for solution-oriented resources and assistance in defining risks. Other outcomes reflected cultural, commodity or market specific variations such as information about farming with horses or specific practices for farmers' markets. Farmers preferred attending trainings that were less than one day, conducted during their commodity off season and within 20-60 miles of their farm.

Significance: Providing fundamental knowledge regarding fresh produce safety that is designed to meet defined needs will ensure that all farmers, despite farm size, commodity grown, geographic location or regulatory pressure will be able to understand and implement practices which reduce food safety risks on the farm. This data will ensure that both farmers and regulatory personnel are exposed to the same information regarding on-farm food safety risks and practices to reduce risks.

T2-06 Tracing Temperature Patterns of Cut Leafy Greens during Transportation

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Introduction: Cut leafy greens (fresh greens that have been chopped, shredded, sliced or torn) have been linked to over 40 outbreaks of foodborne illness since 1990; temperature abuse has been cited as a factor in many of these outbreaks. The 2009 U.S. FDA Model Food Code states that fresh-cut leafy greens be recognized as a time/temperature control for safety (TCS) food and are to be received at food service establishments at or below 5°C.

Purpose: This study determined whether a cost-effective method using commonly available coolers was suitable for transporting cut leafy greens to meet Food Code requirements. Evaluating effectiveness of these transport methods is important for small-scale farmers and processors attempting to meet the transport/delivery requirements.

Methods: Cut leafy greens with a starting temperature of 4°C were held in three different types and sizes of coolers with either ice or ice packs over four hours in simulated transport system to mimic delivery conditions. Temperatures of cut leafy greens were recorded using data loggers. The experiment was replicated in triplicate for each cooler/cooling method. Costs of systems were also evaluated.

Results: All coolers containing ice remained under 5°C for the four hours. Styrofoam coolers with ice packs reached above 5°C after 40 minutes of simulated transport. Cut leafy greens in small coolers with ice packs reached above 5°C after 90 minutes. Cut leafy greens in large coolers with ice packs reached above 5°C after 220 minutes.

Significance: Transporting leafy greens with ice in any of the tested coolers is an effective method to meet Food Code requirements. Transporting using ice packs is suitable if destinations are within 40 minutes of origin. This information is crucial for small farmers as a cost-effective measure to transport fresh-cut leafy greens. Farmers can use this information to make the best decision about transportation method based on their own amount of product and transportation time.

T2-07 Validation of Chlorine Efficacy to Disinfect Process Wash Water Simulating Industrial Conditions

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Introduction: Disinfected water is necessary to avoid cross-contamination during washing in the fresh-cut industry. Chlorination is the most used method but excessive doses, particularly hyperchlorination, have several potential negative effects. Alternative disinfection methods have been tested but although they can be just as effective as chlorine, they are always more expensive. The main goal for the fresh-cut industry should be the management of the optimal use of chlorine, based on its antimicrobial efficacy.

Purpose: The purpose of this study was to validate the efficacy of chlorine to inactivate *E. coli* O157:H7 in process wash water simulating the conditions in the fresh-cut industry.

Methods: A cocktail of *E. coli* O157:H7 strains was used. To simulate an industrial process, a washing tank containing tap potable water was continuously filled with concentrated process wash water with high organic matter and inoculated with the *E. coli* cocktail (5 log CFU/ml) at a flow rate within the range 3.6 – 7.5 l/h. A peristaltic pump dosed a ca. 2 mg/l free chlorine solution to the washing tank during the whole duration of the test. Free chlorine concentration was adjusted to ca. 0.5 - 3 mg/l.

Results: Maintenance of a concentration of free chlorine of ca. 0.5 mg/l significantly reduced the accumulation of *E. coli* O157:H7 in process wash water by 2.5 logs compare to untreated process wash water. However, free chlorine concentrations of ca. 3 mg/l, were enough to completely inactivate *E. coli* in process wash water.

Significance: Results indicate that chlorine is an effective treatment to inactivate *E. coli* O157:H7 under industrial conditions using around 3 mg/l free chlorine, with no effect on COD removal and a very low generation of THM. Validation of the chlorine efficacy by using a dynamic system might facilitate the implementation of selected treatments in the fresh-cut industry.

T2-08 Inactivation of Human Enteric Virus Surrogates on Stainless Steel Surfaces by Non-thermal Plasma

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Introduction: Non-thermal atmospheric plasma (cold-plasma) technology can effectively treat thermally-sensitive food products to reduce the incidence of foodborne bacterial disease outbreaks. When a gas passes through cold-plasma, the resulting reactive species (oxygen, ozone, and free radicals) formed by electron collision have antimicrobial effects against bacteria. However, cold-plasma application for the inactivation of foodborne noroviruses, the leading cause of non-bacterial gastroenteritis, needs to be investigated.

Purpose: The goal of this research was to determine the ability of non-thermal plasma to inactivate human enteric virus surrogates on stainless steel surfaces.

Methods: Cultivable human norovirus surrogates, feline calicivirus (FCV-F9) and murine norovirus (MNV-1), and bacteriophage MS2 at ~7 log plaque forming units (PFU)/ml were inoculated and dried on sterile stainless steel coupons. These coupons were treated with the patented One Atmosphere Uniform Glow Discharge Plasma for 0, 1, 2, 5, and 10 min. Recovered viruses were evaluated by plaque assays using their respective host cells and compared to untreated controls. Each experiment was replicated thrice in duplicate. Data was statistically analyzed using SAS at $P < 0.05$.

Results: Data obtained showed that FCV-F9 was the most sensitive among the tested viruses to cold-plasma treatment, followed by MS2 and MNV-1. FCV-F9 showed reduction of 2.34 and 3.55 log PFU after 1 and 2 min, respectively and to non-detectable levels after 5 and 10 min. MS2 was reduced by 2.03 and 5.46 log PFU after 2 and 5 min, respectively and to non-detectable levels after 10 min. MNV-1 was reduced by 0.56, 1.61, 1.95, and 3.16 log PFU after 1, 2, 5 and 10 min, respectively.

Significance: Non-thermal atmospheric plasma shows promise for use as an alternative disinfection technology against human enteric virus surrogates on stainless steel surfaces to prevent viral transmission. The technology has potential application for microbial inactivation on produce and other thermally-sensitive food products to prevent foodborne viral outbreaks.

T2-09 Evaluation of Deep Cleans in Retail Delis as a *Listeria monocytogenes* Control Strategy

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Developing Scientist Competitor

Introduction: Delicatessen meats sliced at retail are estimated to cause 83% of listeriosis cases resulting from consumption of contaminated deli meat annually. Previously tested daily SSOPs by our group did not reduce *Listeria monocytogenes* (LM) contamination in delis with evidence of highly prevalent and persistent LM contamination thus warranting alternative aggressive mitigation strategies.

Purpose: The purpose of this study was to assess the efficacy of an aggressive twelve hour deep clean SSOP aimed to reduce persistent LM environmental contamination in retail delis.

Methods: We tested the efficacy of a deep clean SSOP in nine delis in three states. The developed SSOP protocol combined Food Marketing Institute recommended daily SSOPs and input from experts in *Listeria*control from food manufacturing and sanitation. The SSOP was executed by a trained professional cleaning service during a 12 hour shut-down period. A modified BAM protocol was used to detect LM in 28 food and non-food contact surfaces samples taken immediately before and after each cleaning, and in samples taken monthly for 3 months.

Results: Delis (n = 5) with historically low LM prevalence (<5% samples; >300 samples tested) had no statistically significant changes in LM positive samples after the deep clean. Deep cleans in four delis with historically high LM prevalence (≥10%) had varying efficacy. In two delis, deep cleaning reduced LM positive samples by 50% and 75%; one deli had no change and one store had increased LM positive samples immediately post-deep clean. LM positive samples returned to pre-deep-clean levels in highly prevalent delis based on monthly sampling.

Significance: Deep cleaning delis can be immediately effective in delis with high LM prevalence and persistence and do not increase LM prevalence in stores with historically low LM prevalence. However, a single deep clean may not be sufficient to mitigate LM in some retail delis with evidence of persistence.

T2-10 *Listeria monocytogenes* in Smoked Fish Production

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Introduction: Reported cases of listeriosis have increased in the UK over the last decade. Although the overall incidence of listeriosis is small (4 cases /mil in 2007), the disease affects especially vulnerable groups (e.g., elderly people) with high mortality (up to 40%). The majority of cases appear to be sporadic and therefore it is difficult to identify the source of disease. Smoked fish (salmon, mackerel, herring and white fish) are a potential source of listeriosis, because the multiplication of pathogens (*Listeria monocytogenes*) can occur even under vacuum packaging conditions at refrigeration temperatures.

Purpose: This study investigates management practices for reducing the risk of *L. monocytogenes* in the Scottish smoked salmon industry by gathering questionnaire information from factory and farm visits as well as collation and analysis of *Listeria*data along the processing chain.

Methods: Sixteen visits to smoked salmon premises were conducted between June and November 2011, interviews were carried out based on a questionnaire.

Results: The results indicate that most processors carry out appropriate food safety practices, but some improvements are needed in order to minimize the risk of *Listeria* contamination. Improvements in refrigerated storage were required for some producers because of the risk of condensation dripping onto product from the ceiling. Small food business operators require additional information on how cleaning and sanitation throughout the process can reduce contamination of the final product. Furthermore, guidance describing the best way of determining shelf life was requested by small processors. Fifty-six percent of the smoked salmon producers (mostly large and medium size) tested the product for *Listeria monocytogenes* and the prevalence ranged between (0 to 12%) between producers. Most processors rarely exceeded (i.e., once every several years) the statutory limit set by the European Union (>100 CFU/g or presence in 25 g). The small producers did not undertake product testing for *Listeria*because of high test costs and lack of technical expertise.

Significance: This study obtained a snapshot of the levels of *Listeria* in smoked salmon as well as industry practices. It was concluded that sharing expertise between producers, especially to smaller processors would be beneficial in terms of consumer protection. The study highlighted the need to provide training through workshops and information by an interactive web-based tool.

T2-11 Assessing Efficacy of Commercially Available Antimicrobial Compounds for Controlling Growth of *Listeria monocytogenes* in Modified BHI Broth and Cold-smoked Salmon

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Introduction : Presence of *Listeria monocytogenes* on foods can lead to a serious foodborne illness. Control of *L. monocytogenes* on cold-smoked salmon remains a considerable challenge, as recalls of cold-smoked salmon due to *L. monocytogenes* contamination continue to occur.

Purpose : This study evaluated antimicrobial compounds nisin, lauric arginate (LAE), epsilon-polylysine (EPL), and chitosan for their effectiveness in reducing initial levels as well as inhibiting growth of *L. monocytogenes* in a laboratory medium and on cold-smoked salmon.

Methods : Brain Heart Infusion broth (4.65% wp-NaCl, pH 6.1) and cold-smoked salmon slices containing nisin (10 ppm), LAE (10 ppm), EPL (250 ppm), or chitosan (200 ppm) were inoculated with *L. monocytogenes* and incubated at 7°C. Viable cells were enumerated over time and growth parameters lag phase (λ), growth rate (μ_{max}), initial cell density (N_0), maximum cell density (N_{max}), and maximum log reduction (N_{maxR}) were calculated.

Results : In broth, nisin and LAE caused significantly greater N_{maxR} than the rest of the treatments, achieving reductions of 5.13 log CFU/ml and 4.02 log CFU/ml, respectively ($P < 0.05$). Regrowth of LAE-treated *L. monocytogenes* was significantly delayed (23.42 days) ($P < 0.05$). In addition, μ_{max} and N_{max} of LAE-treated cells were significantly lower than most treatments (0.12 log CFU/ml/day and 6.33 log CFU/ml, respectively) ($P < 0.05$), with nisin being the only exception. Preliminary results in inoculated salmon found no evidence for antimicrobial activity of LAE against *L. monocytogenes*. The reduction in efficacy compared to broth is hypothesized to be due to partitioning of LAE molecules into the lipid phase of salmon.

Significance : Despite strong antimicrobial activities exhibited by LAE in broth, its application to foods that are high in fat may be limited. This highlights the importance of validating antimicrobial efficacy in the target food matrix.

T2-12 Detection of Histamine-producing Bacteria in Scombrotoxin-forming Fish from the Gulf of Mexico

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Introduction: Previous research has identified *Morganella morganii* as the main histamine-producing bacteria (HPB) in scombrotoxin-forming (SF) fish. Control measures are frequently targeted for the mitigation of this HPB. Recently, molecular based methods have advanced the detection of high-HPB. These new methods may help in understanding the role of *M. morganii* and other high-HPB in scombrotoxin poisoning.

Purpose: The objective of this study was to examine the prevalence and distribution of high-HPB in SF fish from the Gulf of Mexico.

Methods: Gill, skin, and intestine swabs from 54 SF fish were enriched in histidine broth (tryptic soy broth with 1% histidine, 1.5 % NaCl) for 24 h at 30°C. Enrichments were analyzed for total and species-specific HPB by real-time PCR. HPB were isolated and confirmed from positive samples and their histamine production was measured. *Photobacterium damselae* and *M. morganii* histamine production was further compared in ground tuna inoculated at 5.5 log MPN/g for 8 h at 30°C.

Results: *P. damselae* (102/162) was most frequently detected followed by *M. morganii* (34/162), *Enterobacter aerogenes* (10/162), and *Raoultella planticola* (4/162). *P. damselae* and *M. morganii* were detected in gills (30/54, 6/54), intestine (31/54, 11/54), and skin (41/54, 17/54) but *E. aerogenes* and *R. planticola* were infrequently detected on skin (6/54, 2/54) and gills (4/54, 2/54). *P. damselae*, *M. morganii*, *E. aerogenes*, and *R. planticola* isolates produced on average 3063, 4111, 4687, and 4872 ppm histamine after 48 h in histidine broth at 30°C. Finally, *P. damselae* produced significantly higher histamine (1031 ppm, $P = 0.041$) and grew to a significantly higher density (8.4 log MPN/g, $P = 0.050$) in ground tuna compared to *M. morganii* (98.5 ppm; 7.3 log MPN/g) after 8 h at 30°C.

Significance: Using newly developed molecular methods, we found that *P. damselae* was more frequently detected in SF fish from Gulf of Mexico and produced more histamine in tuna muscle than *M. morganii*. This HPB is a significant concern and should be accounted for in the control of scombrotoxin poisoning.

T3-01 Assessing Country Food Safety Risk for Bovine Spongiform Encephalopathy

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Introduction: In 2010, the Australian Government implemented a revised policy for assessment of Bovine Spongiform Encephalopathy (BSE) food safety risk from overseas beef products. The policy, consistent with WTO rules, allows countries, including those that have reported BSE cases, to apply for access to the Australian beef market. Access is dependent on provision of comprehensive data on BSE controls within the exporting country, to allow a desk-based food safety risk assessment to be conducted. In addition, an in-country verification process is used to evaluate the effectiveness of systems and controls.

Purpose: To develop an evidence-based, transparent and consistent risk assessment and country categorization process comparable to that used by the World Organization for Animal Health (OIE) that incorporates additional criteria around animal traceability and slaughtering practices.

Methods: A documented risk assessment process, including an audit protocol for in-country inspections, was developed.

Results: Eight countries have been assessed utilizing methodology developed by Food Standards Australia New Zealand. The country risk levels established have been consistent with or more favorable to those established by OIE. The in-country verification component has derived benefits in better understanding country systems and their oversight, observing the implementation of and compliance with legislation and standards and appreciating animal husbandry and agricultural practices within the country that support the mitigation of BSE risk.

Significance: The Australian process for assessing the BSE risk level of a country incorporates additional criteria around animal traceability and cattle slaughtering practices and is supplemented by an in-country inspection and verification component. The Australian process embraces transparency by inviting countries to comment on and/or challenge risk assessment findings, and at the same time provides flexibility for countries to provide additional data to justify a favorable BSE risk status. The in-country evaluation of systems has supported, and in some cases, elevated the BSE status of countries.

T3-02 Development of Dynamic and Probabilistic Models to Predict *Listeria monocytogenes* Growth

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Developing Scientist Competitor

Introduction: Dynamic temperature and storage time are critical factors for *Listeria monocytogenes* growth on fresh meat.

Purpose: This study developed dynamic and probabilistic models to predict the fates of *L. monocytogenes* on fresh pork.

Methods: A 10-strain mixture of *L. monocytogenes* was inoculated on fresh pork skin (3×5 cm) at 4 log CFU/cm^2. The inoculated samples were then stored aerobically at 4 (10 days), 7 (10 days), 10 (10 days), 15 (4 days), 20 (4 days), 25 (12 h), and 30°C (12 h). The microbiological data were fitted to the Baranyi model to calculate maximum specific growth rate (μ_{max}; log CFU/cm^2/h) and lag phase duration (LPD; h). The μ_{max} and LPD were then fitted to a polynomial equation and the Davey model, respectively. Accordingly, *L. monocytogenes* growth was simulated under constant and dynamic temperatures. Of 49 combinations (temperature (7)×sampling time (7)), the combinations with significant growth ($P < 0.05$) were designated '1', and the combinations with non-significant growth were given '0'. These growth response data were analyzed with the logistic regression. The model performances for dynamic and probabilistic models were evaluated, using observed data.

Results: *L. monocytogenes* growth was not observed before 12 h of storage at 4-10°C, and the pathogen started to grow ($P < 0.05$) after 6, 4, 2, and 2 h at 15, 20, 25, and 30°C, respectively. After a primary model was developed, μ_{max} gradually increased, but LPD decreased as storage temperature increased. The developed secondary models were also acceptable (R^2=0.974). Growth/no growth interfaces of *L. monocytogenes* were produced by the probabilistic model at P = 0.1, 0.5, and 0.9. In addition, the model performance was acceptable for kinetic model (RMSE=0.282) and probabilistic model (concordance index=99.2%).

Significance: The results indicate that the developed models should be useful in predicting kinetic behavior and growth probabilities of *L. monocytogenes* on fresh pork.

T3-03 *Escherichia coli* O157:H7 and *Salmonella* Biofilms Formation and Removal Using Various Sanitizers

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Introduction: Biofilms formed by pathogenic organisms are of great concern in food processing environments. Biofilm allows resistance of bacteria to antimicrobials facilitating their spread and persistence.

Purpose: This study investigates biofilm formation by *Escherichia coli* O157:H7 and *Salmonella* at different temperatures and media concentrations on glass and cement coupons and tested commercially available sanitizers and hot water for biofilm removal.

Methods: Biofilms of *E. coli* O157:H7 (ECH7C and ECH7P), *Salmonella* Enteritidis (SEP) and *Salmonella* spp. (SSP) were developed on glass and cement coupons in Tryptone soy broth (TSB), TSB + 0.5% glucose, TSB + 1% glucose, TSB + 2% sheep blood and TSB + 5% sheep blood for 5 days at 11°C and 28 ± 2°C. Biofilm quantification was done using crystal violet binding assay. Biofilms removal was tested by exposing biofilms to 20 ml of sanitizers, Diskol/Morigad and Hot water (85°C) for 5 mins. The experiment was done in triplicates.

Results: Significantly ($P < 0.05$) more biofilms (OD=optical density) were formed at 28 ± 2°C than 11°C on cement (0.451-0.565) than on glass (0.277-0.358) for all strains. Addition of 1% glucose or 2% sheep blood significantly enhanced biofilm formation. For biofilms developed at 11°C, hot water removed more *E. coli* O157:H7 biofilms on cement (0.007-0.118) than glass (0.004-0.030). *Salmonella* biofilms removal on cement (0.001-0.148) was higher than glass (0.001-0.058). Diskol was most active for *E. coli* O157:H7 biofilm reduction especially those formed (28 ± 2°C) in TSB broth (0.010–0.076) and TSB broths with supplements (0.007–0.145) on glass. On cement, Morigad was most effective (0.009-0.216). *Salmonella* biofilms were more resistant than *E. coli* O157:H7 biofilms at 28 ± 2°C. SEP biofilms grown in TSB+0.5%glucose was resistant to Diskol/Morigad and hot water but susceptible when grown in TSB+5% sheep blood (0.044-0.046).

Significance: Understanding of the influence of temperature, anti-biofilm agent type and contact surface on *E. coli* O157:H7 and *Salmonella* biofilms may provide effective strategies for biofilm control.

T3-04 Inhibition of *Listeria monocytogenes* in Deli-style Turkey Breast Using Alternate Curing Systems and Clean Label Antimicrobials

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Introduction: Fermentation-derived nitrite (NO_2) from vegetable sources is increasingly used as a "clean label" alternative to conventional $NaNO_2$. While some studies suggested that processed meats produced in this manner had lower inhibitory properties than conventionally cured meats, the differences are likely attributed to NO_2 concentration rather than the source.

Purpose: Compare the antilisterial properties of traditional and "clean label" fermentation-derived curing approaches when combined with antimicrobials in deli-style turkey.

Methods: *Listeria monocytogenes* inhibition by NO_2 from synthetic and natural sources was validated in deli-style turkey (73-74% moisture, 1.8% salt, pH 6.4). Products were prepared with 0, 80 or 120 ppm $NaNO_2$ equivalent using synthetic $NaNO_2$ or cultured celery powder; treatments with 0 or 80 ppm nitrite were further supplemented with 3.8% lactate-diacetate (LD) or 1% commercial fermentate (DF). Sliced cooked products were surface inoculated with 3 log CFU/g of *L. monocytogenes*, vacuum packaged, stored at 4°C and triplicate samples assayed through 12 weeks; study was replicated twice.

Results: Results revealed an average 2.4 log *L. monocytogenes* increase at 3 weeks in the control without antimicrobials, 1.3 log increase at 4 weeks for both 80 ppm NO_2 treatments and a 1.5 log increase at 6 weeks for the 120 ppm NO_2 treatments; there was no significant difference in growth inhibition between the two NO_2 sources when equivalent concentrations were added. Uncured turkey with 3.8% LD or 1% DF both delayed growth until 6 weeks, whereas supplementing LD or DF with 80 ppm nitrite from either source delayed listerial growth through 12 weeks.

Significance: This study confirmed that the concentration of NO_2, rather than source, is the primary factor in enhancing the safety of ready-to-eat meats. A clean label solution consisting of a fermentation-derived antimicrobial in combination with 80 ppm naturally-derived NO_2 was shown to inhibit *L. monocytogenes* through 12 weeks storage at 4°C as well as conventional-NO_2 versions.

T3-05 Effectiveness of Bacteriophage Listex™ P100 in Reducing *Listeria monocytogenes* on Roast Beef and Cooked Turkey

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Developing Scientist Competitor

Introduction: The use of phages as biocontrol agents in food and food animals has been successfully applied to poultry, pigs, cattle, cheese, sprouts, melons and other foods. Previous studies have shown that successful phage-based pathogen intervention in food greatly depends on the chemical composition of the food and its specific matrix. It has thus been suggested that there is need to individually optimize protocols for the application of phages with respect to the phages and the target bacteria as well as putting into consideration specifications for the food matrix.

Purpose: To verify the effectiveness of commercially available anti-Listeria phage LISTEX™ P100 in reducing *Listeria monocytogenes* on ready-to-eat (RTE) roast beef and cooked turkey.

Methods: A proof-of-concept study examined the activity of commercial anti-Listeria phage preparation, LISTEX™ P100, on a four-strain *L. monocytogenes* cocktail in two RTE food products; roast beef and cooked turkey. Foods at 4°C and 10°C were inoculated with cold-adapted *L. monocytogenes* to result in surface a contamination level of 10^3 CFU/cm^2. LISTEX™ P100 was applied at manufacturer recommended dose (10^7 PFU/cm^2) and samples taken at regular time intervals during the RTE product's shelf life (t=30 mins, 24 hours, 48 hours, 72 hours, 7 days, 10 days, 14 days, 20 days and 28 days) were treated with a virucide (tea infusion containing 4.3 mmol $FeSO_4$) to neutralize free phages and plated on Oxford media to recover viable *L. monocytogenes*. Controls comprised of RTE meat samples un-inoculated with *L. monocytogenes* with no phage treatment and RTE meat samples inoculated with four strain *L. monocytogenes* cocktail with no phage treatment.

Results: LISTEX™P100 was effective during incubation at 4°C with initial reductions of *L. monocytogenes* of 2.2 log CFU/cm^2 and 1.7 log CFU/cm^2, respectively for cooked turkey and roast beef. For the phage treated samples, the *L. monocytogenes* cell numbers remained below those of the untreated control with a reduction of between 0.9-2.8 log CFU/cm^2 and 1.9-4.1 CFU/cm^2, respectively for cooked turkey and roast beef. An initial *L. monocytogenes* cell reduction of 1.6 log CFU/cm^2 and 1.7 log CFU/cm^2, for cooked turkey and roast beef was achieved by the phage at 10°C (abusive temperature), respectively. At this temperature, the *L. monocytogenes* cell numbers remained below those of the untreated control only during the first 14 days of the experiment for roast beef samples after which there was no difference between the treated and untreated controls.

Significance: An overall reduction of at least 2 logs/cm^2 has an impact on the growth of *L. monocytogenes* on RTE roast beef and cooked turkey. This represents a hurdle which can be used to enhance safety in foods contaminated with *L. monocytogenes*.

T3-06 Phenotypic and Genotypic Characterization of *Salmonella enterica* serovar Dublin Isolates from Cattle and Humans

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Introduction: Antimicrobial resistance (AR), particularly multi-drug resistance (MDR), is of global concern. Nontyphoidal *Salmonella* causes approximately 93.8 million human infections worldwide/year including 1.2 million cases in the US. An increase in both AR and MDR among some serotypes has been observed. *Salmonella*Dublin is the second most common serotype isolated from cattle (2007 to 2011; animal arm of the National Antimicrobial Resistance Monitoring System (NARMS)), is among the most invasive serotypes in humans, and an increase in MDR has been observed.

Purpose: Determine AR, the presence of integrons and replicon types of plasmids in Dublin isolated from cattle and humans submitted to NARMS (1999-2011).

Methods: AR was determined to 15 antimicrobials using a semi-automated broth microdilution system (Sensitire®, Trek Diagnostic Systems, Inc., Cleveland, Ohio) for 272 cattle (C) and 69 human (H) isolates. Resistance was determined using Clinical and Laboratory Standards Institute breakpoints, when available. Isolates were screened for integrons (class 1, 2 and 4) and incompatibility (Inc) replicon types (Carattoli et al. scheme) using standard PCR protocols.

Results: Percent resistance was observed most often to: Ampicillin (63% C; 41% H), Ceftriaxone (19%C; 20%H), Chloramphenicol (60% C; 43% H), Kanamycin (51% C; 39% H), Streptomycin (69% C, 43% H), Sulfa antimicrobials (65% C; 49% H), and Tetracycline (67%C; 46% H). No resistance to Amikacin or Ciprofloxacin was observed. MDR (>5 antimicrobials) was observed in 43% of both cattle and humans isolates. The

most common MDR pattern in cattle was Amoxicillin – Clavulanic Acid/Ampicillin/Cefoxitin/Ceftiofur/Ceftriaxone/Chloramphenicol/Kanamycin/Streptomycin/Sulfa/Tetracyline (n = 27) and Ampicillin/Chloramphenicol/Kanamycin/Streptomycin/Sulfa/Tetracycline (n=11) in humans. Only class 1 integrons were identified in 30% (n = 82) and 25% (n = 17) of the cattle and human isolates, respectively. The replicon types identified from both MDR and susceptible isolates were: FIIS (80% C; 91% H), A/C (52% C; 29% H), H1 (17% C;16% H), FIA (17% C; 3% H), 11 (7% C; 1% H), FIB (6% C; 3% H), P (3% C; 1% H) and N (1% C; 0% H).

Significance: The presence of integrons and the "epidemic resistance plasmids" Inc FIIS, Inc A/C, Inc 11 and Inc N may be useful in addition to phenotypic susceptibility testing when studying the transmission of resistance genes and developing mitigation strategies.

T3-07 Prevalence and Characterization of Cefotaxime-Resistant Microbes in Animal Farms

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Developing Scientist Competitor

Introduction: Antimicrobial resistance is a growing concern in animal and public health. The number of antibiotic resistant microorganisms (ARMs) is increasing and will continue to increase due to the slow development of new antibiotics and lack of alternative therapies for bacterial diseases. Extended spectrum β-lactamase (ESBL) producing microorganisms, which are resistant to third-generation cephalosporins, present a new challenge to the food animal industry.

Purpose: The purpose of this study was to identify the origin of cefotaxime-resistant microorganisms and decipher the mechanisms of ESBL transmission in food animals.

Methods: Cattle fecal swab samples from nine locations and were collected, plated on MacConkey agar plates containing cefotaxime (4 mg/l), and incubated at 37°C for 48 hours to isolate cefotaxime-resistant colonies. After colony purification, 16S rRNA gene sequencing was conducted to identify the resistant microorganisms using the 16S universal primers (27F and 519R).

Results : We isolated cefotaxime-resistant microorganisms in farm animals, which have never been exposed to antibiotics through their entire life span. The prevalence of cefotaxime-resistant microorganisms in cattle was varying among farms, ranging from 5.2% to 100%. Animals reared in loose housing systems show lower prevalence of ARMs compared to animals in intensive housing systems, indicating animal-to-animal transmission plays a key role in ARM transmission. Seventeen different species of microbes including animal, human and plant pathogens as well as soil bacteria were identified by 16S rRNA sequencing.

Significance: This study reports the first occurrence of cefotaxime resistance in animals. The development of cefotaxime resistance microorganisms might have originated from nature as well as the use of antibiotics.

T3-08 Efficacy of *Pseudomonas fluorescens* for Biocontrol of *Escherichia coli* O157:H7 on Spinach

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Introduction: Foodborne outbreaks of *Escherichia coli* O157:H7 associated with the consumption of contaminated leafy vegetables have occurred with increased frequency. Control of foodborne pathogens on leafy green vegetables is crucial for consumer food safety. Biocontrol microbes to suppress foodborne pathogens on leafy vegetables have been proposed.

Purpose: The purpose of this research was to determine the efficacy of *Pseudomonas fluorescens* strains 2-79, Q2-87, and Q8R-1 (non-pectolytic and non-plant pathogenic) for biocontrol of *Escherichia coli*O157:H7 on baby spinach.

Methods: *P. fluorescens* strains 2-79, Q2-87, and Q8R-1 were applied on spinach using the dip inoculation method, prior to inoculation with *E. coli* O157:H7 strains 43894, 43895, and 35150 individually or in a cocktail mixture. The inoculated spinach was stored at 20°C for 24 and 48 h, and then evaluated for *E. coli* O157:H7 populations using Restaino and Frampton *E. coli* O157:H7 chromogenic medium. The effects of storage temperatures (5, 10, 15, 20, 25, and 30°C) on biocontrol efficacy were determined. The reduction of *E. coli* O157:H7 was computed relative to the control.

Results: The efficacy of biocontrol was significantly ($P < 0.05$) affected by storage temperature as suppressive effects were greater at 15°C (1.5-2.4 log CFU/g) than at other temperatures (< 0.93 log CFU/g). The mean reduction of *E. coli* O157:H7 by *P. fluorescens* varied between storage times and ranged from 0.6-2.1 log and 0.5-0.9 log CFU/g of spinach at 24 and 48 h, respectively. The low to moderate reductions of the pathogen may be attributed to the equal ratios (1:1) of *P. fluorescens* to *E. coli* O157:H7 inoculum.

Significance: These results imply that *P. fluorescens* may provide low to moderate reductions of *E. coli* O157:H7 populations on spinach. Biocontrol efficacy may be improved, by increasing the ratios of biocontrol agent to pathogen and combining it with other post-harvest intervention measures.

T3-09 The Effect of NaCl on Antibiotic Sensitivity and Biofilm Formation of *Salmonella*

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Introduction: *Salmonella* cells are possibly exposed to various NaCl levels formulated in processed foods for a long time. The antibiotic resistance and biofilm formation of the pathogen have been observed, and they have also caused critical issues for food safety.

Purpose: Therefore, the objective of this study was to evaluate the effect of NaCl on antibiotic sensitivity and biofilm formation of *Salmonella*.

Methods: Of 10 *Salmonella* strains, two strains of *Salmonella* were selected for the highest antibiotic resistance (ciprofloxacin)/biofilm formation (*Salmonella* Enteritidis NCCP12243) and the lowest antibiotic resistance/biofilm formation (*Salmonella* Typhimurium NCCP10812). The two strains were exposed to 0, 2, and 4% NaCl supplemented in tryptic soy broth (10 ml) for 24 h at 35°C. *Salmonella* cells were also subjected to sequentially increased NaCl concentrations up to 4% NaCl. The *Salmonella* cells were then examined for antibiotic sensitivity by a disc diffusion assay; amoxycillin, chloramphenicol, ciprofloxacin, gentamicin, neomycin, oxytetracycline, streptomycin, and tigecycline. Biofilms of the NaCl-habituated *Salmonella* cells were quantified by crystal violet, and the biofilm cells were visualized by confocal laser scanning microscope. Major components of the biofilm matrix were also examined by Congo red assay.

Results: The relationship between antibiotic sensitivity and NaCl (0, 2, and 4%) was not observed. However, the pathogen had decreased ($P < 0.05$) antibiotic sensitivity to chloramphenicol, gentamicin, and oxytetracycline when *S.* Typhimurium NCCP10812 was exposed to sequentially increased NaCl concentration. *S.* Typhimurium NCCP10812 had increased ($P < 0.05$) biofilms, but *S.* Enteritidis NCCP12243 had decreased ($P < 0.05$) biofilms as NaCl concentration increased. Moreover, major components of *Salmonella* biofilms were cellulose and curli, and these components were not influenced by NaCl exposure.

Significance: These results indicate that NaCl in foods may decrease antibiotic sensitivity and biofilm formation of *Salmonella*.

T3-10 Influence of Illumination Temperature and Wavelength on Bactericidal Effect of Light Emitting Diodes

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Introduction: The preservation of raw food products is of prime importance to the food industry because of their high propensity to pathogenic contamination. The current method of preservation, refrigeration, does not kill bacteria, but only inhibits their growth, with psychrotrophs such as *Listeria monocytogenes* retaining their ability to grow. Hence, another hurdle is needed to ensure the microbial safety of these foods.

Purpose: The aim of this study was to investigate the effect of temperature and wavelength of light emitting diodes (LEDs) on the inactivation of foodborne pathogens.

Methods: *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Listeria monocytogenes* or *Staphylococcus aureus* in tryptone soy broth were illuminated for 7.5 h with 10-W blue (461-nm), green (521-nm) and red (642-nm) LEDs at 20, 15 and 10°C. The distance between the LED and bacterial culture was 4.5 cm and the irradiances of the LEDs were 22.1 (461-nm), 15.97 (521-nm) and 25.48 mW/cm^2(642-nm). D-values were calculated based on survivor curves and compared using ANOVA.

Results: Regardless of the bacterial strains, bacterial inactivation was observed with the range of 4.6 – 5.2 and 1.0 – 2.0 log reductions at 10 and 15°C during illumination with 461- and 521-nm LEDs, respectively. No bactericidal effect was observed during the 642-nm LED treatment and the illumination at 20°C with 461- and 521-nm LEDs. D-values for four pathogens at 10 and 15°C after the illumination of 461-nm LED ranged from 1.29 to 1.74 h, indicating insignificant ($P > 0.05$) difference among the pathogens, except for *L. monocytogenes*. Thus these results indicate that the bactericidal effect of LEDs was highly dependent on the wavelength and the illumination temperature.

Significance: These data suggest that 461- and 521-nm LEDs, in combination with refrigeration, have the potential to act as a novel food preservation technology.

T3-11 What is the Best Surrogate for Human Norovirus?

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Introduction: Human norovirus (HuNoV) is the leading cause of foodborne disease outbreaks in the US. Because HuNoV cannot be grown in cell culture, feline calicivirus (FCV) and murine calicivirus (MNV) have been employed as cultivable HuNoV surrogate viruses. Recently, the usefulness of several different new surrogate viruses has been demonstrated.

Purpose: To compare characteristics of five HuNoV surrogate viruses including FCV, MNV, porcine enteric calicivirus (PEC), Tulane virus (TuV) and aichivirus (AiV).

Methods: Viruses dried on stainless steel disks for 90 min were exposed to 200 and 1000 ppm sodium hypochlorite for 5 min at RT. Viruses were diluted in acid (pH 2, 3) and alkaline (pH 9, 10) conditions for 30 min at 37°C. Sensitivity to heat (56°C, 63°C) was evaluated by dilution in PBS and sensitivity to alcohols by dilution in indicated alcohols. Effects of High Hydrostatic Pressure (HHP) was evaluated at 100-800MPa for 1min at 4°C. Remaining infectious virus was assayed by plaque assay; PEC by an antigen end-point assay.

Results: FCV, Aichi, Tulane and MNV were reduced by 4, 0.5 and 0.1 log, respectively, after exposure to 1000 ppm chlorine. MNV, TuV, AiV and PEC were reduced less than 0.5 log by pH 2 and 3 treatment; FCV, 5.5 and 3.5 log FCV at pH 2 and 3; All viruses were reduced 0.5 log or less at pH 9; only AiV was not inactivated at pH 10. PEC was most susceptible to alcohols followed by MNV; FCV was reduced by 2 log with 70% ethanol for 5 minutes. After 5 minutes at 56°C, MNV, TuV and AiV were reduced by 4 log, but 10 min was required for FCV. HHP reduced MNV, FCV and TuV by at least 4 log at 400 MPa, but AiV was resistant to 800MPa.

Significance: This is the first study to uniformly compare the characteristics of several established and new HuNoV surrogate viruses.

T3-12 Safety of *Enterococcus faecium* NRRL B-2354 (ATCC 8459) for Use as a Surrogate in Thermal Process Validation

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Developing Scientist Competitor

Introduction: *Enterococcus faecium* strain NRRL B-2354 (ATCC8459) has been used for over 40 years by the food industry. It is now most commonly applied as a surrogate for *Salmonella* during challenge testing of thermal processing methods used in the production of dry foods, most notably almonds. However, recent increases in nosocomial infections caused by *E. faecium* and a rise in antibiotic resistance among some strains have raised questions about the safety of strain NRRL B-2354 (ATCC8459).

Purpose: The purpose of this study was to evaluate the safety of *E. faecium* NRRL B-2354 (ATCC8459) using both genomic and phenotypic approaches.

Methods: The genomes of *E. faecium* NRRL B-2354 and the highly related strain ATCC8459 were sequenced and annotated. The strains were also examined and compared to clinical isolates for their capacity to grow in the presence of medically-relevant antibiotics, produce gelatinase and hemolysin, form biofilms on polystyrene, adhere to extracellular matrix proteins, and survive at high temperatures (>45°C) and at low pH (pH 2.4).

Results: The genomes of *E. faecium* NRRL B-2354 and ATCC8459 are nearly identical, are approximately 2.8 Mbp, and include one large (>200 kb) extra-chromosomal plasmid. Hierarchical clustering of genome content showed their distinction from clinical isolates. Both strains lack the majority of virulence factors (*acm, cyl, ebp, esp, gelE, hyl,* and *IS16*) and antibiotic resistance loci known for this species. Neither strain produced gelatinase, hemolysin, or pili nor were they able to adhere to collagen, fibronectin, or fibrinogen or form biofilms. NRRL B-2354 and ATCC8459 are also tolerant to adverse environmental conditions (heat and pH) and sensitive to antibiotics used to treat *Enterococcus* infections.

Significance: The genetic and phenotypic characteristics of *E. faecium* NRRL B-2354 (ATCC8459) are distinct from clinical strains and support its use as a surrogate in thermal challenge tests.

T4-01 Development of a Label-free Surface Enhanced Raman Scattering Method for the Detection and Differentiation of Foodborne Pathogenic Bacteria in Mung Bean Sprouts

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Developing Scientist Competitor

Introduction: Mung bean sprouts are commonly consumed raw or with minimal thermal processing and have acted as a vector for several outbreaks, linked to contamination by *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*. Surface enhanced Raman scattering (SERS) signals is a spectroscopic method able to detect trace amount of chemical and biological samples.

Purpose: The primary objective of this study is to develop optimized vancomycin functionalized silver nanorod (AgNR) arrays substrates to detect foodborne pathogens in mung bean sprout samples based on their SERS spectra. The goal of this study is also to differentiate pathogens from different species and different serotypes of the same species.

Methods: Six different bacteria *Salmonella* Anatum, *Salmonella* Cubana, *Salmonella* Stanley, *Salmonella* Enteritidis, *E. coli* O157:H7, and *Staphylococcus epidermidis*, were inoculated in 10 g of mung bean sprout, and recovered by a two-step filtration process. The vancomycin-treated AgNR substrate was immersed in the bacteria solution for 2 hrs before the SERS spectra of the bacteria were acquired using both bench top and handheld Raman systems.

Results: We established that 1mM vancomycin-coated AgNR arrays substrate with thickness (QCM reading) of 600 nm yielded the most intense bacteria signal. The limit of detection (LOD) of the six aforementioned pathogens in mung bean sprouts samples was 10^2 CFU/ml or 10^3CFU/g of mung bean sprouts when combined with a two-step filtration process in less than 4 hrs. The SERS spectra were acquired by both a bench top and a handheld Raman system. The LOD was confirmed by principle component analysis (PCA) and partial least-squares discriminatory analysis (PLS-DA) with 100% sensitivity and specificity. These six different pathogens were also differentiated by their species and serotypes using PCA.

Significance: This label-free SERS detection technique, based on functionalized AgNR arrays substrates, is a powerful platform to detect low amounts of foodborne pathogens in a short time from real food samples. It has the potential to be used as an on-site pathogen detection method in the food industry.

T4-02 Rapid Identification of *Salmonella* Serovars by Flow Cytometry-based Multiplexing Analysis System

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Developing Scientist Competitor

Introduction: *Salmonella* is the leading cause of foodborne illnesses in the United States. Because of deleterious effects of *Salmonella* on public health and economy, it is highly desirable to develop a detection method that can identify *Salmonella* in food before they reach the consumers. While conventional detection methods using culture or biochemical tests can identify pathogens with good sensitivity, they are laborious and time-consuming. Alternative methods (e.g., ELISA, PCR) suffer from a lack of specificity and require repetitive tests to detect multiple pathogens from one sample.

Purpose: The goal of this study is to develop a sensitive, rapid, and specific bead-based multiplexing array system to detect and identify *Salmonella* serotypes using pattern recognition analysis.

Methods: For this goal, bead-based suspension array of high multiplexing ability was combined with simple multiplex PCR. In the developed assay, the mixture of 14 different types of beads, each functionalized with different oligonucleotide probes, were loaded into 96-well microplate and used as a bead-suspension array platform. Probes and primers were designed using sequences from virulence genes and or serovar-specific regions, and presence of targets was determined by reading fluorescent signals from hybridization between probes and fluorescently labeled PCR products using Bioplex system.

Results: The developed bead-based multiplex array was able to detect synthetic target DNA of complementary sequence at the concentration as low as 1 pM, and when combined with PCR, it could detect *Salmonella* at 10 CFU/ml within 6 h without any pre-enrichment. Additionally, this assay was able to distinguish 7 different serovars (Anatum, Enteritidis, Gaminara, Infantis, Motevideo, Stanely, and Typhimurium) by pattern recognition analysis.

Significance: Our results indicate the developed bead-suspension array can be a rapid and reliable method for simultaneous detection and identification of multiple *Salmonella* serotypes. This array shows a great potential to be adapted for detection of multiple foodborne pathogens in foods.

T4-03 Evaluation of Molecular Alternatives to Traditional Serotyping for *Salmonella enterica* subs. *enterica*

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Developing Scientist Competitor

Introduction: Serotyping of *S. enterica* isolates is essential for risk management strategies and corrective actions to be implemented during outbreaks or food production. Currently, serotyping of *S. enterica* is based on the traditional method of immunological-based agglutination reactions. This procedure is laborious, often difficult to interpret, and not amenable to high throughput workflows.

Purpose: Compare traditional serotyping to three molecular typing strategies: riboprinting, pulsed field gel electrophoresis (PFGE), and Luminex xMAP *Salmonella* Serotyping Assay (SSA) to characterize *S. enterica*.

Methods: A diverse panel of food, outbreak, and environmental *S. enterica* isolates (n >150) were evaluated by Riboprinting using EcoRI and PvuII restriction enzymes, by PFGE using XbaI and BlnI, and by SSA. The results were compared to traditional serotyping.

Results: Strong agreement of serotyping results were observed for food- and outbreak-related *S. enterica* isolates when comparing molecular typing strategies to traditional serotyping. Riboprinting and PFGE were in strong agreement with outbreak isolates of *S.* Enteritidis and *S.* Typhimurium. Molecular serotyping outperformed traditional serotyping in several instances. For example, traditional serotyping identified one food-related isolate as *S.* Senftenberg, but both riboprinting and SSA independently identified the isolate as *S.* Johannesburg or Urbana. Characterization of environmental isolates proved more problematic, with frequent discrepancies between molecular serotyping methods. Results from

two of the three typing strategies were frequently identical for a particular isolate, and by combining the outputs of the different typing strategies, extremely accurate serotyping was possible.

Significance: The majority of *S. enterica* serotypes can be effectively typed using molecular serotyping methods, and combining methods improves typing efficacy for environmental isolates, which may be difficult to characterize using traditional serotyping. Among the molecular methods evaluated, PFGE offers the advantage of lower cost, SSA enables high throughput analyses, and riboprinting is easier to perform and more rapid.

T4-04 Mining of the Specific Molecular Detection Targets of *Salmonella enterica* and Genotyping of Its Isolates

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Introduction: *Salmonella* is one of the most important foodborne pathogens, which represents a significant public health problem and economic burden in many countries and regions of the world.

Purpose: To develop improved fast laboratory diagnostic techniques with the combination of bioinformatics analysis and biological experiment validation for the control of this pathogen. And to develop genotyping method for determining the sources of outbreaks and for understanding the epidemiology of sporadic cases acquired from various sources to meet the increasing demand of *Salmonella* surveillance.

Methods: Specific molecular detection targets for the species of *Salmonella enterica*, its serogroups A to D, and even 8 serotypes were identified based on the analysis results from bioinformatics platform (SMM-system) and PCR assays. Using the multilocus sequence typing (MLST), 25 *Salmonella* isolates from 5 chicken farms, 121 *Salmonella* food isolates from 13 countries or regions, and 353 *Salmonella* clinic isolates from Shanghai CDC were subtyped.

Results: Based on these targets, several multiplex and real-time PCR systems have been developed for the identification and differentiation of this bacterium. Further functional analysis showed that *Salmonella enterica*-specific target genes were related with its survival and pathogenesis; *Salmonella* serogroup-specific target genes were associated with sugar synthesis and metabolism or O-actetyl transfer; and *Salmonella* serovar-specific target genes were involved in DNA repair or phage encoded protein. Using MLST, the farm isolates, isolates from different countries or regions, and clinic isolates demonstrated 6, 42 and 12 sequence types, respectively. In addition, the discriminatory power of MLST (D = 0.947) was close to that of the serotyping method. The close evolutionary relationship between food and clinical isolates suggested that the patients might have been infected by the contaminated food. It was concluded that MLST has been an alternative method for the analysis of *Salmonella* population structure, an evolutionary and epidemiological tool for this pathogen.

Significance: The methods developed in this study can help to the fast laboratory diagnosing of *Salmonella*, to determining the sources of outbreaks and to understanding the epidemiology of sporadic cases acquired from various sources.

T4-05 Inter-laboratory Validation of an Enhanced Multiple-locus Variable-number Tandem Repeat Analysis (MLVA) Protocol for Subtyping *Listeria monocytogenes* from Food, Clinical and Environmental Sources

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Introduction: *Listeria monocytogenes* is the causative agent for human listeriosis. Subtyping data are used to support contamination source tracking and outbreak investigations.

Purpose: This study aims to refine MLVA primers and validate the MLVA assay using an inter-laboratory approach. The aim is to further expand a 2500 strain database containing MLVA information with an additional 2000 strains in order to allow for their implementation in routine analysis.

Methods: *L. monocytogenes* DNA was amplified in two multiplex PCR reactions using eight primer combinations targeting seven specific VNTR loci. Fluorescent PCR fragments were separated using an ABI 3730 Genetic Analyzer. The fragment data were analyzed using GeneMapper and BioNumerics software to determine isolate relationships. Method comparison between MLVA and PFGE was conducted using Comparative Partitions analysis.

Results: The MLVA protocol provided high discriminatory power, enhanced amplification efficiency and data quality. Inter-laboratory validation of the protocol using 60 strains of different subtypes/sources indicated that the MLVA protocol was reproducible and repeatable among three participating laboratories. Comparative Partitions analysis involving 378 strains with 215 PFGE types yielded Simpson's Diversity index values of 0.998 and 0.991 for MLVA and PFGE, respectively. The adjusted Wallace coefficients were 0.292 when MLVA was used as a primary typing method and 0.075 when PFGE was a primary subtyping method, indicating that the MLVA protocol was more discriminatory in some cases. Data analysis of ~4000 strains revealed predominant/persistent *L. monocytogenes* genotypes and their potential epidemiological links to clinically important strains.

Significance: The inter-laboratory validated MLVA protocol, along with the updated *L. monocytogenes* MLVA database, allows for implementation of MLVA for real-time subtyping of this pathogen to minimize listeriosis.

T4-06 Evaluation of Several Drag Sampling Techniques for Isolation of *Salmonella enterica* from Agricultural Environments

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Introduction: *Salmonella enterica* represents a significant public health concern, and is responsible for many cases of foodborne illness each year. There is a need to develop effective sampling strategies to evaluate the prevalence of *Salmonella* in the farm to fork continuum.

Purpose: Two alternative methods employing paint rollers or tampons were compared to conventional gauze drag swabs to evaluate their ability to recover *Salmonella* in agricultural environments.

Methods: Sampling was conducted in a field used to grow cantaloupe and on a small adjacent cattle ranch. Both sites were known to be contaminated with *Salmonella*. Tampons**,** paint rollers, and conventional gauze drag swabs were attached to 1 m of cotton string on one end. Individual drag swabs were bagged and sterilized by autoclaving. At the sampling location, each drag swab was aseptically pre-moistened with 400 g of sterile, canned evaporated milk. At each sampling site, 10 swabs of each type (paint roller, tampons, and gauze) were dragged in a U or W pattern.

One hundred and four environmental grab, cotton tip, and sponge stick samples were also collected from the farm sites. Drag swabs and the environmental samples were shipped to the laboratory, enriched, and analyzed by culture and molecular typing.

Results: Six of 10 (60%) paint roller samples were positive for *Salmonella* from the cantaloupe field, and 1 of 10 (10%) conventional drag swabs were positive. From the ranch samples, 4 of 10 (40%) paint rollers, 1 of 10 (10%) tampons, and 4 of 10 (40%) drag swabs tested positive for *Salmonella*. Multiple different serovars were isolated, though the diversity of serovars isolated from the drag sampling was not as high as that observed in the environmental samples.

Significance: The results of this study indicate the efficacy of using paint rollers as an effective way to sample agricultural environments for the presence of *Salmonella*.

T4-07 Comparison of Microbial Methods to Detect Fecal Coliforms, E. coli, and Salmonella spp. in Finished Compost

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Introduction: Compost provides nutrients for produce crops. Improperly composted feedstocks can harbor pathogens which can be transferred to produce crops. The US Environmental Protection Agency (EPA) and US Composting Council (USCC) provide methods to test biosolids and compost, respectively, for fecal coliforms, *E. coli,* and *Salmonella spp.*

Purpose: To compare existing EPA and USCC methods for the recovery of low levels of fecal coliforms, *E. coli,* and*Salmonella spp*. from composts containing a variety of feedstocks (biosolids, manure, and/or yard wastes).

Methods: Twenty-nine USCC-certified compost samples were collected from across the United States. *Salmonella spp.* and non-pathogenic *E. coli* were inoculated into three 400 g aliquots from each sample at 10^1-2 CFU/g. Each inoculated aliquot was processed using EPA Method 1680 (fecal coliforms), EPA Method 1682 (*Salmonella spp.*), TMECC Method 0701 (fecal coliforms and generic *E. coli*), and TMECC Method 0702 (*Salmonella spp.*).

Results: Statistical significance was reported using alpha = 0.10. EPA methods had significantly higher recovery efficiencies (RE) of *E. coli* ($P < 0.0001$) and *Salmonella* spp. ($P = 0.0596$) than USCC methods from biosolids composts, and for *E. coli* ($P= 0.0318$) from manure composts. Both methods had statistically equivalent REs of *Salmonella* and *E. coli* from manure and yard waste composts (*E. coli* ($P = 0.5164$) and *Salmonella* ($P = 0.6738$)). EPA methods had a significantly higher RE of *E. coli* from biosolids compost compared to RE for yard waste composts. USCC had significantly higher RE for *E. coli* ($P = 0.0187$) and *Salmonella* ($P = 0.0975$) from yard waste composts compared to those from biosolids composts.

Significance: Overall, EPA methods were more efficient in recovering low levels of both *Salmonella* and *E. coli* across all compost types compared to USCC methods. USCC methods were consistently more likely to have higher RE of target organisms from yard waste composts compared to manure or biosolids composts.

T4-08 Automation in a High Throughput Food Processing Laboratory to Facilitate Rapid Turnaround and Regulatory Compliance

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Introduction: As a leading meat processer, John Morrell continually seeks ways to improve their quality, process and productivity. The microbiology laboratory sought to eliminate transcription errors, shorten its analysis turnaround time and reporting time through automation.

Purpose: The current system was labor intensive and required that all processes be re-checked for accuracy prior to data release. Automation reduces transcription errors, increases productivity, enhancing data quality and accelerates result delivery. Faster turnaround translates into faster product release, longer shelf-life and cost savings.

Methods: The microbiology laboratory leveraged the automated VIDAS system to test for *Listeria* spp., *Salmonella* spp. and *E. coli* 0157:H7 on various sample types. Prior to the automation, the manual steps of loading the sample IDs, scanning the print outs from the instruments and then entering the data into reports with secondary review required 40-45 min per batch of 60 samples. This time has been reduced to only 5 min for report review. Since the plants now email sample submittal forms, the data is received by the laboratory data management system called TITAN® (Accelerated Technology Laboratories, Inc., West End, NC.) and parsed and ready to receive the samples. Once the samples are loaded on the VIDAS instrument to match the worklist from TITAN®, they are run, the data is sent back to the data management solution and the final analysis report is completed automatically.

Results: The primary enhancements are enhanced data quality and significant time savings. The automation has saved 35-40 min of work per batch (a batch contains 60 samples), and a typical day includes 10-12 batches or up to 720 test results/day. Conservatively, if we use 35 min per batch, and 10 batches per day, the time savings are in the range of 5.83 hours/day.

Significance: The major significance of this automation project is the unrivaled speed, accuracy and cost savings of the integrated automation solution.

T4-09 A Comparison of a Nested Two-Step qPCR and a Non-Nested One-Step RT-qPCR for Detection of Genogroup II Noroviruses in Diluted Clinical Fecal Samples

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Developing Scientist Competitor

Introduction: Human Noroviruses (NoV) are a major source of foodborne illness in the United States. Molecular assays such as reverse transcription real time PCR (RT-qPCR) are considered the gold standard for detection of NoV. Broadly reactive RT-qPCR assays are generally used to detect NoV in samples where the virus load is expected to be high. When the virus load is expected to be low (as is the case in food and environmental samples), nested PCRs have been used. Unfortunately, a major disadvantage of nested methods is their propensity for cross-contamination.

Purpose: We conducted a controlled comparison of two widely used NoV genogroup II detection assays: a broadly reactive one-step RT-qPCR assay and a nested two-step qPCR assay.

Methods: A human fecal specimen containing GII.2 NoV was serially diluted in DEPC treated water. Total nucleic acids were extracted from each dilution, and a one-step RT-qPCR assay targeting conserved region at the ORF1-ORF2 junction of the genome was performed. In parallel,

a nested two-step qPCR targeting the viral RNA-dependent RNA polymerase gene was also performed. For both assay designs, confirmation of sample positivity was performed using a dot blot hybridization. PCR assays were repeated four times, while hybridization assays were repeated eight times. Detection limits and PCR efficiencies of the assays were compared.

Results: Both assays displayed similar standard curves, however, the nested assay consistently detected one $\log_{10}$ lower virus titer. Dot blot hybridization increased the sensitivity of the nested qPCR by one $\log_{10}$ virus titer, but decreased sensitivity of the one-step RT-qPCR by two $\log_{10}$ virus titer.

Significance: These results confirm that nested two-step qPCR assay for NoV detection is best suited for situations where sensitivity is valued over rapidity, for example with naturally contaminated samples, such as shellfish. However, despite increased analytical sensitivity, the nested two-step qPCR assays suffers from delayed time to results as well as increased chances for cross-contamination.

T4-10 Virus Titer and Suspension Matrix Impacts Estimates of Human Norovirus Infectivity following Thermal Inactivation by Enzyme Pre-Treatment with Proteinase K and RNase Prior to RT-qPCR

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Introduction: Human norovirus (HuNoV) causes the majority of foodborne illnesses in the U.S., but cannot be grown in cell culture. To predict HuNoV infectivity, enzymatic treatment of HuNoV prior to RT-PCR is commonly performed, but false positive results and study variability are often reported.

Purpose: This study evaluates the impact of virus inoculum matrix and titer when enzymatic pre-treatment is employed prior to RT-PCR to predict HuNoV infectivity after heat inactivation. Plaque assay and negative staining electron microscopy (EM) of thermally inactivated murine norovirus (MNV-1) is also included for comparison.

Methods: MNV-1 (5.5-6.4 log PFU/ml) in 12 x 32 mm vials was subjected to 50, 56 and 60°C temperatures for time points between 30 sec to 2 hr. Virus inactivation was visualized by EM and D-values were determined after plaque assay. Low and high titer (approx. 1.5 and 5.5 log PFU/ml, respectively) untreated or heat-inactivated (99°C for 5 mins) virus stocks were subjected to enzymatic pre-treatment. The impact of inoculum matrix, was also evaluated using stocks that were sucrose purified, ultrafiltered, and syringe-filtered (SP/UF/SF), just (SP/UF), or SP/UF with the addition of 5% fetal bovine serum (FBS). Ct values were determined by RT-qPCR.

Results: We report D-values of 77.5, 11.3 and 2.1 min for 50, 56, and 60°C, respectively. For thermally inactivated high titer virus stocks, Average Ct value RDs between the enzyme-treated and untreated samples were 10.18 ± 6.03, 9.73 ±7.41 and 2.31 ±2.04 , for SP/UF/SF, SP/UF, and SP/UF+5% FBS stocks, respectively. EM results revealed intact viral particles in untreated samples, but no discernible intact particles after thermal inactivation.

Significance: This study suggests that both the virus suspension matrix and the virus titer impact RT-PCR results following enzyme pretreatment in thermal inactivation studies. Therefore, these factors should be considered before applying this method in HuNoV detection studies.

T4-11 Evaluation of Automating a Novel Biochemical Freshness Assay for Quantitative Measurement of ATP Degradation Products as a Potential Preventative Control of Fish Intended for the Human Food Market

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Introduction: *Post-mortem* ATP (adenosine triphosphate) degradation products in muscle tissue are mainly produced due to autolytic enzymes rather than microbial flora, and can therefore be used as a criterion for tissue freshness measurement and storage age determination, before microbial spoilage starts and the corresponding traditional techniques for spoilage measurement become relevant. Based on the principle that higher freshness is an indicator of lower risk of microbial spoilage, a novel assay has been developed that quantifies freshness of fish and meat tissue.

Purpose: Evaluate potential workflow and throughput solutions of automating a biochemical assay for quantification of inosine monophosphate (IMP), inosine (Ino) and hypoxanthine (Hx) as an indication of freshness in fish samples.

Methods: The assay was evaluated in 96-well microplate format for automated and non-automated modes validated over multiple runs using 3 dilutions of 3 enzymes and the assay blank. Then, a predetermined 7-fold dilution of 5 to 10 grams of fish tissue from 5 commercially available product matrices for the same species were subjected to 4 different storage conditions and analyzed over time in duplicate for all 3 co-factors. Detection of enzymatic conversion to NADH was read at absorbance 340 nm using a monochromator equipped microplate reader. Freshness was quantified based on K (Saito *et al.* 1959) and K_i (Karube *et al.* 1984) values calculated from corresponding absorbance of each fish extract.

Results: Data demonstrates that lower % IMP (n=10) and higher % Hx (n=10) are indicative of decreasing sample freshness and can be matrix dependent, concluding that freshness calibration indices should be individualized per biologic material and test environment. Assay results are comparative for automated and manual modes using several sample prep options, with inter-assay CV slightly favoring automated results by a margin of 2.15%. Enzyme dilutions produced inter assay average CV of 5.13% regardless of method, with none higher than 7.33%.

Significance: Applications that could benefit from a preventative control for rapid, easy quantification of fish tissue freshness may find this assay useful, such as HACCP management on fish products intended for the human food market.

T4-12 A Simple qRT-PCR Method for Distinguishing Potentially Infectious and Inactivated Norovirus

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Introduction: Norovirus strains have not been reproducibly replicated in the laboratory and there is no suitable small animal model for evaluating infectivity of this virus. To date, this has limited the ability to evaluate intervention methods and inactivation protocols.

Purpose: Because RT-PCR based methods only detect the presence of norovirus RNA sequences, RT-PCR by itself cannot give much information about whether the amplified nucleotide sequence was originally derived from an infectious or an inactivated virion.

Methods: To initiate an infection, the norovirus virion must attach to its host cell via interactions with histo-blood group antigens (HBGAs). Porcine gastric mucin, which chemically mimics HBGA receptors, can, when linked to magnetic beads, be used to bind and extract virions.

Results: Results described here, using GI.1 Norovirus (Norwalk strain) show that after ultra-violet light, high pressure, and thermal treatments, the ability of this virus to bind to swine gastric mucin is substantially abrogated.

Significance: These results indicate that the loss of NoV binding to porcine gastric mucin can be used as a means to preferentially exclude non-infectious virus particles from subsequent RT-PCR detection.

T5-01 Using a Risk-based Approach to Evaluate Mitigation Options for Fresh Produce and Propose Microbiological Sampling Strategies in the Growing Field

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Introduction: Fresh produce can become contaminated along the farm-to-fork (F2F) continuum due to contact with different hazards. Examples of contamination sources are irrigation water, soil amendment, wild and domestic animals, and worker's health and hygiene among others. Given the wide range of contamination sources, an F2F modeling approach is advisable to provide a systematic way to (1) characterize the potential for contamination along F2F stages, and (2) compare the efficacy of different mitigation options to reduce contamination and, therefore, reduce foodborne illness.

Purpose: The purpose of this project was to develop and demonstrate a methodology that could (1) support the investigation of "contamination scenarios" using probabilistic simulation techniques, (2) allow for the comparison of different intervention strategies, and (3) offer a risk-based sampling approach for microbiological contamination in the growing field.

Methods: A pilot study evaluated *Escherichia Coli* O157:H7 contamination of romaine lettuce during the production and harvest stages. An Agent-Based Modeling framework was used to predict the contamination prevalence and levels in the growing field. Input values were derived using a combination of data from literature review, field trials, and expert judgment for stage-specific contamination sources.

Results: The notional results from the hypothetical case studies suggested that contamination levels could be significantly reduced by (1) limiting wild animal access to the growing field, and (2) assigning sufficient buffer zones between the field and the neighboring cattle farm. Results also suggested that, improving the quality of irrigation water could reduce contamination prevalence. Furthermore, the application of a risk-based sampling approach indicated that contaminated units (i.e., lettuce heads) could be identified with a higher probability than standard "Z" sampling patterns.

Significance: This methodology offers a transparent, practical, and robust modeling approach with which to evaluate the efficacy of different mitigation options and identify areas in the growing field for targeted sampling activities.

T5-02 Assessing Soil Sample Methodology for *Salmonella enterica* and Enterohemorrhagic *Escherichia coli* Testing in Commercial Fields

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Introduction: Typical commercial, risk-based soil testing procedures to qualify a field for fresh produce production relies on 10 gram samples for screening. Practical evidence indicated this was inadequate. To improve preventive control standards, assessment of sample mass and pathogen detection protocols are necessary.

Purpose: To assess the effect of sample size on the reliability of pathogen detection schemes in agricultural soils.

Methods: Soil samples were screened for *Salmonella enterica* and enterohemorrhagic *Escherichia coli* (EHEC), respectively. Thirteen fields from one ranch were monitored for persistence of a natural *S. enterica* contamination following application of inadequately managed compost. A single field affected by animal intrusion was screened for EHEC presence. Standard testing was performed using 10 g of soil, enrichment, and PCR screening. Soil samples from initially positive fields were comparatively analyzed using 10 g and 100 g directly- enriched and 100 g soil extraction with sodium phosphate supplemented with 0.1% Tween 20 (NPT). Selected negative fields, based on 10 g samples, were retested with 100 g from the same retained soil sample.

Results: Detection using standard 10 g was achieved in 45 of 252 samples, from 5 of 13 fields. *S. enterica* detection using 10 g and 100 g directly enriched had 29.2% and 62.5% positives, respectively. Contrary to other applications of NPT to soil, pre-enrichment extraction on 100 g resulted in 4.2% *S. enterica* positives. All 10 g positives tested at 100 g sample size increased the frequency of positives among replicates and previously negative lots had positive outcomes among the replicate samples. Detection of EHEC in a different soil using 10 g direct, 100 g NPT extraction, and 100 g direct-enrichment had 20%, 25% and 35% positives, respectively.

Significance: As part of a valid soil sampling plan, sample mass is a significant determinant for field risk assessment and pre-planting standards. Increasing the standard sample size to 100 grams may increase the chance of detecting low level pathogen contamination.

T5-03 Modeling Survival of *Escherichia coli* O157:H7 in Lettuce as a Function of Chlorine Concentration

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Introduction: Produce can become contaminated by fecal pathogens during primary production or processing. *Escherichia coli* O157:H7 is a major risk concern for the fresh-cut vegetable industry. Washing with chlorinated water is the most common treatment used to control microbial risks in processed vegetables.

Purpose: The objective was to develop a mathematical model that describes the reduction of *E. coli* O157:H7 as a function of chlorine level (ppm).

Methods: Iceberg lettuce pieces of 1 x 4 cm were inoculated with 4 log CFU *E. coli* O157:H7. Sodium hypochlorite solutions were prepared in sterilized water to obtain different concentrations of free chlorine (0, 25, 50, 100, 150, and 200 ppm). Inoculated samples were then introduced into 20-ml tubes with different chlorine concentrations and analyzed at different treatment times (0, 10, 30, 60, 150 and 300 s). The surviving *E. coli* O157:H7 cells were enumerated by using Sorbitol MacConkey Agar (Oxoid). Counts were log-transformed and statistical modeling and analysis was performed by using Matlab™ software (Mathwork).

Results: Results indicated that *E. coli* O157 was able to survive at all assayed free Cl levels, with a maximum log-decrease corresponding to 2 logarithms observed in replicates treated with 150 and 200 ppm free Cl. The greatest log-decrease rate was found in the first 30 seconds, and followed by a gradual reduction until the end of the treatment (5 min). Low levels of free Cl (0-50 ppm) yielded similar log-decrease values (0.5 log). The survival pattern was described by a two-phase log-linear model (R^2>0.7) which considered the two observed survival rates. The mathematical relationship between survival rates and Cl levels could be modeled using an exponential family function.

Significance: Disinfection models for *E. coli* O157:H7 on lettuce are valuable tools for the validation of control measures in the fresh-cut vegetable industry and contribute to the improvement of quantitative risk assessments.

T5-04 Quantifying Bacterial Cross-contamination Rates between Fresh Cut Produce and Hands

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Developing Scientist Competitor

Introduction: Fresh-cut produce consumption has increased in the United States over the past few decades due to both increased consumer demand and product availability. Fresh-cut produce is not typically cooked prior to consumption, and the possible risk of cross contamination by hands or kitchen surfaces is a concern.

Purpose: This study aims to quantify the cross contamination rates of transient bacteria between fresh-cut produce and hands.

Methods: A food-grade strain of nalidixic acid-resistant *Enterobacter aerogenes* was used as a surrogate for transient hand-associated pathogens. A validation study showed no significant difference between *E. aerogenes* and *Salmonella* cross contamination ($P > 0.05$). Samples were collected using the glove juice method, where volunteers' hands were massaged for ~1 min inside a nitrile glove containing 20 ml of phosphate buffered saline (PBS). Produce was sampled by homogenization in PBS. Samples were plated onto MacConkey agar containing nalidixic acid. Data were compiled, log transformed, and plotted as frequency distribution histograms. The log percent transfer rates were also analyzed using a Tukey range test to determine if multiple means were significantly different.

Results: Inoculated hands will transfer ~30% of the bacteria to carrots, and ~10-30% to celery and to cantaloupe samples. The Tukey range test showed a significant difference between the transfer rates to carrot and to cantaloupe. Samples of inoculated carrots, inoculated celery, and inoculated cantaloupe transferred ~0.3-1% of bacteria to hands. The Tukey range test showed no significant difference in transfer rates from the three items to hands.

Significance: Direction of transfer plays the largest role in determining transfer rates, followed by difference in produce type. Understanding transfer rates to and from fresh-cut produce will allow for better assessment and management of microbial food safety risks in the home.

T5-05 Quantitative Risk Assessment for *Escherichia coli* O157:H7 in Fresh-cut Lettuce

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Developing Scientist Competitor

Introduction: Leafy green vegetables, including lettuce, are of serious food safety concern, as those are recognized as vehicles for foodborne pathogens such as *Escherichia coli* O157:H7 that could cause severe human illnesses. Development and application of quantitative risk assessment models have been recognized as a strong tool to identify and minimize potential risks associated with foodborne pathogens

Purpose: The objectives of this study were to develop a quantitative microbial risk assessment model to evaluate the public health risks associated with consumption of fresh-cut lettuce contaminated with *Escherichia coli* O157:H7 in the U.S., and to evaluate the impact of potential intervention strategies on public health risks.

Methods: The supply chain of fresh-cut lettuce was modeled from in field production until consumption at home. Using @RISK software a simulation model was developed for exposure and health outcome assessment. The developed model was simulated using Latin Hypercube Sampling for 100,000 iterations to estimate the number of illnesses due to consumption of fresh-cut lettuce in the U.S.

Results: With a prevalence of 0.1% of incoming lettuce to the processing plants, the baseline model (with no inclusion of intervention strategies) predicted 76 cases per million people in the U.S. Among four different intervention strategies evaluated (consumer washing, irradiation, ultrasound and bacteriophages), spraying the lettuce with *Escherichia coli* O157:H7 specific lytic bacteriophages cocktail was the most effective in reducing the public health risks. Sensitivity analysis results indicated that washing with chlorine is the most important factor affecting the number of cases per year predicted.

Significance: The developed risk model can be used to characterize and estimate the microbiological risks associated with *Escherichia coli* O157:H7 in fresh-cut lettuce and to evaluate potential intervention strategies to mitigate such risks.

T5-06 Probabilistic Model of Norovirus Transmission during Handling and Preparation of Fresh Produce at School Food Services

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Introduction: Norovirus (NoV) contamination during school foodservice fresh vegetable preparation or handling can pose serious risks to children and the food handlers since contaminated servings can reach susceptible population groups without further pathogen reduction treatments.

Purpose: The objective was to develop a probabilistic model accounting for NoV transmission during vegetable preparation at school cafeterias.

Methods: A probabilistic risk model was constructed using Visual Basic program for Excel (Microsoft®, Redmond) and @Risk, add-in for Excel (Palisade©, New York). Different contamination scenarios were constructed based on structured food handling observations from 14 secondary school cafeterias in Kansas. Transfer rates, survival rates, disinfection treatment and hand washing efficacy were considered among other variables by defining probability with data collected from literature. The safety criterion was established to 10 PFU/serving. The output of the model corresponded to plaque-forming units (PFU) NoV per serving. In addition, a sensitivity analysis was conducted to assess the effect of the different variables on the final risk.

Results: Results for the pathway describing transfer from contaminated surface or handler to foods (P1) indicated that levels of ≤4 log PFU/fomite did not result in any servings with values >10 PFU/serving .When initial levels were higher, around 20% servings contained levels >10 PFU/serving (for a 7 log PFU/fomite scenario). In the pathway modeling transfer from contaminated vegetables to uncontaminated vegetables, prevalence was lower than the previous pathway, obtaining 10% of servings with >10 PFU/serving (when 7 log PFU/vegetable was the initial contamination level). However, concentration in this pathway was higher than in P1. Also, results indicated that contamination by contaminated work surfaces was more significant than by hands.

Significance: Based on sensitivity analysis, effective training programs specifically addressing food and non-food contact work area sanitation to minimize NoV transfers to fresh produce may be implemented to ensure food safety at school cafeterias.

T5-07 Quantitative Exposure Model for the Transmission of Norovirus in Deli Sandwich Bars

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Introduction: Noroviruses (NoV) are a major cause of foodborne gastroenteritis worldwide and are often transmitted via infected and shedding food handlers manipulating foods such as deli sandwiches.

Purpose: The presented study simulated NoV transmission during the preparation of deli sandwiches in a sandwich bar.

Methods: A simulation model was built by combining the GoldSim and @Risk software packages. The working situation involved three food handlers working during a 3 hour shift on a common working surface. The model consisted of three modules. A first module simulated preparation of the deli sandwiches and contained the NoV reservoirs, locations within the model allowing the accumulation of NoV and the working of intervention measures. NoV reservoirs included (1) hands of all food handlers, (2) prepared sandwiches and (3) food contact surfaces. A second module covered the contamination sources being (1) initial contamination of the lettuce used on the sandwiches and (2) contamination of the sandwich if one of the food handlers was a NoV shedder. A third module included four intervention measures: hand/surface disinfection during preparation of the sandwiches, wearing/changing of gloves and hand washing after a restroom visit.

Results: A NoV shedding food handler could – in the absence of NoV transmission intervention measures – cause mean levels of 31 ± 11, 61 ± 17 and 5 ± 1 NoV particles present on the foods, hands and working surfaces, respectively. In this scenario, 90.2% of all deli sandwiches contained >18 NoV particles, the assumed NoV infectious dose. The inclusion of hand/surface disinfection and hand gloving as single intervention measures resulted only in marginal reductions of NoV levels in the reservoirs while a high compliance of hand washing after a restroom visit did reduce NoV presence on all reservoirs.

Significance: The inclusion of hand/surface disinfection and glove wearing/changing as single intervention measures was not effective in the model, as only marginal reductions of NoV levels were noticeable in NoV reservoirs. On the other hand, a high compliance of hand washing after a restroom visit severely reduced NoV presence on all NoV reservoirs. The presented model showed that good handling practices in deli sandwich bars is an efficient way to prevent NoV transmission.

T5-08 Widthdrawn

T5-09 Prediction of *Bacillus weihenstephanensis* Acid Resistance Using Gene Expression Quantification as Molecular Biomarkers

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Introduction: The physiological state of vegetative cells has a great impact on bacterial resistance. The Omics data allow understanding of microbiological behavior to environmental change due to the process, or storage conditions. The next step is to integrate these data in a quantitative approach.

Purpose: In this study, an integrative approach was followed to select molecular biomarkers of bacterial resistance to further predict the acid inactivation of *Bacillus weihenstephanensis*.

Methods: Combining gene expression and survival ability, potential biomarkers of acid resistance of *B. weihenstephanensis* were identified. RT-qPCR gene expression of 31 genes was quantified (i) during exposure to sublethal conditions and correlated to a subsequent acid-resistance (3D values *i.e.* the time necessary to lose 99.9% of the bacterial population) and (ii) throughout bacterial inactivation and correlated to an instant first bacterial decrease, *i.e.* the time necessary to lose 90% of the bacterial population at time *t*. An individual gene selection was made, and a selection of gene set was also made using a Partial Least Square (PLS) regression. The main advantage of this method is to take gene expression interactions into account.

Results: In sublethal conditions, 4 genes exhibited a linear correlation between their expression and subsequent bacterial resistance. They could be selected as direct biomarkers. While 9 genes, named long-acting biomarkers, showed an up-regulation during short adaptation time and were correlated to an increased acid-resistance over time. It highlighted the importance of non-linear correlation particularly when focusing at transcriptional level. In lethal conditions a set of 8 genes were selected to predict acid-resistance. Using this PLS model, the bacterial resistance of two independent samples was predicted at 2.1 h and 3.3 h whereas the resistances were observed at 2.8 h and 3.7 h, respectively. Further investigations are running to validate the mathematical model in process like conditions.

Significance: This study underlines the possibility to integrate the bacterial physiology state, using molecular biomarkers, into bacterial behavior modeling and thereby further improve microbial risk assessment.

T5-10 An Expert-based Multi-criteria Ranking of Global Foodborne Parasites

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Introduction: Foodborne parasites cause a high burden of infectious disease globally, yet generally do not receive the same amount of attention as other microbiological and chemical hazards in food. Data on disease incidence and transmission routes are lacking, a problem exasperated by symptoms that may be latent or chronic.

Purpose: In December 2010, the Codex Committee on Food Hygiene (CCFH) requested that FAO and WHO provide the Committee with "guidance on the parasite-commodity combinations of particular concern." FAO and WHO initiated a series of activities to provide this guidance, culminating in an expert workshop in September 2012.

Methods: During a weeklong expert workshop, a multi-criteria decision analytic approach was developed and applied. Experts screened an initial list of 95 parasites down to 24 and identified food pathways for each. A tool was designed interactively with the experts to score the importance of each parasite-commodity combination along seven criteria, including disease prevalence, global spread, trends, severity, case-fatality ratio, trade relevance, and socio-economic impact. Each parasite was scored by groups of five along these criteria, with revisions following full-group discussions. Groups provided weights for combining criteria into scores, which were then computed, averaged across groups, and ranked.

Results: Experts ranked *Taenia solium*, *Echinococcus granulosus*, *Echinococcus multilocaris*, *Toxoplasma gondii*, and *Cryptosporidium* as the top five parasites from a global foodborne perspective, followed by *Entamoeba histolytica*, *Trichinella spiralis*, *Opisthorchiidae*, *Ascaris*, and *Trypanosoma cruzi*. Rankings were largely driven by public health impact over other criteria.

Significance: This multi-criteria ranking is the first of its kind for global foodborne parasites, and served as a useful, systematic, and open approach to providing policy guidance. The approach itself has broader applications, as it could be adapted for regional or national use, or expanded to other infectious diseases.

T5-11 Development of a Mobile-based and Web-based Authentication Services Software for Imported Food Safety

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Introduction: Food safety becomes a topic of worldwide concern due to multiple countries' ability to import and export food products. Providing safe food for a world that is increasing in population and decreasing in resources becomes increasingly difficult.

Purpose: To present a mobile-based and web-based authentication services software enabling consumers of imported foods seamlessly connect with legitimate producers worldwide to authenticate and guarantee 'fitness for consumption' at the purchase point.

Methods: The authentication services enabling technologies used included Mobile Phone, Short Message Service (SMS), SMS Gateway (Kannel), Ozeki SMS Gateway, and Web Server. The software used 3-tier architecture - HTML (front-end – user interface); PHP (middleware); and MySQL (back-end running on apache database using phpMyAdmin). UML tools were used to model the system. The server is called by Kannel, processes requests via HTTP, stores what is necessary, and calls Kannel to issue a response SMS in a process that could also be done by Ozeki SMS Gateway. Apache uses a database system/engine to verify and store data while the data is passed via http get parameter responses.

Results: Result obtained by scratching a label on the food container to reveal its pin; entering the pin on a specific mobile or web application and submitting or, texting the pin using mobile phone to a particular shortcode; and getting a response confirming the authenticity of the product. Once used, the pin is sent encrypted to cloud computing servers where it is computed and the response is sent to the user for decision-making.

Significance: Reduces the mortality and morbidity or extreme health challenges caused by the intake of irresistible imported foods that are substandard/spoiled, improperly labeled, and containing harmful levels of preservatives, thereby improving the consumers' health status for less susceptibility to foodborne pathogens.

T5-12 Modeling the Impact of Climatic Variables on *Vibrio parahaemolyticus* Outbreaks in Taiwan (2000-2011)

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Introduction: Climate change is increasingly receiving attention on its potential for bacterial contamination of food and water, which consequently may result in a change of risks related to water- and foodborne infection diseases. However, the quantification on direct consequences of climate change on food poisoning has received less attention. In Taiwan, *Vibrio parahaemolyticus* has been the major threat of food poisoning over the past ten years; moreover, there seems to be an increasing trend in outbreak cases.

Purpose: This study aims to investigate and quantify the relationship between climate variation and incidences of *V. parahaemolyticus* in Taiwan.

Methods: Time periods for analysis were from 2000 to 2011. The climatic variables data of temperature, rainfall, relative humidity and *V. parahaemolyticus* incidences were collected from government organizations, including Department of Health and Center Weather Bureau etc. This study applied time series analysis to develop a model to predict the dynamics of *V. parahaemolyticus* incidences by EViews software.

Results: Results indicated that monthly average maximum temperature, monthly relative humidity and monthly average rainfall have significant impacts on *V. parahaemolyticus* outbreaks during the period of 2000 to 2011. The level of model fit is over 60% and the probability of F-statistic shows significance. Among climatic variables, the monthly average maximum temperature has the greatest influence on *V. parahaemolyticus* outbreaks.

Significance: The findings offer a novel view of quantitative relationship between climate change and food poisoning of *V. parahaemolyticus*. Moreover, our results suggest that it is necessary to develop an early warning system based on the climatic variation information for disease control management.

T6-01 Foodborne Viruses: Integration of Viral Risk in the HACCP Plan of a Food Company

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Introduction: Foodborne viruses are recognized as the major cause of foodborne diseases. A large number of outbreaks have implicated soft fruits.

Purpose: Following several alerts and to address this food safety issue, a fruit company chose to integrate viruses in its HACCP plan.

Methods: To set up the HACPP plan, several steps were as follows: 1) theoretical education of food safety managers; 2) working with food safety managers to identify the critical points of the production and transformation processes; 3) visits on the field with agricultural and food safety managers to confirm the identified critical control points; 4) analyses on fruit and water samples to set up the initial level of contamination.

Results: The potential sources of contamination were: food handlers and the environment (irrigation water, phytosanitary treatments, process water). Identified sources of contamination were confirmed by norovirus analyses: level of contamination ranging from 10^4 to 10^7 genome copies/l in water and from 100 to 10^4 genome copies/25 g in fruits. The complete details of corrective actions put in place will be detailed. Briefly, to educate operators, actions have been implemented via the creation of a fact sheet detailing the problem and the importance of good hygiene practices. Restrooms near the fields were installed and wearing gloves mandated for manipulators. A control plan has been set up to evaluate the persistence of norovirus in environmental samples before use in production and on the fruits after harvest and before transformation. In case of a European alert, a communication plan has been established to inform the company and track the potential origin of contamination.

Significance: Implementing actions have helped to effectively reduce norovirus risk as no contamination has been found in food products since that time. These data demonstrate that by introduction of simple actions, it is possible to integrate viral risk in a HACCP plan to ensure consumer safety.

T6-02 Role of Curli and Contamination Level on *Escherichia coli* O157:H7 Internalization into Organic Spinach Plants Grown on Hydroponics and in Soil

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Introduction: *Escherichia coli* O157:H7 may be internalized into organic leafy greens via root uptake. Understanding the mechanisms of *E. coli* O157:H7 internalization into organic leafy greens is important as produce wash treatment may not remove internalized pathogens.

Purpose: The internalization potential of *Escherichia coli* O157:H7 (EHEC) into organic spinach roots and subsequent transfer to the edible portions of the plant was evaluated. Further, the effect of curli, spinach cultivar, and contamination level on EHEC internalization was studied.

Methods: Spinach cultivars Space and Waitiki were grown in soil and hydroponically under controlled conditions. After emergence of four true leaves, soil and hydroponics solution were inoculated with curli-expressing or curli-deficient EHEC mutants to obtain 5 or 7 log CFU/ml. Spinach leaves, stems, and roots were sampled on day 0, 7, 14, 21 and 35 and surface-disinfected by mercury chloride (0.1%). Incidence and populations of internalized bacteria were determined by spiral planting of tissue homogenate and 8-tube MPN enrichment procedure.

Results: EHEC internalized into hydroponically-grown spinach roots and dispersed to the stem and leaf level. A significantly ($P \leq 0.05$) higher internalization incidence, 27.7% (n = 216) was observed in hydroponically-grown spinach inoculated with 7 log CFU/ml compared to that inoculated with 5 log CFU/ml (17.3%, n = 108). Internalization incidence was significantly greater (42.4%, n = 144) in soil-grown spinach (5 log CFU/g inoculation) than in hydroponically grown spinach; probably due to extensive root damage in plants grown in soil. Spinach cultivars did not influence EHEC internalization as evidenced from 49.1% (n = 216) and 50.9% (n = 216) internalization incidence for Space and Waitiki cultivars, respectively. Curli expression did not influence EHEC internalization into spinach.

Significance: Current study demonstrates that internalization is influenced by the contamination level and farming practices, necessitating the pre-harvest interventions for controlling pathogens in composted manure and irrigation water.

T6-03 Survival of Generic *Escherichia coli* and Surrogate *Escherichia coli* O157:H7 in Manure-amended Soils

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Introduction: Recently released U.S. FDA standards state that untreated biological soil amendments must be applied to soil 9 months before harvest to reduce the risk of pathogen contamination on fresh produce. Manure and soil type may impact survival of bacterial pathogens and risk of produce contamination.

Purpose: To determine survival of three non-pathogenic *Escherichia coli* (gEc) and two attenuated *E. coli* O157:H7 (attEc) strains in various untreated animal manures as influenced by surface application to soils.

Methods: *E. coli* strains cultured separately in poultry litter extract, were composited to produce a high (5.8 log CFU/ml), and a low (3.8 log CFU/ml) population inocula, which were surface-sprayed onto conventional and organic field plots (2 m^2) amended with poultry litter (PL), solid (DS) or liquid (DL) dairy manure, horse manure (HM), or no manure (NM). Survival was determined over 56 days post-inoculation (dpi) by enumeration of colonies on sorbitol MacConkey agar with rifampicin, or by mini-MPN procedure.

Results: Low and high inocula of all isolates decreased ~2.5 and 4.5 log CFU(MPN)/g respectively in conventional soils by 56-dpi. In organic soils, high inocula for all isolates decreased from ~4.5 log CFU/g to non-countable, whereas low inocula gEc declined from ~2.9 log CFU/g to zero and attEc decreased from 2.6 CFU/g to zero. Populations of both gEc and attEc in low and high inocula declined more slowly in PL, DS, and HM compared to populations in DL and NM. Interestingly, both low and high inocula from all manure treatments were unable to be quantitatively recovered on day 28.

Significance: Results indicate that manure type influences the survival of both generic and *E. coli* O157:H7 strains in manure-amended soils. Environmental factors (e.g., soil moisture fluctuations) and inocula conditioning also may influence the rate of bacterial population decline in manure-amended soils and should be examined more closely.

T6-04 Survival of *Salmonella*, *Escherichia coli* O157:H7, Non-O157 Shiga Toxin-producing *Escherichia coli*, and Potential Surrogate Bacteria in Soil as Affected by the Addition of Fast Pyrolysis-generated Switchgrass Biochar

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Introduction: Fast pyrolysis of switchgrass (and resultant biochar) can be used for bio-fuel production, soil amendments for fertilizing crops, binding heavy metals, and sequestering environmental biocarbon.

Purpose: To determine the influence of fast pyrolysis-generated switchgrass biochar on survival of foodborne pathogens and potential surrogate bacteria in soil.

Methods: Soil was amended with 7.5% biochar and inoculated with one of the following seven bacterial composites: *E. coli* O157:H7 (EHEC), *Salmonella enterica*, non-O157 Shiga toxin *E. coli* (STEC), ATCC nonpathogenic *E. coli* (BSL-1), attenuated EHEC-A, ATCC-attenuated EHEC-B, and attenuated *Salmonella* at 6.9 – 8.0 log CFU/g of soil, 6.9 percent moisture, sealed, and stored at 22°C.

Results: Twenty-four hours after inoculation, all biochar-amended soil populations were 0.33 – 1.40 log CFU/g lower than biochar-free soils, except for STEC, which declined by 1.51 log CFU in both soils. By day 5, populations of STEC and EHEC-A in biochar-amended soil were only detectable by enrichment. Pathogens, detectable by spread plating, declined 1.77-3.56 log in biochar-free soil and 2.58-4.36 log in biochar-amended soil, while surrogates were reduced 1.81-2.77 and 4.55-5.80 log in biochar-free and biochar-amended soils, respectively. By day 11, populations of STEC, EHEC-A and BSL-1 *E. coli* in biochar-amended soil were only detected by enrichment. Pathogens, detectable by direct plating, declined 2.12-5.03 log in biochar-free soil and 3.64-5.77 log in biochar-amended soil, while plating-detectable surrogates declined by 2.73-4.49 and 4.28 -4.94 log in biochar-free and biochar-amended soils, respectively. The lowest 11-day (log CFU) reductions in biochar-free soils for pathogens and surrogates, respectively, were O157:H7 (2.12) and EHEC-B (2.73), while the lowest declines in biochar-amended soils were *Salmonella* (3.64), and attenuated-*Salmonella* (4.28). By day 11, pathogen populations were 0.81-3.65 log lower in biochar-amended soil when compared with biochar-free soil.

Significance: Biochar is effective for inactivating pathogenic EHEC, STEC and *Salmonella* in soil. Attenuated *Salmonella* and EHEC-B composites may be effective soil surrogate microorganisms.

T6-05 Effects of Agricultural Practices on *Salmonella* Contamination in Tomato Fields

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Introduction: Human foodborne diseases associated with fresh produce, like *Salmonella enterica* contamination on tomatoes, is of interest due to recent outbreaks. However, the exact risks associated with agricultural practices are still unclear.

Purpose: To investigate the effects of irrigation, fertilization and planting practices with/without stakes or polyethylene mulch on *Salmonella* contamination of tomatoes in the field.

Methods: Experiment A was conducted by split plot design with irrigation sources as main plots and planting practices as sub-plots with 720 plants in total. Subplot planting practices included staked with mulch (SP), staked without mulch (SW) and non-staked without mulch (NW). For experiment B, a randomized complete block design was used with four fertilization/planting practices (conventional/SP, poultry litter ash/SP, poultry litter/SP and poultry litter/NW) in each of the four blocks with 960 plants in total. Irrigation water, plant rhizosphere, branches, and fruits were sampled for *Salmonella* detection. Bacterial population was measured by the most probable number method (MPN).

Results: In experiment A, no *Salmonella* were isolated from well irrigation water or the corresponding field plants. *Salmonella* populations (MPN/l) in pond irrigation water were higher in August and September (0.34 and 0.54) than October and November (0.068 and <0.033). Similarly, *Salmonella* population/contamination ratio of plant rhizosphere/branches increased from August to September and fell thereafter. In experiment B, no *Salmonella* were isolated from conventional or ash amended soils or the plants grown on them. The bacterial population in the rhizosphere of plants grown on poultry litter amended soils decreased from 480 to 0.34 MPN/kg during the study. In both experiments, rhizosphere *Salmonella* population was highest in SP plots, while *Salmonella* was only isolated from the branches of NW plants. No contaminated fruits were detected.

Significance: These field experiments provide insights into the control of *Salmonella* contamination on tomatoes and statistical data for risk analysis.

T6-06 Identifying Field-level Risk Factors Associated with *Listeria monocytogenes* and *Salmonella* Contamination in Produce Fields

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Developing Scientist Competitor

Introduction: The identification of management practices that pose the greatest risk for preharvest contamination of produce fields is crucial to the development of Good Agricultural Practices (GAPs).

Purpose: A cross-sectional study was conducted to determine the associations between field management practices and the likelihood of a *Salmonella* or *Listeria monocytogenes* positive field.

Methods: Over five weeks, 21 produce farms in New York State were visited. Information about field-level management practices were recorded for 263 fields and 600 environmental samples (1 drag swab and soil sample per field, and a water sample when available) were collected and analyzed for *Salmonella* and *L. monocytogenes*. Management practices were evaluated by logistic regression analysis for their association with the likelihood of a pathogen positive field, defined by a soil or drag swab sample from a field testing culture positive for the respective pathogen.

Results: *Salmonella* and *L. monocytogenes* were detected in 6.1% and 17.5% of fields (n=263), respectively. Additionally, *Salmonella* and *L. monocytogenes* were detected in 11% and 30% of water samples collected (n=74), respectively; the majority of positive samples were from surface water within 50 m of a field. Manure application within a year was observed to increase the odds of a *Salmonella* positive field 15 times, when compared to a field where manure was applied more than a year ago, or not at all. Presence of a buffer zone (at least 10 m) was shown to decrease the likelihood of a *Salmonella* positive field (odds ratio,[OR]0.2). Irrigation (within 3d, [OR]7.2), wildlife presence (within 3d, [OR]6.0), and soil cultivation (within 7d, [OR]3.4) in the field were all identified as important management practices increasing the likelihood of an *L. monocytogenes* positive field.

Significance: This study has identified management practices that are associated with the likelihood of field-level pathogen contamination. These findings will help growers evaluate their current practices and implement GAPs that reduce the risk of preharvest field contamination.

T6-07 Survival of *Listeria innocua*, *Listeria monocytogenes* and *Salmonella enterica* on Watermelon Surfaces during Storage and Postharvest Washing

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Introduction: Recent *Listeria* outbreaks and recalls associated with cantaloupe, honeydew, and watermelon, support the expansion of comparative studies to evaluate this pathogen in relation to more extensively characterized pathogens associated with cantaloupe outbreaks towards better identification of critical preventive controls.

Purpose: To evaluate the survival of various strains of *L. monocytogenes, L. innocua* and *Salmonella enterica* on watermelon surfaces under varying conditions of storage temperature and pathogen dose as well as their persistence after post-harvest processing including washing and disinfection.

Methods: The effect of storage conditions (15 to 37°C for up to 10 days) on the survival of *Listeria spp.* and *Salmonella* at various inoculation doses (log 1 to log 6 CFU/cm^2) was evaluated on watermelon upper rind exterior and the yellow ground spot following a factorial design. Additionally, disinfection by total immersion or swabbing in commercial solutions of sodium hypochlorite and peracetic acid were evaluated to determine the log-reduction of *Listeria spp*. on watermelon rinds following attachment.

Results: Immediate drop in population densities was determined for *Listeria* and *Salmonella* within the first 24 h and persistent viability was highly dependent on storage temperature and inoculum dose ($P < 0.05$), however loss of viability after 10 d was substantial at temperatures greater than 20°C. The behavior of *L. monocytogenes* in comparison to *L. innocua or Salmonella* was not significant ($P > 0.05$) thus *L. innocua* was judged to be an acceptable surrogate for rind survival under BSL 1 conditions. Population behavior was not different between the upper rind and the yellow ground spot. Disinfection by immersion in 30 mg/l peracetic acid or 100 mg/l of sodium hypochlorite products resulted in 2-2.5 log-reduction of attached cells.

Significance: Persistence of *L. monocytogenes* on watermelon could represent a risk for food safety, however proper handling and effective postharvest washing could be implemented as preventive control strategies and achieve significant risk reduction.

T6-08 Survival of *Salmonella enterica* in Manure Dust on Spinach Leaves

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Developing Scientist Competitor

Introduction: Microbiological safety of fresh produce has attracted a lot of attention in the past three decades due to pathogen contamination along the farm to fork continuum resulting in outbreaks. Although animal manure has been identified as a major pre-harvest pathogen source, there is little research on the potential role of airborne manure dust as a vehicle for pathogen transmission.

Purpose: This study assessed the survival capabilities of *Salmonella* in dry turkey manure particles (of a size capable of being airborne) when present on spinach leaves, as well as effect of UV radiation on that survival.

Methods: Turkey manure dust (125 µm) at 5% moisture level inoculated with a cocktail of *Salmonella* was lightly dusted onto spinach leaves under growth chamber conditions and survival monitored over 21 days. Effect of UV light on survival was factored into the experimental design such that the abaxial and adaxial sides of the leaves were compared for *Salmonella* survival. Treatment controls consisted of plants grown under UV filter. Outcomes were compared with trials where plants were inoculated with *Salmonella* via aerosol spray. Survival curves were generated and transformed data analyzed using SAS.

Results: Under UV light, particulate manure dust significantly ($P < 0.05$) reduced *Salmonella* inactivation on spinach leaves. Deposition site on leaves also influenced survival as *Salmonella* survived preferentially ($P < 0.05$) on the abaxial surface. By day 7 post-inoculation, *Salmonella* cells in manure dust on leaves had achieved only a 2 log reduction, while population in control samples declined by approximately 5 logs. By day 14, recovery of viable *Salmonella* from abaxial and adaxial leaf surfaces was 33% (n = 6) versus 0% respectively on XLD agar.

Significance: This research supports the hypothesis that dust generated from poultry manure increases the likelihood that *Salmonella* can persist on leafy green surfaces, especially in arid areas where unintended aerosolization could frequently occur.

T6-09 Airborne Transport of Foodborne Pathogens from Bovine Manure to Lettuce and Tomato

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Developing Scientist Competitor

Introduction: Contamination of produce is a critical food safety issue. Such contamination may result from airborne transmitted bacteria via nearby manure application. The California Leafy Greens Marketing Agreement recommends planting vegetables 122 m away from a manure source, but lacks supporting scientific evidence.

Purpose: We conducted a field experiment to determine the distance coliforms can be transferred by air during manure application, and the resulting rate of contamination on vegetables.

Methods: Romaine lettuce and tomato were planted in a field at the OARDC. Main plots (8 m^2) were arranged in a randomized complete block design. Liquid bovine manure was spread in a 5-m-wide band perpendicular to expected wind direction, next to the first row of plots. Agar plates located 24 cm above the ground and at the point nearest the manure spreading, 15, 30, and 122 m downwind, and 15 m upwind were left open for 5-15 min after spreading. Lettuce leaves and tomato fruits were collected before and immediately after manure spreading, then on post application days 1, 3, 5, and 7. Air temperature, wind speed and direction were recorded on each sampling day. Dilutions were prepared from homogenized vegetable samples and plated; all plates were incubated at 37°C for 36 hours, and colony counts (CFU/ml) were determined.

Results: The number of airborne bacteria on open agar plates was highest at the point nearest the manure spreading. Counts decreased significantly at 15 m and 30 m downwind ($P < 0.05$), but after 30 m, the number of bacteria on open plates stayed constant ($P = 0.6$). Number of bacteria on vegetable samples did not vary with distance ($P = 0.3$). Wind speed and direction were not correlated with number of bacteria ($P = 0.4$).

Significance: This study's findings can be used as evidence to support farm food safety policy and to develop future strategies to prevent and control microbial hazards.

T6-10 Impact of Extreme Climatic Events on Microbial Safety of Leafy Greens: Flooding

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Introduction: Growing field used for leafy greens may periodically be subject to flooding, which affect the safety of fresh produce mainly through the spread of faecal contamination. However, information on the impact of flooding on the microbial safety of vegetables is still needed. Within Europe, the south-east region of Spain represents the main vegetable producer. This area is frequently affected by extreme climatic conditions such as flooding at the end of the summer season.

Purpose: The purpose of this study was to evaluate the impact of environmental flooding on the microbial safety of lettuce.

Methods: Last year, according to the State Meteorological Agency (AEMET), there was a rainfall of 105 L/24 hours the last two days of September, which is almost 800% above the historic average of the Southeast of Spain. One week after this flooding event, samples were taken to evaluate the microbial safety of irrigation water, soil and lettuce in four different growing fields. Samples were taken up to 45 ays after the climatic event. The microbial parameters evaluated were *Escherichia. coli* spp., *Enterococcus* spp., *E. coli* O157:H7, VTEC and *Salmonella*.

Results: The analysis of the soil and lettuce after one week of the flooding event revealed that *E. coli* spp. was found in all the tested samples, including lettuce (3.3 ± 0.2 log CFU/g). Higher values were observed for irrigation water and soil samples (3.6 ± 0.1 and 4.0 ± 0.1 log CFU/g, respectively). *Enterococcus* were found in water samples (3.5 ± .1 log CFU/g). Several samples (water, soil and plant) were positive for *Salmonella* and VTEC. However, samples taken after 15 or more days of the flooding event were negative for *E. coli* spp. or pathogenic microorganisms.

Significance: These data suggest that flooding represents a main source of contamination but, depending on the climatic conditions, survival of pathogenic microorganisms might be very low.

T6-11 Assessing the Microbial Risk of Soil, Irrigation Water, and Farm Worker Hands to Produce Contamination on Farms and Packing Sheds Near the U.S.-Mexico Border

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Introduction: Produce associated enteric disease outbreaks are responsible for serious economic losses, morbidity, and mortality. Few studies have directly identified routes of contamination at the farm or packing shed.

Purpose: The goal of our study was to quantify microbial levels in potential environmental sources of contamination (soil, irrigation water, farm worker hands) and identify their role in produce contamination.

Methods: From 2011-2012, 237 produce composite (cantaloupe, jalapeño, tomato) and 121 matched irrigation water, 79 soil, and 158 farm worker hand rinse composite samples were collected from 10 farms on the Mexican side of the U.S.-Mexico border. From each sample, generic *Escherichia coli*, enterococci, fecal coliforms, and coliphages were enumerated. The magnitude of association between produce and environmental sample contamination was assessed by chi-square tests and logistic regression models (prevalence data), Spearman's correlations (non-normal concentration data), and linear regression models (concentrations). Human ethical research approval (IRB) was secured.

Results: In general, farm worker hand samples had higher concentrations of fecal indicators than soil or water samples. Soil had the lowest prevalence of fecal indicators compared to hands and water samples. Pooling data across the two years of sample collection, the concentrations of *E. coli* (rho = 0.9), enterococci (rho = 0.5), coliforms (rho = 0.3), and coliphage (rho = 0.7) were significantly and highly correlated between hands and produce, but not between soil or water and produce. The presence of *E. coli*, enterococci, and coliphage was significantly associated between hands and produce (odds ratios ranged between 2.3 - 8.5 across indicators), but not between soil or water and produce. These trends were also observed when analyses were adjusted for crop type and processing.

Significance: These results suggest that decreasing farm worker hand contamination would be an effective intervention to decrease microbial contamination of produce in the study region.

T6-12 Impact of Riparian Forests on the Prevalence of Non-Pathogenic *Escherichia coli* Contamination in Produce Fields

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Introduction: Agricultural water and soils contaminated with animal feces are identified routes of produce microbial contamination. While fecal deposition by wild and domestic animals may facilitate the introduction of pathogens into the agricultural landscape, environmental conditions (i.e., soil properties, precipitation, and temperature) can constrain microbial survival and persistence in the pre-harvest environment. Limited data exists on the role of riparian forests bordering croplands, which provide natural habitat for wildlife, in fecal bacteria dispersal onto adjacent fields.

Purpose: The prevalence and distribution of generic *Escherichia coli* in proximity to riparian forest buffer zones in the pre-harvest environment were investigated.

Methods: Riparian forests and adjacent produce fields located along the Flint Creek (FC; n = 9) and the Hoosick River (HR; n = 10) were sampled between September and October 2012. *E. coli* was cultivated in samples collected from soil (n = 279), drag swab (n = 115), fecal (n = 157), and water (n = 20). Variances in the frequency of positive cultures were analyzed using a chi-square test. Isolates were confirmed by PCR and *mdh* gene sequencing.

Results: The overall prevalence of *E. coli* cultivated from all samples was 65% (372/571). *E. coli* was widely distributed in all sample types collected from both field and forest sites in the two riparian zones: soil (53%, 149/279), drag swab (73%, 84/115), fecal (76%, 119/157), and water (100%, 20/20). HR riparian sites (forest and fields) were more likely to yield positive samples (79%, 219/276) compared to FC sites (52%, 153/142) ($P < 0.007$). In general, forest soils were significantly associated with *E. coli* compared to field soils in both riparian zones ($P < 0.007$).

Significance: In support of FSMA prevention-based standards, our study will be used to develop tools to provide science-based environment-specific recommendations for preventive strategies to mitigate microbial contamination risk in the pre-harvest environment.

T7-01 Assessment of Microbial Quality of Cooked Ready-to-Eat Street Foods Vended in Calabar Metropolis, Nigeria

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Introduction: Microbial load in ready-to-eat (RTE) food is a key factor in assessing the quality and safety of food. It also reveals the level of hygiene adopted by food handlers in the course of preparation of such foods.

Purpose: The microbiological quality of ready-to-eat (RTE) street cooked foods vended in Calabar Metropolis were investigated with the aim of assessing the risk of foodborne illness

Methods: Six types of cooked foods samples were collected from six different locations in Calabar Metropolis from three vendor categories; stationary with shade (SWS), stationary without shade (SWOS), and mobile vendors (MV). The samples (432) were analyzed for aerobic plate counts (APC), *Enterobacteriaceae* counts, *Salmonella aureus* counts, yeast and mold counts, coliform and *Escherichia coli* counts.

Results: The food samples from all the vendors had a high level of enteropathogen contamination. MV RTE foods had the highest ($P < 0.05$) level of contamination, with counts that ranged from 1.70×10^6 to 5.0×10^6 CFU/g (APC), 0.80×10^6 to 4.00×10^6 CFU/g (coliform), 1.20×10^6 to 4.00×10^6 (yeast and mold), 1.20×10^6 to 4.20×10^6 CFU/g (*E. coli*), 1.60×10^6 to 5.80×10^6 CFU/g (*S. aureus*) and 1.10×10^6 to 3.40×10^6 CFU/g (*Enterobacteriaceae*), respectively. SWS RTE foods had the lowest ($P < 0.05$) counts in coliform (1.30×10^6 to 2.50×10^6 CFU/g), yeast and molds (1.30×10^6 to 2.80×10^6 CFU/g) and *S. aureus* (1.20×10^6 to 3.60×10^6CFU/g).

Significance: The findings indicated that there was high risk of contracting foodborne illness from the street foods vended in Calabar Metropolis, as the microbial counts of most of the foods were within unacceptable limits, hence the need for implementation of sanitation code and licensing of street food vendors.

T7-02 Optimal Food Safety Sampling under a Budget Constraint

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Introduction: Most of the literature regarding food safety sampling plans implicitly assumes that all lots entering commerce are tested. In practice, however, only a fraction of lots may be tested due to a budget constraint. In such a case, there is a tradeoff between the number of lots tested and the number of samples per lot.

Purpose: To illustrate this tradeoff, a simple model is presented in which the optimal number of samples per lot depends on the prevalence of contaminated sample units and the relative costs of sampling a lot and of drawing and testing a sample unit from a lot.

Methods: The assumed objective is to maximize the number of contaminated lots that are rejected subject to an overall food safety sampling budget constraint. The optimization problem is solved using the Lagrangian and numerical methods.

Results: Under a budget constraint, the optimal sample size depends only on prevalence and the ratio of the cost per lot to the cost per sample unit, not on the size of the budget or number of lots inspected. If the ratio of the cost per lot to the cost per sample unit is substantial, the optimal number of samples per lot increases as prevalence decreases. However, if the ratio of the cost per lot to the cost per sample unit is sufficiently small, the optimal number of samples per lot reduces to one (i.e., simple random sampling), regardless of prevalence.

Significance: In practice, the cost per sample unit may be large relative to the cost per lot due to the expense of laboratory testing and the presence of natural bottlenecks in the food production and distribution system (e.g., ports of entry) through which many lots must pass. In the food safety domain, sampling plans with few samples per lot are commonly criticized for their lack of statistical rigor; however, the need to balance the tradeoffs between the number and size of clusters has long been appreciated in the field of experimental and survey design.

T7-03 Quantitative Risk Assessment for Campylobacteriosis in New Zealand by the Bayesian Approach

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Introduction: New Zealand has the highest rate of reported campylobacteriosis in the developed world. Due to the large economic and health consequences of campylobacteriosis, intervention programs to reduce the disease rates are required to be designed and implemented. Quantitative microbial risk assessments (QMRA) are used to identify all the risk pathways in the food chain and to examine the most effective interventions to reduce the rate of foodborne illness. Many of these risk assessment studies have been conducted using the infeasible Monte Carlo approach.

Purpose: The purpose of this study was to conduct a QMRA by Bayesian inference using a Bayesian Belief Network model, which has many advantages.

Methods: A simplified model was used to describe the entire food chain from farm to fork with all the variates, parameters and variables of interest. The microbiological data of two New Zealand poultry processing plants for the last two years were incorporated. Numerical computations were performed using WinBugs software.

Results: The QMRA indicated that hygiene has a significant impact on the total probability of illness. An increase in the poor hygiene percentage by approximately 50% reflected an increase of approximately 50% in the probability of developing illness; the impact of increasing the contamination prevalence on farms and after plant processing was similar to the hygiene impact. However, the estimated probability of contracting campylobacteriosis by consuming poultry predicted 2,000 more cases than the actual notified number from all the sources. This is a more plausible estimate than the QMRA estimate which used the Monte Carlo method, given the number of unreported cases and the number of campylobacteriosis cases acquired by sources other than poultry consumption.

Significance: The results of this study provide an attractive and reliable tool for risk management in selecting the best and most effective intervention (e.g. education of consumers) for reducing campylobacteriosis, given the impossibility of producing *Campylobacter*-free chicken under the current chemical interventions applied at processing plants.

T7-04 Modeling the Influence of Temperature, Water Activity and Water Mobility on the Persistence of *Salmonella* in Low-moisture Food

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Developing Scientist Competitor

Introduction: *Salmonella* is able to survive in low-moisture foods for many weeks or months. Heat resistance is affected by many factors including water activity (a_w). Low a_w environments protect *Salmonella* from thermal inactivation. Water mobility is different from a_w, and is a measure of the ability of water to translocate in a food. Little is known about the role of water mobility in influencing the survival of *Salmonella* in low-moisture foods.

Purpose: This study developed mathematical models that predict the behavior of *Salmonella* in low-moisture foods as influenced by a_w, temperature and water mobility.

Methods: Whey protein powders of differing water mobilities were equilibrated to various a_w levels between 0.19 and 0.54. Powders were inoculated with a four-strain cocktail of *Salmonella* (all previously involved in outbreaks in dry foods). Powders were vacuum-sealed and stored at temperatures ranging from 21°C to 80°C. Survival data was fitted to the log-linear, Geeraerd-tail, Weibull, biphasic-linear and Baranyi models. Secondary linear models relating the time required for first decimal reduction (δ) and shape factor values (β) to a_w, temperature and water mobility were fit using multiple linear regression. The models were validated in dry non-fat dairy and grain products, as well as low-fat peanut and cocoa products.

Results: Water activity significantly influenced the survival of *Salmonella* in low-moisture food ($P < 0.05$) at all temperatures while water mobility had no effect independent of a_w ($P > 0.05$). The Weibull model provided the best description of survival kinetics. Secondary models were useful in predicting the survival of *Salmonella* in tested low-moisture foods (R = 0.94), providing a more accurate prediction in non-fat food (R = 0.95) as compared to food containing low-fat levels (R = 0.91).

Significance: The models developed in this study provide baseline information to be used for research on risk mitigation strategies for low-moisture foods.

T7-05 Risk Assessment of *Escherichia coli* O157 in Burgers Made from Australian Beef Trim

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Introduction: *Escherichia coli* O157 is an enteric pathogen which can result in serious illness. Cattle are a known reservoir for this organism and there is potential that meat can become contaminated during slaughter. For this reason, all beef trim destined for grinding in the US has to be sampled and tested using the "robust" N-60 sampling program.

Purpose: Australia is a major supplier of manufacturing meat intended for grinding in the USA and the purpose of this study was to assess the risk posed by *E. coli* O157 from the consumption of burgers made only from Australian beef trim and to compare the effects of sampling and cooking interventions on illness estimates.

Methods: A quantitative risk assessment model was developed for *E. coli* O157. Lots of 700 cartons, each 27.2 kg, were modeled from the time the beef trim was packed into cartons through to consumption as 100 g burgers. The number of illnesses from burgers consumed was calculated for each of the following scenarios: cooking at home (to variable internal temperature), cooking by a quick service restaurant to internal temperature of 68°C, and sampling of raw beef trim using various sampling plans in combination with home-cooked burgers.

Results: Using only Australian beef, burgers cooked and consumed at home resulted in 3.0 illnesses per 10,000,000 burgers while from quick service restaurants the rate was 7.3 illnesses per 100,000,000,000 burgers consumed. By contrast, sampling of raw beef trim prior to grinding reduced the illness rate to 1.5 per 10,000,000 burgers consumed under N-60 sampling. This rate dropped further to 1.1 per 10,000,000 when N-60 is doubled to N-120.

Significance: In this risk assessment the rate of illness from burger consumption was modeled for 700 × 27.2 kg carton lots of beef trim. The results of this work show that the rate of illness from the consumption of burgers made only from Australian beef trim are low. In addition, the results confirm the lack of effectiveness of sampling beef trim for *E. coli* O157 relative to thorough cooking of burgers.

T7-06 Comparison in the Reduction of Patulin Content under Different High Pressure Processing Conditions with the Use of Hydrogen Peroxide

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Introduction: Patulin is a mycotoxin commonly encountered in juices and is produced by certain fungi within the genera of *Penicillium*, *Aspergillus* and *Byssochlamys*. The mycotoxin can cause a broad spectrum of toxicity (including carcinogenicity and teratogenicity) and hence the regulatory limit was set at 50 ppb. Although patulin is reactive, it exhibits high stability under acidic pH and cannot be significantly inactivated by thermal or non-thermal processing. Therefore, more effective methods are required to degrade the mycotoxin when encountered in juices.

Purpose: The purpose of this study was to evaluate if a combination of high hydrostatic pressure and hydrogen peroxide can be applied to degrade patulin encountered in juices.

Methods: Patulin-spiked juices or simulated juice (including malic acid, sorbitol, sucrose, fructose, glucose and water, pH adjusted to 3.5) was treated with different HHP treatments (400 MPa to 600 MPa for 30 – 300 s) in the presence and absence of hydrogen peroxide (H_2O_2 0 – 10% v/v). The patulin was extracted from treated juice samples and levels quantified using RP-HPLC at 276 nm. The data were calculated and statistical analysis performed using ANOVA.

Results: There were statistically significant differences ($P < 0.05$) in the reduction of patulin in patulin-spiked juices after HHP-hydrogen peroxide combined treatment. For instance, after 600 MPa for 300 s, the remaining patulin contents were 61.43 ± 1.74 ppb for H_2O_2-added, 156.81 ± 2.65 ppb for H_2O_2-non added and 179.56 ± 0.78 ppb for the control. However, the levels of patulin in simulated juice were not significantly different between those with or without hydrogen peroxide or treated with high pressure.

Significance: The results demonstrated that HHP can be applied in the presence of a suitable oxidant (e.g., hydrogen peroxide) to decrease patulin levels in juices. It is likely that the HHP degradation of patulin also involves other components within juices given that patulin reduction was negligible in simulated juice.

T7-07 Biofilm Formation and Cell Invasion among Environmentally Persistent *Escherichia coli* Isolates from South Africa Watersheds

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Introduction: The increasing scarcity of freshwater around the world, the concomitant loss of aquatic biological diversity, and the ongoing introduction of invasive species highlight the need for expanding investigations into the effects of altering aquatic habitats on water quality, food safety, and disease transmission. In recent years the use of *Escherichia coli* as in indicator organism has been challenged in the light of various reports that many strains of this bacterium have the ability to survive and proliferate in the external environment, outside of the gastrointestinal tract of the host.

Purpose: This study seeks to clarify whether *E. coli* populations in South Africa aquatic environments are structured according to the habitat, and if so, whether these bacteria still have the ability to circulate through and impact human or animal hosts.

Methods: Environmentally persistent *E. coli* strains (n = 122) were isolated from seven dams in the Gauteng region of South Africa by sampling water, sediment, and aquatic plant surface habitats. Phenotypic analyses of motility, growth kinetics, and biofilm formation were used to investigate bacterial capacity to survive and proliferate. Invasion and adherence assays were then performed in mammalian cell culture to measure their infection capacity in vitro.

Results: Among six *E. coli* populations classified as strong biofilm producers (3 fold increase capacity for biofilm formation over a reference strain DH5a), four were isolated from the surface of aquatic plants. Biofilm production strongly correlated with increased motility and reduced growth rates, suggesting that environmental transition from free living existence to biofilm lifestyle requires a unique growth mode specialized for long-term colonization of surfaces. *E. coli* strains were able to attach and invade cultured mammalian cells, thus having potential to invade into the host cell, induce an inflammatory response, and subsequently, cause disease.

Significance: A greater understanding of *E. coli* biofilm processes in various aquatic habitats should lead to novel, effective control strategies for control of environmentally persistent biofilms and a resulting improvement in water management as well as human and ecosystem health.

T7-08 Survival of *Listeria monocytogenes* in Three Dairy Powders

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Introduction: There is currently limited information available on the survival of *Listeria monocytogenes* in low-moisture dairy products such as dairy powders.

Purpose: The present study examined the survival of *L. monocytogenes* in 34% whey protein concentrate powder, nonfat dried milk and spray-dried cheese powder.

Methods: The three dairy powders were inoculated at a low (1-10 CFU/g) and high (10-100 CFU/g) levels using a three strain cocktail of freeze-dried *L. monocytogenes*. The inoculated products were stored at room temperature in sealed Whirlpak bags. A 4 dilution 3-tube most probable number (MPN) method was used to verify initial inoculation levels and to quantify *L. monocytogenes* at days 3, 7, 14, 21, and 28. Thereafter, 25 g of each powder at both inoculation levels was tested for the presence of *L. monocytogenes* at 30 day intervals using the Biomerieux VIDAS LMO2 assay.

Results: No significant differences ($P > 0.05$) were observed among the levels of *L. monocytogenes* in the low level inoculation of each dairy powder by day 28. No significant differences ($P > 0.05$) were also observed among the levels of *L. monocytogenes* in the high level inoculation of each dairy powder by day 28. *L. monocytogenes* was detected in the low level inoculation of the three dairy powders at days 58, 88, 118 and 148. *L. monocytogenes* was detected in the high level inoculation of the three dairy powders at days 58, 88, 118, 148 and 178. Results from this study indicate that *L. monocytogenes* can survive in these dairy powders for up to six months.

Significance: This data has significant implications for *Listeria* control strategies implemented during the manufacture of dairy powders.

T7-09 Behavioral Beliefs of Consumers Who Consume Either Pasteurized or Unpasteurized Milk: A Preliminary Study

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Introduction: Unpasteurized milk is legally sold in some states and is available through alternative sources in additional states. Consumption of raw milk is considered a risk factor for foodborne illness in all populations, especially highly susceptible groups.

Purpose: This preliminary study was conducted to understand antecedents to unpasteurized milk consumption behavior in the United States.

Methods: Survey methods were used to probe beliefs that may frame subsequent milk consumption behavior. Consumers of unpasteurized milk (n = 36) or pasteurized milk (n = 45) were recruited to participate in the study.

Results: Both groups agreed that they have personal control over whether they will contract a foodborne illness from consuming a milk or dairy product ($P = 0.32$). Neither group believed they were susceptible to a milk-borne illness ($P = 0.95$), but if they did become ill from milk consumption, the illness would be serious ($P = 0.21$). Pasteurized milk consumers had greater trust than unpasteurized milk consumers in institutions (government or science) that strive to protect health and safety of people ($P > 0.001$). Respondents did not differ in their overall political philosophy ($P = 0.86$). There were representatives of all response choices (conservative to liberal), with both groups tending to neutral as the median choice. Anger ($P = 0.43$) and uncertainty ($P = 0.32$) were not affective responses that related to beliefs about possible health risk associated with consuming milk. The affect, worry, differed between pasteurized- and unpasteurized-milk consumers with pasteurized milk consumers expressing greater worry ($P = 0.04$). Unpasteurized milk consumers strongly believed their health was a function of their personal actions ($P = 0.001$). Pasteurized milk consumers also believed in the connection between behavior and health, but the belief lacked the strength expressed by those who drink unpasteurized milk.

Significance: Strength of a belief is theorized as highly predictive of one's eventual behavior, useful information in constructing persuasive educational campaigns to promote the safety of pasteurized milk.

T7-10 Food Safety Certification of a Dairy Farm with ISO 22000 International Standard

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Introduction: Primary production has important influence on the safety of milk products. Microbiological hazards can be introduced both from the farm environment and from the milking animals themselves. Potential also exists for the contamination of milk with residues of veterinary drugs, pesticides and other chemical contaminants. ISO 22000:2005 (Food Safety Management Systems – Requirements for any Organization in the Food Chain) provides an internationally recognized framework for establishing a food safety management system that combines prerequisite programs (PRPs), HACCP principles, system management and interactive communication.

Purpose: The purpose of our study was to evaluate whether a dairy farm can comply with ISO 22000 and gain certification by an independent certification body.

Methods: The study took place in a Mexican dairy farm producing about 60,000 liters a day with 1,700 cows. Under the commitment of the top management, a food safety leader was enrolled and a food safety team was constituted. Its role was to establish the management system according to the requirements of ISO 22000:2005 and ensure its effective implementation in the field by the 47 workers.

Results: The establishment of an ISO-22000 based food safety management system took approximately 1 year and lead to the successful certification in the course of 2012. We show that ISO 22000 is a food safety standard applicable not only to food manufacturing but also to other steps of the food chain, including farming.

Significance: To our knowledge, this is the very first dairy farm certified with ISO 22000 worldwide. To ensure food safety at all stages of the food chain, it is important that clear communication and interactions exist between all parties within an integrated food safety management system. This can be facilitated by ISO 22000 standard.

T7-11 Characterization of the Microbial Population Present on a Short Ripening Mexican Artisanal Cheese

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Developing Scientist Competitor

Introduction: There are a diversity of Mexican artisanal cheeses such as Poro cheese that is usually made with raw milk. However, the use of non-pasteurized milk represents an important risk to consumers' health as it can contain pathogenic microorganisms.

Purpose: To characterize the autochthonous microbial population in Poro cheese using the ribosomal intergenic spacer analysis technique (RISA).

Methods: The bacterial population in Poro cheese obtained of Bejucal Ranch from Tabasco was characterized through total DNA extraction from milk, whey, curd and ripened cheeses (7, 30 and 60 days) in dry and rainy seasons. The DNA extracted from samples were used as templates for the PCR-RISA technique using ITSF (5'-GTCGTAACAAGGTAGCCGTA-3') and ITSReub (5'-GCCAAGGCATCCACC-3') primers. Cycling conditions were 95°C for 7 minutes, followed by 30 cycles at 95°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, ending with 72°C for 7 minutes. The fingerprint was obtained and selected amplified products were purified and sequenced. Sequences were compared with the NCBI database to identify the bacterial population.

Results: In raw milk from dry and rainy season were identified *Staphylococcus aureus*, *Bacillus subtilis* and *Comamonas testosteroni, Lactobacillus paraplantarum*, *Corynebacterium resistens,* respectively. These microorganisms were not found on the later production process. Lactic acid bacteria (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. bulgaricus and *Lactococcus lactis*) and opportunistic pathogen (*Klebsiella pneumoniae*) prevailed in the curd and in cheese samples at 7, 30, and 60 days of ripening in both seasons.

Significance: This study identified the composition of microbial populations during Poro cheese manufacture. Microorganisms such as lactic acid bacteria could be used as starter culture to provide the typical sensorial characteristics of Poro cheese made with pasteurized milk.

T7-12 Concentration of Biogenic Amines in Rainbow Trout (*Oncorhynchus mykiss*) Preserved in Ice and Its Relationship with Physicochemical Parameters of Quality

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Developing Scientist Competitor

Introduction: Biogenic amines are formed as a result of amino acid decarboxylation and is linked to food deterioration. Analysis of these metabolites may be of great importance to determine food quality.

Purpose: The aim of this study was to quantify the biogenic amines (putrescine and cadaverine), and evaluate the physicochemical parameters (pH, ammonia and total volatile bases) of rainbow trout meat (*Oncorhynchus mykiss*).

Methods: Forty-five samples were packed in ice and transported in a styrofoam container to the laboratory. Samples were analyzed for pH (AOAC 2005), ammonia (Nessler reagent) and total volatile bases (Conway's method). Biogenic amines (putrescine and cadaverine) were measured by HPLC-PDA. Analyses were performed daily until the 15th day of storage. All analyses were performed in triplicate during the experiment. The results were subjected to one-way ANOVA followed by Tukey test using the software GraphPad Prisma 5.

Results: Biogenic amines concentrations and pH increased significantly throughout the storage period. No significant differences were observed in total volatile bases values over the time. Ammonia was detected after the 11th day of storage.

Significance: Based on these results, cadaverine and putrescine may be used as a quality index of rainbow trout; however, total volatile bases may not be adequate parameter for this matrix.

T8-01 Multistate Foodborne Disease Outbreaks Associated with Raw Tomatoes, United States, 1973–2010: A Recurring Public Health Problem

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Introduction: Fresh fruits and vegetables are increasingly recognized as sources of foodborne disease outbreaks in the United States. Consumption of raw tomatoes has been linked to several large multistate outbreaks, suggesting that contamination occurs early in tomato production.

Purpose: Describe the frequency and characteristics of multistate foodborne outbreaks attributed to raw tomatoes.

Methods: We reviewed outbreaks, defined as ≥2 persons with a similar illness, reported to CDC's Foodborne Disease Outbreak Surveillance System from 1973-2010. Tomato-associated multistate outbreaks were defined as those resulting from raw tomato consumption in more than one U.S. state or territory. We analyzed demographics, pathogens, geographic distribution, seasonality, tomato type, and contamination source.

Results: From 1973-2010, 15 multistate outbreaks were attributed to raw tomatoes, resulting in a reported 1,952 laboratory-confirmed illnesses (median 86 per outbreak, range 8-429), 384 hospitalizations, and 3 deaths. Most outbreaks (80%) were reported from 2000-2010, and 73% occurred May-September. Thirty-seven states (median 9 per outbreak, range 2-24), predominantly in the eastern U.S., were affected. Outbreaks commonly affected adult (median age 34 years, range <1-97 years) women (58%). All outbreaks were caused by *Salmonella* [serotypes Newport (6 outbreaks), Braenderup (2), Baildon, Enteritidis, Javiana, Montevideo, Thompson, Typhimurium (1 each); one outbreak included multiple serotypes]. Red round (69%), Roma (23%), and Grape (8%) were the tomato types reported. Although 87% of outbreaks were associated with tomatoes served predominantly at restaurants, all reports suggested that contamination likely occurred during production in growing fields, at packinghouses, or at fresh processing (e.g., slicing) facilities located mainly in the southeastern U.S. Only one outbreak resulted in a recall of raw tomatoes.

Significance: Multistate outbreaks attributed to raw tomatoes were large, widespread, and increasingly recognized and reported. Public health interventions should focus on reducing *Salmonella* contamination early in tomato production — on the farm, in packinghouses, and during repacking and fresh processing.

T8-02 Process Analysis of Chlorine Replenishment of Lettuce Washing Water

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Introduction: Chlorine replenishment is a critical operation for fresh produce processing to maintain sanitizing levels of free chlorine in wash water. Systematic multi-factor response data are needed to improve management of the chlorination process of high input organic loads.

Purpose: In this project, we investigated dynamic changes in water quality and sanitation efficacy during chlorine replenishment of fresh-cut lettuce wash water.

Methods: Sodium hypochlorite was incrementally added into simulated lettuce wash water containing preset lettuce latex concentrations. Changes in water quality, including pH, free chlorine, total chlorine, and oxidation reduction potential (ORP), were closely monitored throughout the process. Sanitization efficacy of wash water was evaluated by measuring inactivation of a three-strain cocktail of *Escherichia coli* O157:H7.

Results: A 3-stage sequence in measured total and free chlorine concentrations was observed in response to incremental addition of NaClO to simulated wash water. A typical chlorine-breakpoint curve was composed of 1) an initial peak, 2) a valley, and 3) steadily increasing concentration of measured free chlorine. The boundaries of these three phases define the combined hump and chlorination breakpoint, respectively. Free chlorine concentration and cumulative NaClO input were correlated only during phase 3. The positions of the free chlorine peaks and valleys shifted as a function of the organic content in the wash water and the cumulative NaClO input. When cumulative NaClO input exceeded the breakpoint level, i.e., free chlorine was approximately 5 mg/l. *E. coli* O157:H7 population was reduced to undetectable level (<0.75MPN/ml).

Significance: This study provides critical information in establishing performance standards of produce wash water sanitation and developing improved chlorine dosing systems to maintain stable chlorine concentration during commercial product wash operations.

T8-03 Enhanced Reduction of Microbial Load in Produce Wash Water Using a Non-Oxidizing Disinfectant

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Introduction: Contaminated produce wash water can be responsible for the cross contamination of produce and consequently disinfectant is usually added. The most widely used disinfectant is chlorine in a mildly acidic solution. While chlorine can reduce the numbers of microbes, its efficacy is reduced by the presence of organic matter derived either from soil or the produce itself.

Purpose: A non-oxidizing disinfectant consisting of a synergistic combination of plant extracts and low concentrations of silver and copper ions (MicroPure Technologies LLC, (MPT)) was evaluated for its ability to reduce microbial load. The efficacy of the disinfectant as a produce wash was compared to free chlorine at 50 mg/l (pH 6.0) in over 60 experiments.

Methods: Mixtures of *Salmonella enterica*, *Listeria monocytogenes* and *Escherichia coli* O157:H7 (three strains per species) were used as inocula. Bacteriophage MS2 was tested, alone and in combination with the bacteria. The disinfectants were added to solutions of 5% sterile vegetable juice prior to inoculating with the bacteria or viruses. The suspensions were mixed thoroughly and constant agitation applied. Samples were removed after 1, 2 and 3 minutes of contact time. A solution of 5% juice with inoculum was used as a control.

Results: The chlorine rinse achieved typical reductions of <1 – 2 logs for bacteria and MS2. Conversely the non-oxidizing MPT disinfectant consistently achieved reductions of 4 - 6+ logs for all three bacteria and MS2 at both 4°C and 20°C. Repeated bacterial challenges of wash water containing a single dose of MPT disinfectant demonstrated that its disinfectant efficacy was largely unaffected, thus indicating the ability to reuse the disinfectant multiple times.

Significance: These results suggest that MPT was superior to chlorine in reducing the microbial burden in water, which suggests MPT will reduce the risk of cross contamination of produce during washing operations prior to packaging / distribution.

T8-04 Quantitative Transfer of *Salmonella* during Commercial Slicing of Tomatoes as Impacted by Multiple Processing Variables

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Developing Scientist Competitor

Introduction: Pre-sliced tomatoes were linked to 429 illnesses in a multistate salmonellosis outbreak in 2004. In addition to dump-tank washing, slicing/dicing is another key post-harvest step that can lead to cross-contamination.

Purpose: The objective was to assess the impact of multiple processing variables on *Salmonella* transfer during commercial slicing of tomatoes.

Methods: Red round tomatoes were dip-inoculated to contain avirulent *Salmonella* Typhimurium LT2 at ~ 5 log CFU/g and air-dried for 2 h. After slicing one inoculated tomato with a manual (Prince Castle Model 943) or electric slicer (Edlund Model 350), the blade, back/bottom or back/side-plate was sanitized before slicing 20 uninoculated tomatoes to quantify *Salmonella* transfer. Thereafter, the impact of processing temperature (4, 10 or 23°C), slicer post-contamination hold time (0 or 30 min), tomato surface (wet or dry), slice thickness (3/16, 1/4 or 3/8 inch), and tomato variety (Torero, Rebelski or Bigdena) on *Salmonella* transfer was assessed using the manual slicer. The top, middle, and bottom slice from each tomato and the blade, back-plate, and bottom/side-plate surface samples collected using Kimwipes® were homogenized by stomaching in lactose broth for 1 min and then surface-plated on trypticase soy agar containing 0.6% yeast extract, 0.05% ferric ammonium citrate and 0.03% sodium thiosulfate w/o 0.45 µm membrane filtration to quantify *Salmonella*.

Results: Significantly greater *Salmonella* transfer ($P < 0.05$) was seen using the manual as compared to electric slicer with populations linearly and logarithmically decreasing 2.73 and 4.36 log CFU/tomato, respectively, after slicing 20 tomatoes. The bottom/back-plate of the manual slicer and the blade of the electric slicer were the primary contributors to *Salmonella* transfer. Statistically similar *Salmonella* transfer rates were observed at 4 (0.6%), 10°C (0.1%) and 23°C (1.1%). Increasing the slicer post-contamination hold time from 0 to 30 min prolonged *Salmonella* transfer during slicing. A significantly higher ($P < 0.05$) *Salmonella* transfer rate (12.2%) was observed when tomatoes were wet. Thinner tomato slices resulted in relatively higher *Salmonella* populations on uninoculated tomatoes. Concerning tomato variety, *Salmonella* transfer was significantly greater ($P < 0.05$) for Torero (1.11%) as compared to Rebelski (0.08%) and Bigdena (0.07%) tomatoes.

Significance: Commercial slicing of tomatoes is best conducted using an electric slicer at 4°C to minimize potential transfer of *Salmonella*. These findings will also be useful in the development of science-based transfer models for risk assessments.

T8-05 Inactivation of Aerobic Mesophilic Bacteria and *Escherichia coli* K-12 on Cantaloupe Rind Surface Using Wet Steam Treatments

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Introduction: The presence of human bacterial pathogens on cantaloupe rind surfaces and transfer to fresh-cut pieces during preparation continue to be a microbial safety hazard for the produce industries and consumers alike.

Purpose: A prototype flash steam lab unit was used to treat cantaloupe rind surfaces inoculated with *Escherichia coli* K-12 bacteria at 5.2 log CFU/cm^2.

Methods: The cantaloupe rind surfaces were placed at a distance of 8.9 cm from the outlet of the flash unit and the effect of wet steam treatment at 65 ± 2°C for 60 s, 120 s, and 180 s was investigated. The initial population of aerobic mesophilic bacteria, yeast and mold and lactic acid bacteria on control cantaloupe rind surfaces averaged 6.5 ± 0.22, 2.8 ± 0.12 and 3.3 ± 0.12 log CFU/cm^2, respectively.

Results: Steam treatment at 60s, 120s and 180s reduced the aerobic mesophilic bacteria to 4.2, 3.1 and 2.3 log CFU/cm^2, respectively. At 120 and 180 s, the surviving inoculated populations of *E. coli* bacteria and lactic acid bacteria on cantaloupe rind surfaces averaged 0.6 and 0.9 log CFU/cm^2, respectively, while yeast and mold populations were below detection. *E. coli* bacteria plated on Violet Red Bile Agar, with 5 ml overlay of the same agar containing 4-methylumbelliferyl-beta-D-glucuronide (MUG) increased by 4 CFU only.

Significance: The results of this study suggest that wet steam treatment can be used to reduce microbial populations of cantaloupe rind surfaces and enhance microbial safety of whole and fresh cut cantaloupe pieces to reduce or eliminate illness and costly recalls due to foodborne outbreaks.

T8-06 Commercial Thermal Process for Inactivating *Salmonella* Poona on Surfaces of Whole Fresh Cantaloupes

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Introduction: Outbreaks of salmonellosis by *Salmonella* Poona and listeriosis by *Listeria monocytogenes* have been associated with the consumption of cantaloupes. Commercial washing processes for cantaloupes are limited in their ability to inactivate and/or remove this human pathogen.

Purpose: Our objective was to develop a commercial-scale surface pasteurization process for enhancing the microbiological safety of cantaloupes.

Methods: Whole cantaloupes, surface inoculated with *S.* Poona RM 2350, using the dip method, were stored at 32°C for 24 h prior to processing to allow for strong attachment and biofilm formation. Inoculated cantaloupes were treated in 275 l of hot water at 92°C for 60 and 90 s.

Results: Hot water treatments at 92°C for 60 and 90 s have resulted in excess of 5 log reduction of *S.* Poona per g cantaloupe rind. Cantaloupes that were treated and stored at 4°C for 9 days retained their firmness qualities and maintained non-detectable levels of *S.* Poona as compared to the controls. Also, levels of *S.* Poona on fresh-cut cantaloupes prepared from treated cantaloupes and stored for 9 days at 4°C were non-detectable as compared to the controls. Temperature penetration profiles indicated that the surface temperature of the whole cantaloupe was 26-30°C below the wash water temperature.

Significance: These results indicate that surface pasteurization at 92°C for 90 s will enhance the microbiological safety of cantaloupes and will extend the shelf life of this commodity as well. This process parameters, short time treatment of 90 s or less, fall within the commercial requirements of the whole cantaloupe processors/packers industry.

T8-07 Effectiveness of Calcium Hypochlorite on Bacterial and Viral Contamination of Alfalfa Seeds

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Developing Scientist Competitor

Introduction: Alfalfa sprouts have been involved in numerous foodborne outbreaks increasing awareness for seed and sprout safety. FDA recommends soaking seeds in calcium hypochlorite ($Ca(ClO)_2$) solution before sprouting to reduce pathogens.

Purpose: The objective of this study was to determine the effectiveness of $Ca(ClO)_2$ on inactivation of bacteria and viruses on alfalfa seeds. The efficacy of $Ca(ClO)_2$ in the presence of a simulated organic load during disinfection was also considered.

Methods: Alfalfa seeds were inoculated with human norovirus (hNoV) GII, Tulane virus (TV), *Escherichia coli* O104:H4, and *Salmonella* Agona. Seeds were air-dried for 60 min and treated with $Ca(ClO)_2$ (2000 ppm or 20,000 ppm, pH 7.00) for 20 min at 22°C. Similar treatments were conducted in the presence of artificial organic material (10%, 30%, or 50% fetal bovine serum) in Hanks Balanced Salt Solution. Pathogens recovered from seeds were quantified by real-time RT-PCR, plaque assay, or bacterial enumeration. For each organism and $Ca(ClO)_2$ concentration, three treated seed samples and one recovery/negative control were prepared for each of three trials. One seed sample was also included without virus inoculation for a neutralization/cytotoxicity control.

Results: Significant log reductions were observed in the order of hNoV < TV < *Salmonella* Agona < *E. coli* O104:H4 for both concentrations of $Ca(ClO)_2$; data ranged from 0.86 ± 0.18 to 3.37 ± 0.37 at 2000 ppm, and from 1.66 ± 0.41 to 5.47 ± 0.33 at 20,000 ppm. $Ca(ClO)_2$ at 20,000 ppm was more effective than 2000 ppm for all the organisms tested. This trend was also observed in samples containing an artificial organic material load. $Ca(ClO)_2$ activity on virus inactivation substantially decreased as the organic load increased, especially at FBS levels ≥ 30%; however, little difference were observed on bacteria, as $Ca(ClO)_2$ inactivated all the bacteria completely in the presence of organic loads. Reduction was greater in FBS-containing samples compared to alfalfa seeds, indicating a close relationship between the organisms and alfalfa seeds.

Significance: Results obtained in this research reveal more significant inactivation of bacteria rather than viruses on alfalfa seeds by calcium hypochlorite; indicating the potential risk of virus transmission via sprouted seeds.

T8-08 Thermal Inactivation of Human Norovirus Surrogates in Spinach

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Developing Scientist Competitor

Introduction: Leafy greens, including spinach, have potential for human norovirus transmission through improper handling and/or contact with contaminated water. Inactivation of norovirus prior to consumption is essential to protect public health. Because of the inability to propagate human norovirus *in vitro*, murine norovirus (MNV-1) and feline calicivirus (FCV-F9) have been used as surrogates to model human norovirus behavior under laboratory conditions.

Purpose: The purpose of this study was to (i) determine thermal inactivation kinetics of MNV-1 and FCV-F9 in spinach, (ii) compare first-order and Weibull models in describing the data, and (iii) measure the uncertainty associated with the process.

Methods: Thermal inactivation kinetics (D- and z-values) were determined for various treatment times at 50, 56, 60, 65 and 72°C in 2 ml vials. Weibull and first-order models were compared to describe inactivation kinetics. Statistical evaluation and linear and non-linear regression analyses were performed using SPSS Statistical package. The comparison test (ANOVA, Post Hoc test) was used to analyze the effect of time on survival ratio.

Results: z-values determined for MNV-1 were 11.66 ± 0.42 using the Weibull model and 10.98 ± 0.58 for the First-order model and for FCV-F9 were 10.85 ± 0.67 and 9.89 ± 0.79, respectively. There was no difference in D- or z-value using the two models ($P > 0.05$). Relative uncertainty for dilution factor, personal counting and test volume were 0.005%, 0.0004% and ca. 0.84%, respectively. The major contribution to total uncertainty was from the model selected. Total uncertainties for FCV-F9 for the Weibull and First-order models were 3.53-7.56% and 11.99-21.01%, respectively, and for MNV-1, 13.14-16.94% and 3.10-7.01%, respectively.

Significance: Novel and precise information on thermal inactivation of norovirus surrogates in spinach was generated enabling more reliable thermal process calculations to control the virus. Consideration of uncertainty measurements, which allow quantitative indication of analytic variability for any result, is needed to enhance validity of represented data.

T8-09 Extraction of Hepatitis A Virus from Seawater with Zeolite Granules

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Developing Scientist Competitor

Introduction: Hepatitis A virus is responsible for outbreaks of gastroenteritis among consumers of shellfish harvested from fecal polluted waters. Even point source discharge of human waste can result in viral contamination of approved shellfish beds; therefore, a rapid detection of viral contamination in seawater could prevent economic loss.

Purpose: To investigate the potential of zeolite granules in large-scale concentration of hepatitis A virus from seawater.

Methods: Hepatitis A virus strain HM175/18f was inoculated into 500 ml of artificial seawater and filtered through 2 g of zeolite granules contained in a layer of cheesecloth. The zeolite granules were immediately dried and incubated with 1 ml of eluent for 0.5 h at 42°C to elute the virus. RNA was extracted from 140 µl of the pre-filtered and post-filtered water and the eluate. qRT- PCR was conducted to determine the recovery rate.

Results: Zeolite granules (2g) were able to remove 99.9% of the viruses (5-6 logs) from 50 ml seawater , and 90%~96% of the viruses (7-8 logs) from 500 ml seawater. A variety of eluents were tested for their abilities to elute the virus. Results showed that sodium chloride (0.6M-5.8M, pH 2.6-10.6); calcium chloride (0.1%, 2%, 10%); EDTA disodium (0.1%, 0.4%, 1%, 10%); 0.1M glycine (pH 2 and 9); 0.5M phosphate buffer (pH 1-13); protein denaturants of various types (pepsin, trypsin, proteinase K, urea, and guanidine thiocyanate); ammonium salts (ammonium sulfate, ammonium acetate, ammonium phosphate); surfactants Triton X-100 and Tween-80 all failed in eluting the virus. However, sodium dodecyl sulfate proved to be rather successful; 1 ml of 5% SDS in 0.5M phosphate buffer (pH 12) could elute 5 logs of viruses from zeolite.

Significance: Zeolite can absorb up to 8 logs of hepatitis A virus from 500 ml of seawater in less than 1 minute and has the potential to be developed into a rapid concentration and detection method for hepatitis A virus in seawater.

T8-10 Population Dynamics and Mutability of *Listeria monocytogenes* Derived from the Food Chain

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Developing Scientist Competitor

Introduction: Serotyping and pulsed-field gel electrophoresis (PFGE) have been useful for investigating *Listeria monocytogenes* (*Lm*) strains associated with human disease; however, they lack discriminatory power required to delineate closely related strains. Sequence-based methods can be used to link genotypes with food chain-relevant phenotypes, though the relationship between multilocus sequence typing (MLST) and adaptive mutability and cold growth of *Lm* has not been explored.

Purpose: Assess genetic diversity amongst food chain-derived *Lm* using MLST and PFGE with respect to mutability and cold growth phenotypes.

Methods: *Lm* isolates (n = 54) were analyzed by MLST, PFGE, and serotyping. *InlA* genotypes were determined by sequencing, mutability assessed by plating on agar containing rifampicin (100 µg/ml), and cold adaption determined at 4°C following a downshift from 37°C in BHI broth.

Results: Thirteen sequence types (STs) and 36 pulsotypes were observed, with one novel ST identified. Strains discriminated by PFGE and serotyping were also found to possess different STs. Distinct STs were observed among lineage I (LI) and LII isolates. ST321 (n = 10) isolates possessed type 3 (a.a. 700) premature stop codon *inlA* mutations, whilst type 4 (a.a. 8) and 11 (a.a. 685) were seen in 67% and 22% of ST9 (n = 9) isolates, respectively. Ten STs were observed amongst *Lm* encoding full-length InlA. LI isolates were more mutable than LII ($P = 0.002$), and no correlation with fast, intermediate, and slow cold adaptors was observed amongst STs.

Significance: MLST revealed diversity amongst food chain strains, generally agreeing with serotyping and PFGE data. MLST data, however, allowed a more comprehensive assessment of phylogenetic relationships amongst strains, including an association of some STs with *inlA* genotypes. Also, we showed that STs in LI isolates commonly linked to listeriosis more readily acquire point mutations compared to LII. This suggests positive selection plays a role in the maintenance of wild-type *inlA* sequences in LI strains.

T8-11 Isolation and Molecular Identification of *Cronobacter* spp. from Non-dairy Foods in Indonesia

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Introduction: *Cronobacter* spp. (formerly *Enterobacter sakazakii*) is a group of emerging pathogens that have been implicated as the causative agents of meningitis and necrotizing enterocolitis in certain groups of infants. In Indonesia several isolates have been obtained mostly from powder infant formula and weaning foods.

Purpose: This research aimed to isolate *Cronobacter* spp from non-dairy foods, i.e., flour, starch and spice powder and evaluate the biochemical as well as molecular characteristics using rapid biochemical test and PCR, respectively.

Methods: The biochemical assay was conducted using API 20E test while PCR was done on the 16S rRNA gene using 2 primer pairs, i.e., 16 SUNI-L/Saka 2b (Segment 1) and ESA1/16 SUNI-R (Segment 2). Products of the DNA amplification were sequenced and then analyzed for their relatedness to reference isolates of *C. sakazakii* from GenBank using BLAST program.

Results: In this study, 36 samples of flour, starch and spices were screened for the presence of *Cronobacter* spp. Twelve typical colonies on DFI were obtained and the biochemical test suggested that 11 were *Enterobacter sakazakii* while one was identified as *E. cloacae*. The PCR suggested that the 12 isolates including the one identified as *E. cloacae* were confirmed as *C. sakazakii* and *C. muytjensii*. Relationships between strains for segment 1 depicted in a dendrogram using the neighbor-joining method show that the twelve isolates can be differentiated into two groups. Eleven isolates had 97-99% homology with *C. sakazakii* in the GenBank, while one had high homology with C. *muytjensii* ATCC 51329 (99%).

Significance: This study signifies the importance of non-dairy foods as the source of *Cronobacter* spp.

T8-12 Comparison of Four Different Methods for Detection of Shiga Toxin-producing *Escherichia coli* (STEC) in Environmental Samples

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) is a major concern for food industry and public health due to its deleterious effects. It is highly critical to develop rapid and sensitive detection methods for STEC in environmental or food samples in order to control STEC-related foodborne illnesses. Currently only limited number of STEC detection methods are available, and most of these methods are limited by their low sensitivity or specificity.

Purpose: The objective of this study was to evaluate four different STEC detection methods for their efficiency in identifying STEC in environmental samples.

Methods: For this objective, culturing method on Sorbitol MacConkey agar (CT-SMAC) or rainbow agar, multiplex PCR detecting *stx*1/2 and *rfb* genes for STEC and O157 serotype respectively, immunomagnetic bead-based immuno-fluorescent assay (IFA), and commercial Dryspot *E. coli*Seroscreen kit were compared for their sensitivity and specificity in identifying STEC in various environmental samples including rectal swabs, water, soil and feed.

Results: Of 482 samples tested, 127 were positive for O157 STEC and 196 were positive for non-O157 STEC. All positive samples were detected by multiplex PCR (100%); however, culture methods, IFA, and commercial Dryspot Seroscreen kit could detect only 68%, 86%, and 39% of the positive samples, respectively. Additionally, culture methods showed higher false-positive rates (23%) than other three methods tested. To our knowledge, this is the first study which compared multiple STEC detection methods for environmental samples.

Significance: Our results indicate that PCR is the most reliable method for detection of STEC in complex samples with highest specificity and sensitivity than other methods. Also it is suggested culture method alone is not sufficient to detect STEC in complex samples. Further study will be needed to confirm the efficiency of PCR in STEC detection from food samples. We believe employing PCR as a routine STEC monitoring system will improve food safety.

T9-01 The Long-term Health Outcomes of *Salmonella* Infections: What Do We Know?

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Introduction: Non-typhoidal *Salmonella* infections cause approximately 1.29 million illnesses, 23,000 hospitalizations and 400 deaths annually in the United States, costing $2.6 to $4.4 billion in medical costs, lost productivity and premature deaths. Burden of disease estimates typically reflect costs associated with acute infection and only consider reactive arthritis and/or irritable bowel syndrome as long-term health outcomes (LTHO). Other LTHOs may represent significant disease burden but are poorly understood.

Purpose: The objective of this review was to 1) identify existing peer-reviewed literature on the severity and incidence of LTHO associated with human salmonellosis, 2) qualitatively evaluate the strength of the evidence, and 3) identify potential data gaps.

Methods: LTHO were defined as symptoms that persist at least six months after infection, arise at least six months after infection or sequellae that are not expected to resolve. The online PubMed electronic database was searched using the indexed Medical Subject Headings to identify articles reporting primary research on LTHO from human case-control, case-series and cohort studies. Following a three-stage relevance screening, a full article review was conducted by a single reviewer to qualitatively assess relevance and quality and summarize findings.

Results: Of 121,020 identified articles, 76 were included in the review to date. Ten studies examined an entire population, four used disease registries, and a further 18 were outbreak specific. Several studies involved the same population over multiple time points. Follow-up ranged from none to 10+ years post-infection. 60 specific LTHO were identified and grouped into 14 health categories. The most commonly reported LTHO were reactive arthritis, cardiovascular sequellae, neurological sequellae, bone sequellae and GI sequellae.

Significance: Death, irritable bowel syndrome, reactive arthritis and neurological sequellae are LTHO very well supported by the literature. Other LTHOs have insufficient data to generate incidence estimates and further cohort studies are needed.

T9-02 Risks of Long-term Health Outcomes of Shiga Toxin-producing *Escherichia coli* Infection: An Epidemiologic Review

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) infections are estimated to cost between $136 million and $2.4 billion per year in the United States. However, these estimates are based on incompletely understood risks of long-term health outcomes (LTHO) after STEC infection. Evidence-based quantification of these risks is critical for improved economic cost models, public health priority setting and policy-making.

Purpose: The objective of this review was to 1) identify existing peer-reviewed literature on the severity and incidence of LTHO at least six months after acute STEC infection or post-diarrheal hemolytic uremic syndrome (HUS); 2) qualitatively evaluate the strength of the evidence, and 3) identify potential data gaps.

Methods: An extensive search of the online PubMed electronic database was conducted to identify primary research on LTHO from human case-control and cohort studies. The entire literature from six major authors was also reviewed. Following a two-stage relevance screening, a full article review was conducted to qualitatively assess relevance and quality and extract data to be summarized.

Results: Of 2,270 identified articles, 80 studies were included in the review. The majority of studies (57%) were retrospective reviews of patient records with no comparison group or systematic follow-up. Twenty-three (29%) were from one of two cohorts: the Walkerton Health Study and the Utah HUS Registry. After 5 to 10 years of follow-up, 20% to 30% of pediatric HUS cases showed signs of hypertension or reduced renal function. Other LTHO identified included neurological dysfunction, gastrointestinal disorders, diabetes/pancreatic injury and reactive arthritis.

Significance: Few systematic, prospective, population-based studies with control groups exist to inform our understanding of the risks of LTHOs after STEC infection. Much of the evidence comes from studies that were not designed to estimate risk. Population-based studies and meta-analyses are needed to better understand the incidence and risk of LTHO following STEC infection.

T9-03 A Review of Long-term Health Outcomes following Listeriosis Infection

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Introduction: *Listeria monocytogenes* can cause significant morbidity and mortality. Listeriosis can develop into secondary infections, including sepsis or infections of the central nervous system (CNS), and the mortality rate is near 20%. Most epidemiological studies of listeriosis outcomes focus on acute effects and do not include long-term follow-up. Long-term health outcomes (LTHO) associated with listeriosis contribute significantly to the burden of disease but are not well understood.

Purpose: The objective of this review was to 1) identify existing peer-reviewed literature on the severity and incidence of LTHO associated with human listeriosis; 2) qualitatively evaluate the strength of the evidence, and 3) identify potential data gaps.

Methods: An extensive search of the online PubMed electronic database was conducted to identify primary research on LTHO from human case-control, case-series and cohort studies. LTHO were defined to be symptoms that persist for at least six months after infection. Following a two-stage relevance screening, a full article review was conducted to qualitatively assess relevance and quality and extract data to be summarized.

Results: Of 465 identified articles, the majority of studies were individual cases studies and only 32 met the inclusion/exclusion criteria. Less than half of included studies specified length of follow-up (9% < 1 year; 38% > 1 year). Outcomes most commonly reported were CNS sequelae (93.8%), although 22% of studies mentioned other sequelae. Half of the studies reported paralysis or palsy. Non-CNS sequelae observed included chronic lung disease and increased cancer risk.

Significance: Few studies evaluating outcomes of listeriosis have been conducted, particularly in the last ten years. CNS sequelae are well documented but additional LTHOs are not well understood. Studies featuring long-term follow-up are needed to determine the burden of residual effects following human listeriosis.

T9-04 Long-term Health Effects of *Campylobacter* Infection: A Systematic Literature Review

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Introduction: *Campylobacter* is the most common cause of bacterial gastroenteritis worldwide. Symptoms typically last from three to six or more days but long-term health outcomes (LTHO) such as Guillian-Barre Syndrome (GBS) and reactive arthritis do occur. Published case reports indicate that LTHO may reach beyond these illnesses. Understanding LTHO associated with campylobacteriosis is critical to developing disease burden estimates that are used to rank risks and prioritize resources.

Purpose: The objective of this review was to 1) identify existing peer-reviewed literature on the severity and incidence of LTHO associated with human campylobacteriosis; 2) qualitatively evaluate the strength of the evidence, and 3) identify potential data gaps.

Methods: An extensive search of the online PubMed electronic database was conducted to identify primary research on LTHO from human case-control and cohort studies. LTHO were defined to be symptoms that persist more than six months after infection. Following a two-stage relevance screening, a full article review was conducted to qualitatively assess relevance and quality and extract data to be summarized.

Results: Of 2,224 identified articles, 30 studies were included in the review (12 case-control; 13 population cohort; 5 outbreak cohort). Eight studies (26%) did not report risk estimates for LTHO and seven (23%) examined multiple bacterial pathogens and could not provide results for *Campylobacter* alone. GBS and irritable bowel syndrome (IBS) were the most common LTHO examined; other LTHO included hypertension, renal impairment and reactive arthritis. Nine case-control studies reported 20% - 57% of GBS cases had evidence of prior *Campylobacter* infection while five studies found increased risk of IBS.

Significance: Few studies evaluate the LTHO of *Campylobacter* infection and those that do are limited in scope. Current literature provides evidence to support the existence of LTHOs but, beyond GBS, there is no clear understanding of the risk of disease.

T9-05 Applying Source Attribution to Elucidate the Trend of Human *Campylobacter* Infections

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Introduction: Since 2005 human campylobacteriosis has increased by 38% to c6500 cases pa in Scotland. Previous work has determined that the source of clinical strains is principally retail chicken with a significant proportion of the remainder attributable to ruminants. Further, the of typing clinical isolates from Grampian Region (pop 500,000) has been shown to be representative of the whole of Scotland.

Purpose: This study seeks to determine the current sources of human campylobacteriosis in Scotland.

Methods: All clinical isolates (798) were collected over the twelve month period to March 2011 along with epidemiological data (isolation date, patient gender, age and home address, exposure and foreign travel information). *Campylobacter* isolates from the principal source hosts were also collected: retail chicken (238), cattle (142) and sheep (167). Multi-locus Sequence Typing at ten loci (seven housekeeping, *porA, flaA, flaB*) was carried out. Strain similarity was determined using Nei genetic distance and molecular attribution of source species using modeling by simple proportions (e.g., the Dutch model) and Bayesian stochastic methods (e.g., STRUCTURE and Asymmetric Island (AI) Model). Comparison was made to our large 2005/06 study.

Results: The study showed that there was a significant ($P < 0.05$) increase in prevalence from cattle (21.9% to 33.1%), sheep (33.2 – 52.7%) and retail chicken (64.0-90.3%). Nei's genetic distance showed significant differences ($P < 0.05$) for all animal sources and humans between 2005-6 and 2011. Eight of the top 16 sequence types from clinical isolates changed in abundance between 2005-6 and 2011. Attribution to source in 2011 had the same rank order as previously with chicken most important followed by cattle and sheep, then wild birds and pigs. There was a difference between the source attribution models for attribution to chicken with the Asymmetric Island model attributing approximately 75-81% whilst STRUCTURE attributed 40-54%.

Significance: The findings indicate an increase in the prevalence in the ruminant and retail chicken sources as well as a change in the distribution (and relative importance) of different source and clinical sequence types. Source attribution indicates that retail chicken remains the most important source of human infection.

T9-06 What Was Fishy about the Sushi? The 2012 *Salmonella* Bareilly Cluster Investigation, the Texas Experience

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Introduction: Each year approximately 42,000 cases of salmonellosis are reported to the Centers for Disease Control and Prevention (CDC). The number of actual cases is estimated to be greater than one million. In Texas an average of 5,000 salmonellosis cases are reported per year.

Purpose: In March 2012, an increase in the number of cases of *Salmonella* Bareilly was noted nationally and an investigation was begun to identify the vehicle and source of infections. *Salmonella* Bareilly is a rare serotype, accounting for 0.5% of all sporadic *Salmonella* infections.

Methods: The case definition for this outbreak included laboratory confirmed cases of *Salmonella* Bareilly, Pulsed-field gel electrophoresis (PFGE) XbaI pattern JAPX01.0042 or *Salmonella* Nchanga, PFGE XbaI pattern JRQX01.0004 with illness onset (or isolation date, if onset date was unknown) from January 28, 2012 through July 26, 2012. The Texas Department of State Health Services (DSHS) laboratory identified 14 cases of *Salmonella* Bareilly PFGE XbaI pattern JAPX01.0042. Cases were interviewed using hypothesis generating and supplemental questionnaires.

Results: Eleven of the 14 Texas cases reported eating sushi ($P < 0.0001$); of those 11, 10 different sushi restaurants were named. Two restaurant locations, with multiple cases, provided traceback information that was key to the investigation. The vehicle identified as causing infections in this outbreak was Nakaochi Scrape tuna used in sushi.

Significance: CDC identified a total of 425 cases in this multi-state cluster; 410 persons with *Salmonella* Bareilly and 15 persons with *Salmonella* Nchanga. A successful outcome – identification of the vehicle and source of the outbreak – occurred due to the cooperation and good communication among the laboratories; state, regional, and local health departments; restaurants; and regulatory partners.

T9-07 Source Attribution for Human Cases of Shiga Toxin-producing *Escherichia coli* in New Zealand

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Developing Scientist Competitor

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 and related non-O157 STEC strains are pathogens of public health concern worldwide and can cause life-threatening diseases. Cattle are considered the principal hosts of STEC and have been shown to be a source of infection for both foodborne and environmental outbreaks in humans overseas. Since 1993, when New Zealand's first case of STEC in humans was reported, infections have appeared as sporadic cases and small clusters throughout the country, suggesting highly dispersed animal and/or environmental exposures as the source. No cases associated with regulated foods have been confirmed.

Purpose: The aim of this study was to investigate risk factors associated with STEC infections in humans in New Zealand and provide epidemiological information on the source and exposure pathways.

Methods: During a national prospective case-control study from July 2011 – July 2012, any confirmed case of STEC infection reported to Public Health Units, together with a random selection of controls representative of the national demography, were interviewed for risk factor evaluation using a standardized questionnaire. Questionnaire data were analyzed using multivariable logistic regression with multiple imputations to adjust for the small proportion of missing data in some variables.

Results: Data from 113 eligible cases and 506 controls were evaluated. Animal and environmental risk factors for STEC infections that were statistically significant ($P < 0.05$) were: cattle livestock being present on meshblock (OR 1.89, 1.04 - 3.42 95% CI) (meshblock is smallest geographical unit statistical data are collected for), having contact with animal manure (OR 2.09, 1.12 - 3.90 95% CI), having contact with recreational waters (OR 2.95, 1.30 - 6.70 95% CI). However, food-associated risk factors were not identified as sources of STEC infection.

Significance: These findings are somewhat contrary to source attribution information from other countries, strongly indicating that environmental and animal contacts constitute much more important exposure pathways for human cases of STEC in New Zealand than food pathways.

T9-08 Assessing Bacterial Contamination in Ground Beef from the Saskatchwan Retail Market

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Developing Scientist Competitor

Introduction: Ground beef sold by retail outlets in Saskatchewan, Canada originating from a facility regulated by the federal government or licensed by the province can be identified by the package label legend. However, retailers can also sell product that is from locally licensed facilities or has been further processed and repackaged at the point of sale, which might have no label information identifying the source.

Purpose: The objectives of the study were to collect baseline information on bacterial contamination in retail ground beef offered for sale in Saskatchewan and to assess any differences in bacterial contamination based on information available to the consumer at the point of sale.

Methods: Ground beef samples (n = 309) were purchased from May 2011 through May 2012 based on season, geographic region, and census data. Samples were categorized as being from facilities that were federally regulated or licensed by provincial government (n = 126), licensed by local health regions (n = 80), or unknown (n = 103). Total aerobic plate counts (TAPC) and total *E. coli* plate counts (TEPC) were determined using 3M™ Petrifilm™ methods. Total bacterial contamination (TBC) was estimated using real-time quantitative PCR with a universal 16S RNA bacterial target. The data were analyzed using linear regression to account for season and whether samples were fresh or frozen at purchase.

Results: TAPC and TBC significantly differed among all three study categories ($P < 0.001$). The highest TAPC and highest TBC estimates were observed in repackaged samples with no inspection information on the label legend. TAPC and TEPC in samples from federally regulated or provincially licensed facilities were significantly lower than samples from both locally licensed facilities ($P = 0.002$) and samples with no inspection information on the label legend ($P = 0.01$).

Significance: The lowest average general bacterial contamination measured as TAPC, TEPC, and TBC was observed in samples with label legends indicating they were from federally regulated or provincially licensed production facilities.

T9-09 The Use of Global Trade Item Numbers (GTIN) in the Investigation of a *Salmonella* Newport Outbreak Associated with Blueberries

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Introduction: In August 2010 the Minnesota Department of Agriculture and Minnesota Department of Health investigated an outbreak of 6 cases of *Salmonella* Newport infection occurring in northwestern Minnesota, which identified fresh blueberries as the cause.

Purpose: This investigation demonstrates the use of novel data sources to solve foodborne outbreaks.

Methods: Traditional traceback methods involving the review of invoices and bills-of-lading were used to attempt to identify the source of the outbreak. When these methods failed, novel traceback methods were used.

Results: The use of supplier-specific 12-digit Global Trade Item Numbers (GTINs) and shopper-card information was used to identify a single blueberry grower linked to cases corroborating the results of a case-control study in which consuming fresh blueberries was statistically associated with illness (5 of 5 cases vs. 8 of 19 controls; matched odds ratio [mOR], undefined; $P = 0.02$). Consuming fresh blueberries from retailer A was also statistically associated with illness (3 of 3 cases vs. 3 of 18 controls; mOR, undefined, $P = 0.03$). Based on initially incomplete evidence in this investigation, the invoices pointed to wholesaler A and grower A based on first-in-first-out product rotation. However, when point-of-sale (POS) data were analyzed and linked to shopper-card information, a common GTIN was identified. This information led to an onsite record evaluation at retailer A, and the discovery of additional records at this location documented the supply chain from grower B to wholesaler C to retailer A, shifting the focus of the investigation from grower A to grower B.

Significance: This investigation demonstrates the emerging concepts of Critical Tracking Events (CTEs) and Key Data Elements (KDE) related for food product tracing. The use of these shopper-cased data and the event data that were queried by investigators demonstrates the potential utility of consciously designed CTEs and KDEs at critical points in the supply chain to better facilitate product tracing.

T9-10 Generic *Escherichia Coli* Contamination of Spinach at the Preharvest Level as Affected by Farm Management and Environmental Factors

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Developing Scientist Competitor

Introduction: To reduce incidence of foodborne illnesses attributed to produce, it is of interest to study farm-related risk factors for produce contamination with *Escherichia coli*.

Purpose: The objective of this study was to determine the effect of farm management and environmental factors on the preharvest spinach contamination with generic *E. coli* as an indicator of fecal contamination.

Methods: A repeated cross-sectional study was conducted by visiting spinach farms up to four times per growing season over a period of two years (2010 and 2011). A total of 955 spinach samples were collected from 12 spinach farms in Colorado and Texas as representative states of the Western and Southwestern United States, respectively. Farmers were surveyed about farm-related management and environmental factors using a questionnaire. Associations between the prevalence of generic *E. coli* in spinach and farm-related factors were assessed using a multivariable logistic regression model including random effects for farm and farm visit.

Results: Overall, 6.6% of spinach samples were positive for generic *E. coli*. Significant risk factors for spinach contamination with generic *E. coli* were proximity (within 10 miles) of a poultry farm, the use of pond water for irrigation, the period since planting spinach greater than 66 days, farming on fields previously used for grazing, production of hay before spinach planting, and the farm location in the Southwestern United States. The presence of generic *E. coli* was significantly reduced when the irrigation lapse time was greater than 5 days, by the use of portable toilets, training to use portable toilets, and use of hand-washing stations.

Significance: To our knowledge, this is the first report of an association between field workers' personal hygiene and produce contamination with generic *E. coli* at the preharvest level. Collectively, our findings support that practicing good personal hygiene and other good farm management practices may reduce produce contamination with generic *E. coli* at the preharvest level.

T9-11 A Comparison of Food Vehicles Implicated in Outbreaks and United States Food Consumption Patterns, 2005-2010

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Introduction: Outbreak surveillance data are often used to determine the common sources of foodborne illnesses. One question associated with this use of outbreak data is whether the foods implicated in outbreaks mirror the most common food exposures in the population or are foods associated with higher risks. We investigated whether the types of foods implicated in outbreaks reported to CDC's Foodborne Disease Outbreak Surveillance System (FDOSS) were also those ingested most commonly as reported by participants in the 2007-2008 National Health and Nutrition Examination Survey (NHANES).

Purpose: The purpose of this study was to compare foods implicated in outbreaks with those reported in a nationally-representative survey to determine if some food commodities are relatively more likely to be implicated in outbreaks.

Methods: We matched foods implicated in outbreaks from 2005 through 2010 to those reportedly consumed by NHANES participants using 24-hour dietary recall. FDOSS food commodity categories were used to compare foods in both databases. Analyses were restricted to foods; non-dairy beverages, condiments, and sweeteners were excluded.

Results: During 2005-2010, 1014 foods were implicated in 2828 outbreaks. Among these, 951 (94%) were also foods reportedly consumed by NHANES respondents. Higher proportions of foods implicated in foodborne disease outbreaks belonged to beef (7%), fish (8%), and mollusk (3%) commodities compared with the proportions in meals consumed by NHANES study participants (4%, 1%, and <1%, respectively). In contrast, lower proportions of outbreaks implicated foods in the fruits-nuts (4%) and grains-beans (3%) commodities compared with the proportions in meals reported by NHANES participants (6% and 8%, respectively).

Significance: The results of this study suggest that beef, fish, and mollusks may be more frequently implicated in foodborne disease outbreaks relative to their reported frequency of consumption. This information can be used to target prevention efforts toward riskier food commodities.

T9-12 Revised Estimates of the Burden of Foodborne Illness in Canada

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Introduction: Foodborne illness estimates help set food safety priorities and create public health policies. In 2008, the Public Health Agency of Canada estimated that 11 million episodes of foodborne illness occur each year in Canada. Although the best estimate at the time, it was determined using older methods and data. The Public Health Agency of Canada recently completed revised estimates of foodborne illness for Canada.

Purpose: There were two overall objectives: (1) calculate a more accurate estimate of domestically, acquired foodborne illness in Canada using current data and more robust methods and (2) identify knowledge gaps for further research.

Methods: Estimates for 30 known pathogens and unspecified agents using data from Canadian surveillance systems (for years 2000-2010), relevant international literature and the 2006 Canadian census population were calculated. The analysis accounted for under-ascertainment as public health surveillance systems are subject to under-reporting and under-diagnosis. Estimates on the proportion foodborne and the proportion travel-related were incorporated for each pathogen. Monte Carlo simulations were performed to account for uncertainty using @Risk software generating mean estimates and 90% credible intervals.

Results: There are an estimated 4.0 million episodes of domestically acquired, foodborne illness each year in Canada (1.6 million episodes from 30 known pathogens and 2.4 million episodes from unspecified agents). The top four pathogens are (1) norovirus, (2) *Clostridium perfringens*, (3) *Campylobacter* spp. and (4) non-typhoidal *Salmonella* spp.

Significance: The revised estimates cannot be compared for trends with the 2008 estimate because different methods were used. Although lower, the revised estimates are more accurate than the 2008 estimate because they use current data and more rigorous methods.

Policy makers, industry, academia and other organizations can use the revised estimates to better inform policy, research, food safety risk assessments, education campaigns and other prevention and control activities – ultimately improving the health of Canadians.

T10-01 *Salmonella* Survival and Differential Expression of Membrane-associated Genes in a Low Water Activity Food

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Introduction: Numerous outbreaks of salmonellosis associated with low water activity foods have been reported in recent years. However, the adaptive mechanisms utilized by *Salmonella* to survive in low water activity foods for prolonged periods of time is not fully understood.

Purpose: The purpose of this study was to investigate the survival characteristics and changes in gene expression profiles associated with fatty acid biosynthesis of five serovars of *Salmonella* exposed to a low water activity food (sugar) over a 14-day period.

Methods: Five serovars of stationary phase *Salmonella* (*S.* Typhimurium ATCC 2486, *S.* Enteritidis H4267, *S.* Tennessee ARI-33, *S.* Tennessee S13952 and *S.* Tennessee K4643 [peanut butter outbreak strain]) were each inoculated into granular sugar (treatment; A_w=0.50) or sugar water (0.25% w/v; A_w = 0.99; control) and held aerobically over a 14-day period at 25°C. First, survival of the each strain was tested by serial diluting and spread plating on TSA and XLT-4 plates at selected sampling times (0, 1, 3, 5, 7, and 14 days). Then, gene expression was evaluated by extracting total RNA of each strain using RNeasy Mini Purification Kits, and Real-Time Reverse-Transcriptase Polymerase Chain Reaction (qRT-PCR) was performed to compare gene expression profiles. The Relative Expression Software Tool (REST; 2009) was used to analyze gene expression using *rec*A and *ffh* as reference genes. All experiments were performed in triplicate.

Results: After 14 days incubation at 25°C, there was a 2.5 to 3.9 log reduction for all five strains. *S.* Tennessee ARI-33 and *S.* Tennessee K4643 displayed greater survival than *S.* Typhimurium, *S.* Enteritidis and *S.* Tennessee S13952, which showed no growth on XLT-4 after 14 days incubation. The *fab* A gene (unsaturated fatty acid biosynthesis) was observed to be up-regulated for all strains for at least one sampling time. *S.* Typhimurium and *S.* Enteritidis increased expression of the *cfa* gene (cyclopropane fatty acid biosynthesis) over 14 days and concurrently had a lower survival rate.

Significance: The results suggested that low water activity environments might trigger unsaturated fatty acid biosynthesis of *Salmonella*, and cyclopropane fatty acid synthesis is not favorable for survival of the five serovars tested over 14 days.

T10-02 Roles of Fatty Acid Composition and Cell Membrane Fluidity in Thermal Resistance of *Salmonella* after Desiccation

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Developing Scientist Competitor

Introduction: *Salmonella* cells that have survived in a desiccated state in dry foods demonstrate increased thermal resistance. Our previous microarray work showed that fatty acid (FA) metabolism was *Salmonella*'s most significant physiological response to desiccation. FA profile changes in response to pH and temperature have been studied; however, it is unknown how changes in FA and membrane fluidity during desiccation affect thermal resistance.

Purpose: To understand FA composition and cell membrane fluidity effects in desiccation-induced thermal resistance of *Salmonella*.

Methods: FA extraction and methylation of *S.* Tennessee cells were done by the Miller-Berger method. FA profiles were generated by GC-FID mass spectrometry (Midi Inc.). Anisotropy and membrane fluidity of fresh and desiccated cells (36% ERH, 24 h and 5 days) were measured by fluorimetry during thermal treatment from 36 to 80°C, using 1, 6-diphenyl-1,3,5-hexatriene. Data from three independent experiments were analyzed by one-way ANOVA.

Results: Compared to fresh cells, desiccated cells exhibited significantly increased percentage of FAs that are saturated (+2.8%), cyclic (+4.5%), and decreased unsaturated FAs (-4.5%). Longer desiccation significantly increased these changes (P = 7.4E-05). There was no significant difference between long- and short-chain FAs among all conditions, indicating that FA changes during desiccation did not result from catabolic pathways. Anisotropic measurements during thermal treatment showed that membrane fluidity of fresh cells remained unchanged until a sharp decrease at 58°C, indicating a phase transition. Desiccated cells showed overall lower fluidity with a continual decrease from 36 to 50°C, followed by a gradual increase to 62°C, then a decrease. The reduction of unsaturated and increase of cyclic FAs in desiccated cells suggested an upshift of melting point, which explained the overall downshift of fluidity and its protection from cellular component leakage.

Significance: Thermal resistance of *Salmonella* after desiccation is influenced by changes in cell membrane FA composition and structure.

T10-03 *Listeria monocytogenes* Persistence- and Virulence-associated Mechanisms are Mediated by Lmo0753, a Crp/Fnr Family Transcription Factor

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Introduction: *Listeria monocytogenes* is a foodborne pathogen of public health concern. Most persistence- and virulence-associated mechanisms of this pathogen are controlled by PrfA, a Crp/Fnr transcription factor. Previous studies have identified a second transcription factor of the same family, Lmo0753, which is present in only human-associated outbreak strains.

Purpose: The purpose of this study was to determine if Lmo0753 contributes to persistence- and virulence-related mechanisms of motility, biofilm formation, hemolytic activity, and intracellular survival and growth.

Methods: Motility assays of strains 10403S and EGD-e, along with their respective *Δlmo0753*mutants, were conducted for 48 h at 37, 25, and 4°C on BHI soft-agar plates. Crystal violet biofilm assays were conducted at 37°C and 25°C on glass and plastic surfaces for 48 h in BHI and 1% peptone water. For listeriolysin O-associated hemolytic assays, serial dilutions of supernatants of each strain and mutant were made followed by addition of sheep red blood cells, incubation for 30 min at 37°C and subsequent OD quantification. For intracellular assays, monolayers of mammalian cell cultures J774 and Caco-2 were grown to confluency, infected, and every 2 h bacterial growth was enumerated.

Results: Lmo0753 plays a significant role in flagellar motility (P < 0.05) in 10403S at 37°C and 25°C and EGDe at 4°C. Significant decreases (P < 0.0001) in biofilm production and hemolytic activity (P < 0.05) were also observed for *Δlmo0753*in 10403S and EGDe. Lmo0753 appeared to contribute to the intracellular growth of both 10403S and EGDe in J774 cells but not in Caco-2 cells.

Significance: Findings from this study may help to elucidate the specific role of Lmo0753 in *L. monocytogenes* toward a better understanding of the persistence and virulence mechanisms of this pathogen.

T10-04 The Long-term Survival of *Salmonella* Cells Adhered to Stainless Steel under Various Environmental Conditions and Their Resistance to Disinfectants

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Developing Scientist Competitor

Introduction: Low a_w foods (< 0.85 a_w) do not support pathogen growth, but have been associated with numerous outbreaks of *Salmonella*. Increasing concern over the safety of dry products is encouraging the industry to seek better manufacturing practices, primarily to prevent cross-contamination from the factory environment.

Purpose: The aim of this study was to investigate the persistence of *Salmonella* strains attached to surfaces under various environmental conditions, and their resistance to disinfectants.

Methods: Fifteen *Salmonella* isolates were dried onto stainless steel surfaces, placed in controlled conditions and their viability assessed at times from 1 hour to 30 days. Strains showing high and moderate survival were reassessed using various parameters: temperatures (37°C, 25°C and 15°C); relative humidity (33% and 53%); presence of food debris (infant formula, skimmed milk, milk chocolate and cocoa powders); inoculum levels (10^4, 10^5 and 10^7CFU/surface); and periodic wetting (every 7 days). The strains attached to discs for 30 days were subjected to surface disinfection using sodium hypochlorite and propan-2-ol, following a modified protocol of the EU surface disinfectant test.

Results: The highest survival rate was associated with strains of *S.* Muenchen, *S.* Enteritidis, *S.* Typhimurium DT104 and *S.* Agona, showing, after 30 days, a reduction in viability of 1.5 to 2 log CFU/surface. Prolonged survival was not serotype or time related. Presence of food debris (0.0-1.0 log CFU/surface reduction) and exposure to water (<0.2 log CFU/surface reduction) favored the survival of all isolates over 30 days. Strains survived better at lower temperatures (1.0-1.4 log CFU/surface reduction) and lower humidity (1.5-2.1 log CFU/surface reduction). 30 day

adhered strains were not resistant to disinfectants (500 ppm of sodium hypochlorite and 40 % of propan-2-ol), achieving the required standard 4 log reduction.

Significance: The results provide an insight into *Salmonella* survival in dry processing environments, can help to choose appropriate methods for their control and create a basis for current studies on physiological and molecular mechanisms of *Salmonella* survival.

T10-05 Differentiation of Closely Related *Salmonella enterica* Serotype Heidelberg Isolates by Comparative Genomic Analysis

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Developing Scientist Competitor

Introduction: *Salmonella enterica* serovar Heidelberg is one of the top serovars responsible for numerous human outbreaks, including a 2011 multistate outbreak involving 136 confirmed cases and one death, resulting from consumption of contaminated ground turkey.

Purpose: Our objectives were to explore how whole genome sequencing (WGS) can differentiate outbreak isolates of *Salmonella* Heidelberg from non-outbreak isolates that share the same pulsed-field gel electrophoresis (PFGE) pattern, and provide a better understanding of the evolution and ecology of *Salmonella* Heidelberg.

Methods: DNA from 44 *Salmonella* Heidelberg isolates with 20 having a nearly indistinguishable PFGE *Xba*I and *Bln*I pattern collected from various sources over 30 years, including the 2011 outbreak, was sequenced using the 454 GS FLX (Roche) platform. Phylogenetic analyses were conducted on a matrix of variable single nucleotide polymorphisms (SNPs) identified with the program kSNP. DNA from a clinical 2011 outbreak isolate was sequenced and assembled using the Pacific Biosciences (PacBio) *RS* sequencer and their hierarchical genome assembly process.

Results: SNP analysis found a total of 860/4,053 SNPs informative and distinguished isolates sharing the same PFGE patterns. The outbreak isolates clustered together having only 2 diagnostic SNP differences among them. Furthermore, the outbreak isolates contained a VirB/D4 virulence plasmid that carries the Type IV secretion system, and an incompatibility group (Inc) I antimicrobial resistance plasmid encoding resistance to gentamicin (*aacC2*), beta-lactams (*bl2b*_tem-1), streptomycin (*aadAI*) and tetracycline (*tetA*, *tetR*). Additionally, the complete, closed genome/plasmids sequence from a clinical isolate was rapidly determined using the PacBio system providing more nucleotide sequences for analyses.

Significance: This study shows that WGS, in combination with other methods, is a powerful tool for separating strains considered clonal by indistinguishable PFGE profiles. The timely application of WGS technology will advance investigations to identify bacterial sources of infections and to understand the outbreak transmission dynamics of *Salmonella*.

T10-06 Fate of Infiltrated *Salmonella* Cells in Tomatoes during Storage

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Introduction: Several salmonellosis outbreaks have been traced back to tomatoes that were potentially contaminated during post-harvest handling. The tomato stem scar has been proposed as an important gateway for *Salmonella* infiltration to internal tissues during washing. However, the fate of the internalized cells in the tomato during storage is not well understood.

Purpose: This project evaluated the effect of storage duration and temperature on the survival and growth of *Salmonella* cells internalized through infiltration during simulated tomato dump tank handling.

Methods: Green mature tomatoes were inoculated with *Salmonella enterica*, including *S.* Newport, *S.* Typhimurium, and *S.* Thompson. Batches of tomatoes (90°F) were submerged in cell suspensions (80°F) of individual *Salmonella* strains for 15 min, treated with surface disinfectant and stored at 54°F and 70°F for up to two weeks. Samples of the core tissue (11mm x 15mm disc, 20mm below stem scar surface) were excised on days 0, 7, and 14 to enumerate *Salmonella* cells infiltrating and surviving inside tomato tissues using a microplate MPN.

Results: The frequency of *Salmonella* presence in the core tissues was significantly affected by strain type ($P < 0.05$) and storage time ($P < 0.05$). The cell populations for selected strains of *S.* Thompson and *S.* Typhimurium in tomato core tissue increased by 1.71 and 3.65 log MPN/g after the storage for 14 days, respectively. This indicated a significant proliferation by certain strains in tomato tissues during the storage. The increase in cell counts was significantly affected by strain type ($P < 0.0001$) and storage time ($P < 0.0001$). Although the tested strains responded to the storage differently, they were all capable of survival and even proliferated in the ripening tomato tissues.

Significance: These findings underline the importance of preventing pathogen infiltration during post-harvest processing.

T10-07 Autoinducer-2 Signaling Molecules Produced by *Pediococcus* Suppress Growth and Virulence Gene Regulation in *Salmonella*

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Developing Scientist Competitor

Introduction: Probiotics provide numerous benefits to the host including protection from pathogens by competing for nutrients, space and producing antimicrobial substances. Recently, a novel mechanism of protection is discovered by which *Pediococcus* suppressed the growth of *Salmonella* and interfered with gene regulation in *Salmonella*. These findings were discovered by using sterile spent medium produced by *Pediococcus* in which *Salmonella* was cultured. We hypothesized that the quorum sensing signal Autoinducer-2 (AI-2), present in the sterile medium, was responsible for this gene interference and further experiments confirmed this hypothesis.

Purpose: Further investigate the mechanism by which *Pediococcus* retards the growth of and interferes with the gene regulation in *Salmonella.*

Methods: *Pediococcus* was cultured in MRS broth for 24 h and this medium was filter sterilized to produce the sterile spent medium. Growth curves were conducted by culturing *Salmonella* in the sterile spent medium and measuring optical density every hour for 24 h. To investigate virulence gene regulation interference, total RNA was extracted from *Salmonella* cultured in the sterile spent medium and Real-Time PCR (RT-PCR)

was used to evaluate gene expression. Finally, *Salmonella* was cultured in the medium containing 100 µM or 200 µM of AI-2 and gene expression analysis was repeated.

Results: *Salmonella* growth was inhibited and the expression of the virulence gene *hilA* was down regulated when *Salmonella* was cultured in the sterile spent medium produced by *Pediococcus*. A more targeted investigation confirmed that AI-2, a chemical molecule present in the medium, was responsible for suppression of *hilA* gene expression.

Significance: From these experiments, we found a new mechanism by which probiotics *Pediococcus* may protect the host against pathogen infection; by suppressing growth or interfering with gene regulation. Further studies are needed to refine our knowledge of the interaction between AI-2 and other virulence genes in *Salmonella* and the relationship between quorum sensing and virulence.

T10-08 Relationship between Culture- and Molecular-based Methods in Detecting *Escherichia coli* O157 in Cattle Feces

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Introduction: Detection of *Escherichia coli* O157 in cattle feces has traditionally utilized culture-based methods. Molecular-based PCR methods have recently been reported as alternative detection tools.

Purpose: Our objective was to compare culture- and PCR-based assays for *E. coli* O157 detection in the feces of naturally infected feedlot cattle.

Methods: Fecal samples were collected from pens of feedlot cattle enrolled in an *E. coli* O157 intervention study. Samples considered high shedders (≥ 10^4 CFU/g feces) by a semi-quantitative culture method, culture positive after enrichment (immunomagnetic separation and plating on selective medium), or culture negative for *E. coli* O157 were used. One hundred fecal samples from each category (high shedder, enrichment positive, culture negative) were randomly selected for evaluation by real-time PCR. DNA was extracted pre- and post-enrichment from each sample and subjected to a previously-published *E. coli* O157 real-time assay, based on three genes, *rfb*E, *stx*1, and *stx*2. All real-time assays were run in triplicate, and a sample was considered positive when both the *rfb*E gene and either *stx*1 or *stx*2 had an average CT value ≤ 36.

Results: For fecal samples identified as high shedders, 37% (37 of 100) were positive for *E. coli* O157 by PCR prior to enrichment, and 85% (85 of 100) were positive by PCR post-enrichment. For enrichment-positive cultures, 4% were detected by PCR prior to enrichment and 43% were PCR positive post-enrichment. Seven samples were culture negative and PCR positive prior to enrichment, and 40 were PCR positive but culture negative after enrichment.

Significance: Our data suggest a discrepancy between culture- and molecular-based detection methods for *E. coli* O157 in cattle feces; however, the agreement is best in samples containing high concentrations of *E. coli* O157.

T10-09 Improved Protocol for Isolation of *Campylobacter* spp. from Retail Broiler Meat

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Introduction: Campylobacteriosis continues to be an important bacterial foodborne disease worldwide.

Purpose: The objective of our research was to evaluate a rinse method using buffered peptone water (BPW) to enrich retail broiler meat under aerobic condition for the isolation of *Campylobacter* spp.

Methods: In each of two experiments (Exp1 and Exp2), 120 retail meat samples (breasts = 40, tenderloins = 40, thighs = 40) collected in the states of Alabama and Washington were analyzed for the presence of naturally occurring *Campylobacter* spp. One piece of meat was rinsed in Exp1 (A1) and two pieces in Exp2 (A2). Samples were also tested with a reference (R1, R2) method (enrichment of 25 g of meat in Bolton broth at 42°C under microaerobiosis. Isolation was performed on CCDA plates and identification was done using PCR and characterized by PFGE. Result from reference and alternative methods were analyzed with the McNemar's chi-square.

Results: Samples in Alabama had less prevalence of *Campylobacter* spp. than samples in the state of Washington ($P \leq 0.05$). The percentage of positive was higher in A than in R subsamples ($P \leq 0.05$) and rinsing two pieces of meat yielded the highest percentage of positive subsamples ($P \leq 0.05$). R subsamples showed variations in the prevalence by product ($P \leq 0.05$). However, the alternative subsamples from Exp2 (A2) had similar prevalence of positives among products. A2 subsamples had more *C. coli* isolates and a larger diversity of *Campylobacter* spp. by PFGE.

Significance: Rinsing broiler meats was less time consuming, required less sample preparation and was more sensitive than the reference method for the isolation of naturally occurring *Campylobacter* spp. This new method could help with epidemiological and intervention studies to control *Campylobacter* spp.

T10-10 Effect of Bicarbonate Concentration on Aerobic Growth of *Campylobacter* in a Fumarate-Pyruvate Medium

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Introduction: *Campylobacter* are a major cause of human foodborne illness. These enteropathogens are microaerophilic capnophiles; therefore, cultures are generally grown in modified atmospheres. *Campylobacter* are unable to metabolize carbohydrates; however, the bacteria can metabolize organic acids such as fumarate and pyruvate. Recent research has indicated that *Campylobacter*spp. are capable of aerobic growth in media supplemented with fumarate and pyruvate.

Purpose: The purpose of the present study was to examine the effect of sodium bicarbonate ($NaHCO_3$) concentration on aerobic growth of *Campylobacter*in a fumarate-pyruvate medium.

Methods: Fumarate-pyruvate broth medium was supplemented with 0.00 to 0.10% $NaHCO_3$ and inoculated with *Campylobacter coli* 33559, *Campylobacter fetus* 27349, *Campylobacter jejuni* 33560, or *Campylobacter jejuni* 49349. Aliquots of inoculated media were transferred to wells of a honeycomb plate and placed in a Bioscreen Microbiology Reader. Cultures were incubated aerobically at 37°C for 72 h, and culture optical density (OD) was measured during incubation (n = 5). Additional experiments were conducted to compare CFU/ml of *Campylobacter* recovered from media supplemented with 0.05 % $NaHCO_3$, inoculated with *Campylobacter* spp., and incubated aerobically or microaerophilically for 72 h at 37°C (n = 6).

Results: Results indicated that the OD of cultures of all isolates, except *C. coli*, were significantly ($P < 0.05$) higher when grown in fumarate-pyruvate medium containing added $NaHCO_3$. The addition of $NaHCO_3$ produced significant increases in the OD of most isolates during early periods of growth. Also, there was a 5 to 6 log increase in CFU/ml of all isolates recovered from media supplemented with 0.05% $NaHCO_3$, and there was no significant difference in the number of CFU/ml recovered from media incubated aerobically or anaerobically.

Significance: Findings indicate that supplementing fumarate-pyruvate broth medium with $NaHCO_3$ improves the ability of the medium to support aerobic growth of *Campylobacter*. This medium might provide an alternative to culturing *Campylobacter* under microaerophilic conditions.

T10-11 In-depth Analysis of Chlorine Dioxide Exposure on *Listeria monocytogenes*

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Developing Scientist Competitor

Introduction: As application of novel sanitizers increases, elucidation of stress management systems employed by foodborne pathogens is pivotal. Preliminary work in *Listeria monocytogenes* has shown increased activity of oxidative stress management genes upon exposure to chlorine dioxide, ClO_2. Regulatory genes, *sigB* and *ctsR* are active during oxidative stress response.

Purpose: The purpose of this study was to further investigate the phenotypic and genotypic response of *L. monocytogenes* exposed to sublethal ClO_2 concentrations. This work allowed for a more thorough understanding of *L. monocytogenes*' stress response mechanism and ClO_2 survival strategies.

Methods: *L. monocytogenes* 10403S wildtype, Δ*sigB* and Δ*ctsR* null mutant strains were exposed to 300 mg/l aqueous ClO_2 at 37°C, 230 rpm for up to 20 min, with cell populations enumerated every 2.5 min. RNA was extracted from *L. monocytogenes* 10403S wildtype following exposure to 300 mg/l ClO_2 for 15 min and untreated cells; gene expression levels from untreated and ClO_2 treated cells were compared using qRT-PCR with a total of 6 primer and probe gene sets. All experiments were performed in triplicate.

Results: Following 20 min exposure to 300 mg/l ClO_2, log death of *L. monocytogenes* wildtype, Δ*sigB*, and Δ*ctsR* was 0.491, 0.678 and 0.415 CFU/ml, respectively. Transcript levels of *sigB*, *lmo0669*, *dnaK*, clpC, and *lmo1433* significantly increased upon exposure to ClO_2 compared to unexposed cells ($P < 0.05$). These genes are under direct regulation of *sigB* or *ctsR*, were shown to be over expressed via microarray analysis, and fall under the role categories of cell processes, energy metabolism and protein fate.

Significance: This study provides a more defined picture of the response of *L. monocytogenes* to oxidative stress resulting from ClO_2 exposure. The heightened transcriptional activity of genes related to reductase enzymes and chaperone proteins provides insight into how foodborne pathogens respond and potentially survive sanitizer exposure.

T10-12 Norovirus Cross-contamination Associated with Bare Hands and Gloves during Produce Handling

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Introduction: Norovirus-infected persons can harbor and shed up to 10^{11} genomic copies of norovirus/gram of feces when ill, and also shed pathogens asymptomatically. Gloves are often worn as a protective barrier when handling ready-to-eat foods and produce, but they cannot prevent cross-contamination if the outside of the gloves become contaminated.

Purpose: The objective was to quantify and compare glove and bare-hand contamination of food and food-contact surfaces, using three glove types and porcine skin sections to mimic bare-hand contact.

Methods: Virus transfer from porcine skins and gloves (yellow latex) to stainless steel (n = 12 each), from porcine skins to gloves (yellow latex, blue latex, nitrile) (n = 9 each), and from contaminated skins and gloves to multiple leaves of iceberg lettuce (n = 6 each) was modeled and quantified using a stool suspension-cocktail of human (GII and GI) and murine norovirus (MNV-1), or MNV-1 alone. Donor surfaces were interfaced with recipient surfaces by applying 1000 g /4.4 cm^2 for 10 seconds using a mechanical transfer device. Viruses were eluted from recipient surfaces and recovery was determined following real time RT-qPCR.

Results: Transfer rates (GII, GI, MNV-1) from gloves to stainless steel were higher (53.4%, 61.0% and 56.9%) than from porcine skin to stainless steel (39.3%, 42.4% and 43.0%) ($P < 0.05$). Transfer rates (GII, GI, MNV-1) from porcine skin to gloves were 38.4%, 33.1% and 34.9% (yellow latex), 50.8%, 51.9% and 41.0% (blue latex) and 35.0%, 35.6% and 29.3% (nitrile), showing that gloves can become contaminated when applied with contaminated hands. After sequentially touching lettuce with contaminated gloves or skins, virus could be detected on the 15^{th} leaf touched following a single contamination event.

Significance: Virus cross-contamination associated with glove use and bare-hand contact is modeled and quantified in this study to help in developing quantitative risk assessment models for human norovirus contamination by food handlers.

T11-01 Foodborne Pathogen Persistence in the Meat Processing Environment: Longitudinal Study Results, Training Outcomes, and Additional Investigation

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Developing Scientist Competitor

Introduction: Recent foodborne illness outbreaks attributed to strains of pathogens persisting in food processing facilities emphasize the continual need to address knowledge gaps regarding foodborne pathogen persistence.

Purpose: This study aimed to 1) conduct longitudinal sampling for *Listeria monocytogenes*, *Salmonella enterica*, and *Escherichia coli* O157:H7 in four ready-to-eat or fresh meat production facilities, 2) perform molecular subtyping and statistical analyses to elucidate transmission patterns and sites of persistence, 3) provide in-plant trainings and suggested interventions, and 4) conduct follow-up sampling to determine training and intervention effectiveness.

Methods: Briefly, sponge samples of environmental sites, food contact surfaces, and finished products were collected monthly for six months and processed to isolate pathogens according to modified USDA-MLG protocols 4.05, 5.05, and 8.07; the same methodology was used for follow-up sampling. Isolates were characterized by molecular subtyping and data were analyzed using binomial statistical tests to identify persistent subtypes and harborage sites. Trainings were held after the initial six-month sampling period and covered facts on foodborne pathogens and persistence, facility-specific results, and suggested interventions. A paired t-test was used to compare pre- and post-training exam scores to assess participant knowledge changes.

Results: *Eco*RI ribotyping statistical analysis revealed persistent ribotypes and harborage sites for *L. monocytogenes* in Facility A (DUP-1052A, 116-239-S-2; two sites), Facility B (DUP-1062B, DUP-1062E, DUP-1052E; one site), and Facility C (DUP-1042B; six sites). No persistent *Xba*I PFGE pulsotypes of *S. enterica* or *E. coli* O157:H7 were statistically identified. Exam score means significantly increased ($P < 0.05$) from pre- to post-training for three facilities. Among sites that were positive at least once, point prevalence for *L. monocytogenes* on the midshift follow-up sampling date was less than the six-month mean prevalence for two of the three facilities sampled.

Significance: These data suggest that particular *L. monocytogenes* subtypes may persist in the meat processing environment while subtypes of *S. enterica* and *E. coli* O157:H7 are likely transient. Furthermore, highly targeted interventions and training may be necessary to achieve mitigation of *L. monocytogenes* once persistence is established.

T11-02 The Spatio-temporal Distribution and Geographical Predictors of *Listeria* species in Natural Areas and the Produce Pre-harvest Environment of New York State

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Developing Scientist Competitor

Introduction: *Listeria monocytogenes* can be found in diverse environments, often at considerable prevalence, and is able to persist in food processing/handling facilities. Managing this pathogen thus presents a significant challenge to the produce industry. The presence of *Listeria* species has been suggested as an indicator of potential *L. monocytogenes*contamination.

Purpose: The aim of this study is to gain a more complete understanding of the differences in the environmental distribution and ecology of distinct *Listeria* spp. Data from this study will specifically provide insights on the usefulness of *Listeria* spp. as an indicator of *L. monocytogenes* contamination in the produce preharvest environment.

Methods: Geo-referenced environmental samples from natural areas and produce fields in New York State (NYS) were analyzed for the presence of *L. monocytogenes* and other *Listeria* spp. Classification trees were developed using remotely sensed data to determine spatial and temporal predictors of *Listeria* spp.

Results: In the produce preharvest environment, the prevalence of *L. monocytogenes* and other *Listeria* spp. (e.g., *L. innocua*, *L. seeligeri*, and *L. welshimeri*) were 15% (88/588) and 28% (165/588), respectively. Proximity to water, impervious surfaces, and pastures; soil moisture; and temperature were factors that predicted the prevalence of *L. monocytogenes* in the preharvest environment. Similar factors predicted *L. innocua* (water and pasture proximity; frost/thaw cycle) and *L. seeligeri* (urban, pasture, and water proximity; soil moisture). In natural areas, the prevalence of *L. monocytogenes* and other *Listeria* spp. (i.e., *L. innocua*, *L. marthii*, *L. seeligeri*, and *L. welshimeri*) were 8% (59/734) and 28% (206/734), respectively. Temperature, pasture proximity, and frost cycles predicted the prevalence of *Listeria* species in natural areas.

Significance: Our data demonstrate widespread presence of *Listeria* species in natural areas and the preharvest environment (approaching 30% prevalence) and identify ecological factors that affect the prevalence of different *Listeria* species.

T11-03 Molecular and Genomic Characterization of STEC in The Netherlands

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Introduction: The incidence of STEC O157 disease and the distribution of the three LPSA6 lineages involved differ considerably between The Netherlands and the United States (Franz 2012). The reasons for this are unclear. Besides O157 there is a large group of STEC belonging to other serotypes (non-O157 STEC), displaying a high diversity in the capacity to cause disease.

Purpose: This study had two major goals. First, the genomic comparison of STEC O157 isolates belonging to similar LSPA6 lineages from The Netherlands and the US. Second, developing a molecular risk assessment (MRA) approach to discriminate between STEC of high and lower human health risk.

Methods: Eighteen Dutch human STEC O157 isolates (5 LSPA6 lineage I, 8 lineage I/II, 5 lineage II) were subjected to whole genome sequencing using the Illumina MiSeq platform and compared with STEC O157 Sakai as a reference strain. Based on a published single nucleotide polymorphism (SNPs) list from sequenced U.S. isolates (Bono 2012), phylogenetic comparisons were made. A set of 225 non-O157 strains isolated from humans, food and cattle were screened for a large number of virulence genes. In addition, these strains were phylotyped and grouped in different seropathotypes (Karmali 2003).

Results: Phylogenetic analysis of SNP lists generated from the genome sequences revealed that Dutch and U.S. isolates belonging to the same LSPA6 lineage cluster together, suggesting no major differences between isolates from both geographic locations. Further analysis of the genome sequences and SNPs of the Dutch strains is ongoing. With respect to the non-O157 STEC, cluster analysis based on the presence/absence of virulence genes showed that STEC seropathotype A and B (including the top 5 relevant STEC serotypes in the EU) clustered together and were separated from seropathotype D and E. The majority of the isolates belonged to *Escherichia coli* phylogroup B1 (60%) and A (20%). Genes responsible for the differentiation between the seropathotypes include *eae, efa1,* and *nleB.* The latter determinant was strongly associated with isolates from hospitalized patients.

Significance: These results increase the understanding in cross-boundary STEC epidemiology and provide a framework for STEC risk assessment.

T11-05 Morphological Characterisation of *Bacillus sporothermodurans* Spores Using Various Microscopic Techniques

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Introduction: *Bacillus sporothermodurans* are gram positive, mesophilic sporeformers that are able to produce highly-heat resistant spores (HRS). Information on the nature and origin of these spores may contribute to reduction in contamination and therefore improvement in the quality of dairy products. $(GTG)_5$ fingerprinting is known to be a promising genotypic tool that allows for more rapid and reliable results and has not yet been used in strain identification of *B. sporothermodurans*.

Purpose: In this study, $(GTG)_5$ fingerprinting was evaluated for *B. sporothermodurans*. In addition, the isolation of various strains from UHT milk as well as from the dairy farm were compared using REP PCR and $(GTG)_5$ PCR.

Methods: In this report, a collection of 9 *B. sporothermodurans* strains were obtained from UHT milk or farm sources (i.e., feed concentrate, silage and raw milk) from different countries. These strains were identified using the general *B. sporothermodurans* PCR as well as the more specific HRS-PCR method identifying the HRS-clone. REP PCR and $(GTG)_5$ PCR fingerprinting were performed on all 9 isolates and thereafter analyzed using a dendrogram.

Results: The two new UHT milk strains (i.e., QA1 from Belgium and F3 from South Africa) were positive for the HRS-PCR. Four of the 9 isolates from raw milk or feed concentrate were tested negative for the HRS-PCR. The farm strain MB 1505 sourced from silage was positive for the HRS-PCR. From the dendrogram, *B. sporothermodurans* strains showed an overall similarity of 40%. The three strains (QA1, F3 and MB 372) showed slight differences in similarity to the other strains. This would be expected as all the three strains were isolated form UHT milk. It was observed that even though the feed concentrate strains all originated from Belgium, the similarity between them was not more than 80% in both molecular methods.

Significance: $(GTG)_5$ PCR has been used to produce DNA fingerprints for a number of species and in this present study, we showed that this method could be used to fingerprint *B. sporothermodurans* strains.

T11-06 Prevalence and Survival of Foodborne Pathogens and Indicator Bacteria in Raw Cookie Dough

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Introduction: Raw cookie dough has recently been recognized as a new food vehicle for foodborne pathogens. Risks associated with cookie dough have not been fully studied, and therefore it is critical to study the pathogen prevalence in cookie dough products.

Purpose: The objective of this study was to evaluate microbiological safety of commercial ready-to-bake cookie dough products by studying prevalence of indicator bacteria and pathogens in these products.

Methods: Chocolate chip cookie dough products of three different brands were purchased from retail stores, and analyzed for the presence of coliform, generic *Escherichia coli, Salmonella* and *STEC*. Petrifilm was used to determine the presence of coliform and *E. coli*, and multiplex PCR for *inv*A gene and *stx*1/*stx* 2 genes were used for detection of *Salmonella* and STEC.

Results: Out of 90 samples tested, 7 samples contained detectable coliforms with counts ranging from 30 to 250 CFU/g, and only 2 samples contained generic *E. coli* at the level of higher than 10 CFU/g, which is the tolerance limit for refrigerated cookie dough products. Interestingly, 86% of coliform and 100% of *E. coli* were detected in products from two brands. Neither *Salmonella* nor *E. coli* O157:H7 were detected in any of the tested samples. It was noted that 96.7% and 97.8% of the tested cookie dough samples were within standards required by the regulation for coliform and *E. coli*, respectively. Additionally, even though all tested product samples were analyzed before their best by dates, prevalence of coliform and *E. coli*counts showed significant positive correlation with the storage time.

Significance: These results suggest the microbiological risk of cookie dough products could increase with longer storage time. The results also indicate the brands, or more precisely, ingredients and/or the entire manufacturing process of each brand could have correlation with microbiological risks of the final products.

T11-07 Molecular Characterisation of *Bacillus sporothermodurans* Using (GTG)5 and REP PCR Fingerprinting

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Introduction: *Bacillus sporothermodurans* are gram positive, mesophilic sporeformers that are able to produce highly-heat resistant spores (HRS). Information on the nature and origin of these spores may contribute to reduction in contamination and therefore improvement in the quality of dairy products. $(GTG)_5$ fingerprinting is known to be a promising genotypic tool that allows for more rapid and reliable results and has not yet been used in strain identification of *B. sporothermodurans*.

Purpose: In this study, $(GTG)_5$ fingerpringting was evaluated for *B. sporothermodurans*. In addition, the isolation of various strains from UHT milk as well as from the dairy farm were compared using REP PCR and $(GTG)_5$PCR.

Methods: In this report, a collection of 9 *B. sporothermodurans* strains were obtained from UHT milk or farm sources (i.e. feed concentrate, silage and raw milk) from different countries. These strains were identified using *B. sporothermodurans* PCR as well as the HRS-PCR method. REP PCR and $(GTG)_5$PCR fingerprinting were performed on all 9 isolates and thereafter analysed using a dendogram.

Results: The two new UHT milk strains (i.e. QA1 from Belgium and F3 from South Africa) were positive for HRS. Four of the 9 isolates from raw milk or feed concentrate were tested negative for HRS. The farm strain MB 1505 sourced from silage was positive for HRS. From the dendogram, *B. sporothermodurans* strains showed an overall similarity of 40%. The three strains (QA1, F3 and MB 372) showed slight differences in similarity. This would be expected as all the three strains were isolated form UHT milk. It was observed that even though the feed concentrate strains originated from Belgium, the similarity was not more than 80% in both molecular methods.

Significance: $(GTG)_5$ PCR has been used to produce DNA fingerprints for a number of species and in this present study, we showed that this method could be used to fingerprint *B. sporothermodurans* strains.

T11-08 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 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 identify starter cultures that minimize the growth of *B. cereus*.

Methods: The influence of the 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 seed 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 seed broth. However, incorporation of ground baobab seeds with 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 seed broth did not cause any antimicrobial activity. *B. cereus* NVH391-98 grew well in all three substrates in mono-culture. Further, all three *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 seed broth and during cooked baobab seed 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.

Poster Abstracts

P1-01 Foodborne Illness Attribution of USDA-Regulated Products

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Introduction: Outbreak-associated foodborne illnesses linked to meat and poultry were analyzed to determine their impact on consumers, to identify areas where industry or regulatory practices need improvement, and to highlight the need for proper food handling by consumers.

Purpose: This study provides important information to frame risk analysis communication to consumers and food handlers regarding meat and poultry products, and to provide guidance to policy makers and industry leaders.

Methods: Foodborne outbreaks reported by CDC attributed to meat and poultry products from 1998-2010 were collected; outbreak-associated foods were placed into 14 distinct consumer-identifiable groups: BBQ, Chicken, Chicken Nuggets, Deli Meat, Ground Beef, Ham, Multiple Meats, Other Beef, Other Meats, Pork, Roast Beef, Sausage, Steak, and Turkey. Each outbreak was analyzed by the number of illnesses and the types of pathogens involved. A severity metric weighted each illness based on the pathogen's estimated hospitalization rate, and the 14 food groups were assigned a severity index number based on the aggregate.

Results: Food groups were placed into one of four risk categories: Highest, High, Medium and Low Risk, based on outbreaks, illnesses and their corresponding severity index. Multiple Meat and Other Meat categories were removed from the final ranking due to their ambiguity. Those with the greatest numbers of illnesses and the most severe pathogens fall into the highest risk categories (Highest and High), while those with fewer illnesses and less-severe pathogens fall into the lower risk categories (Medium and Low).

Significance: These results have direct public health implications for consumers, providing guidance to government and industry on where food safety improvement resources should be focused.

P1-02 Modeling Risks to Sensitive Subpopulations from *Listeria monocytogenes*

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Introduction: *Listeria monocytogenes* is a foodborne pathogen which causes invasive listeriosis within susceptible subpopulations. An outbreak in Canada occurred in 2008, causing 57 cases of listeriosis and 23 deaths from consumption of ready-to-eat (RTE) meats.

Purpose: To create a quantitative microbial risk assessment (QMRA) model to rapidly generate risk estimates for invasive listeriosis in susceptible Canadian subpopulations given contamination of RTE meats with *L. monocytogenes*.

Methods: A QMRA model using Monte Carlo simulation was developed with the Microsoft Excel add-on, @Risk 5.5. National and provincial prevalence data for immunocompromised subpopulations were gathered. A sub-model was created to simulate the overall prevalence of general and susceptible subpopulations, accounting for random overlapping of conditions. Relative risks to susceptible subpopulations were determined by adjustment of the general population dose-response model with relative susceptibility factors. Test simulations were run using default model parameters to simulate a hypothetical contamination event.

Results: The proportion of illnesses attributed to each subpopulation indicated that individuals with liver disease (28%) and cancer (24%), followed by alcoholics (11%) and the elderly (7%), were most at risk. However, cases of listeriosis are infrequently reported in the liver disease and alcoholic subpopulations, which may indicate misdiagnoses or lack of dietary exposure to such foods.

Significance: The QMRA was designed to be a rapid risk assessment tool with the ability to predict the number of cases of listeriosis that may occur following a contamination event when combined with information on the population that may be exposed to a RTE meat product. The QMRA can be used to identify key subpopulations for targeted messaging efforts in the event of a real or suspected *L. monocytogenes* contamination event.

P1-03 Retail-to-Fork Risk Modeling to Predict the Risks Associated with *Escherichia coli* O157:H7 from the Consumption of Fresh-cut Salads and Sprouts in Korea

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Introduction: Shiga toxin-producing *Escherichia coli,* including *E. coli* O157:H7 in fresh-cut vegetables and salads is a major food safety and public health issue worldwide. However, little information has been available on the quantitative microbial risk assessment of *E. coli* O157:H7 through fresh-cut salads and sprouts in Korea.

Purpose: The objective of this study was to develop a quantitative model along the retail-to-fork steps to predict the risk associated with *E. coli* O157:H7 from the consumption of fresh-cut salads and sprouts and to identify the important steps in risk management.

Methods: The objective of this study was to develop a quantitative model along the retail-to-fork steps to predict the risk associated with *E. coli* O157:H7 from the consumption of fresh-cut salads and sprouts and to identify the important steps in risk management.

Results: Current prevalence data showed no contamination of *E. coli* O157:H7 among 1,174 samples in Korea, indicating no risk from the consumption of fresh-cut salads and sprouts. However, scenario analysis on the contamination in initial products (up to 0.14% of contamination ratio) suggested that mean P_{inf} per serving were estimated 2.65×10^{-4} and 2.54×10^{-4} for fresh-cut salads and sprouts, respectively. Using contamination data of generic *E. coli* as a surrogate for *E. coli* O157:H7, the highest growth of *E. coli* (3.2 log CFU/g for 99thpercentile value) was estimated during retail step.

Significance: The risk model developed in this study along the retail-to-fork steps as well as scenario analysis provided scientific background of management options to prevent human exposure to *E. coli* O157:H7.

Poster

P1-04 Development of a Facility-level Quantitative Microbial Risk Assessment Model for *Listeria monocytogenes* in Cold Smoked Salmon

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Developing Scientist Competitor

Introduction: Cold smoked salmon (CSS) has traditionally been challenging in regard to the development of Hazard Analysis Critical Control Point (HACCP) plans as the process lacks a definitive inactivation step. Instead control is established through various steps, each contributing to partial control of microbiological hazards. To determine if quantitative microbial risk assessment (QMRA) techniques can be used to develop risk-based HACCP plans, a manufacturing facility-level risk assessment was undertaken for *Listeria monocytogenes*.

Purpose: This work undertook development of a QMRA to identify key factors contributing to the risk of *L. monocytogenes* in CSS. The objectives were to develop a facility-specific modular product pathogen pathway risk assessment model for CSS and determine if sensitivity analysis can be used to identify risk-based critical control points (CCPs).

Methods: An extensive literature search was performed; relevant data was reviewed, including identification of appropriate predictive microbiology models describing *L. monocytogenes* behavior in CSS. The exposure assessment for the CSS process was based on seven modules (Primary Production, Raw Product Processing, Brining, Cold Smoked Product Processing, Post-Cold Smoking Processing, Distribution/Marketing, and Consumer). The output of the exposure assessment was then linked to the dose response module. The model was simulated using Latin Hypercube Sampling for 100,000 iterations with @Risk software.

Results: Predicted contamination levels at retail were similar to those from the published literature. The sensitivity analysis indicated slicing, the incoming concentration of *L. monocytogenes* in raw salmon, and cold smoking as the three main critical factors affecting the concentration of *L. monocytogenes* at the end of the manufacturing process.

Significance: This study provides a QMRA at a facility level that can be used to evaluate risk mitigation strategies for *L. monocytogenes*. This model is being used to identify CCPs and the impact of critical limits for the control of *L. monocytogenes* in CSS.

P1-05 Prevalence and Counts of *Salmonella* and Enterohemorrhagic *Escherichia coli* in Raw, Shelled Runner Peanuts

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Introduction: Three major outbreaks of salmonellosis linked to consumption of peanut butter during the last 6 years have underscored the need to investigate potential sources of *Salmonella* contamination in the production process flow. Possibilities include initial contamination of raw peanuts which survives roasting and post-roasting environmental contamination. The partition is important to manufacturers of peanut butter who wish to protect public health while preserving a range of options when roasting peanuts.

Purpose: In order to develop prevalence and level information for raw runner peanuts used for the production of peanut butter a total of 10,162 samples (350 g) spanning three crop years (2009 – 2011) were analyzed for *Salmonella* and enterohemorrhagic *Escherichia coli* (EHEC).

Methods: Double-blinded study samples (1,500 g) were obtained from backup 22-kg samples representing lots of runner peanuts (all grades) where a primary sample had tested negative for aflatoxin. The 350 g portions were analyzed using a PCR multiplex assay targeting specific DNA markers unique to *Salmonella*, *E. coli* O157 and enterohemorrhagic *E. coli*. Positive samples were culturally confirmed, and pathogenic organism levels determined using a most probable number (MPN) dilution series.

Results: *Salmonella* was detected in 68 samples, with the highest prevalence rates observed for the crop year 2009 and among market grades for "splits". Only 3 samples were positive for EHEC. For most samples only the original enrichment was positive, yielding an MPN result of 0.74 CFU/350 g. For 11 samples MPN values ranged from 1.7 to 5.3 CFU/350 g.

Significance: It is generally acknowledged that *Salmonella* in raw peanuts can be significantly reduced by heat treatment applied by dry- or oil-roasting, as it is routinely done prior to the production of peanut butter. The information from the this study can be used to develop quantitative microbial risk assessments which would assist manufacturers in understanding what level of microbial intervention should be provided by the roasting process to protect public health.

P1-06 Risk Assessment of Salmonellosis from Raw Shelled Runner Peanuts

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Introduction: Consumption of peanut butter has been linked to three outbreaks of salmonellosis in the last 6 years which sickened nearly 1,500 people and possibly caused the deaths of 8 individuals. Guidance issued by the Food and Drug Administration recommends that manufacturers have in place a validated intervention process capable of adequately reducing the presence of *Salmonella* spp.

Purpose: A Monte Carlo risk assessment was performed in order to assess the risk of salmonellosis presented by commercial raw peanuts used for production of peanut butter, and the degree to which it would be controlled by application of intervention processes validated to defined performance standards.

Methods: The Monte Carlo risk assessment was performed using available *Salmonella* prevalence and level data for raw runner peanuts, the primary feedstock for peanut butter production. The impact of microbial interventions was computed, and the risk of illness from any residual *Salmonella* was estimated using two dose response models, one commonly cited in *Salmonella* risk assessments and one designed to better model *Salmonella* infectivity in low water activity foods.

Results: In the absence of an intervention it was estimated that an arithmetic average of 244 individuals per billion servings of raw peanuts would experience salmonellosis. In the absence of an episodic contamination event a roasting process validated to achieve a reduction of 3 logs or more would be sufficient to produce very low risk, defined as less than 1 hospitalization per billion servings of shelled runner peanut products. Under a worst case scenario in which *Salmonella* is amplified in a railcar of peanuts due to the presence of moisture from condensation, introducing a "slug" of contamination to a roasting process, the reduction required to produce a very low risk product would increase to 4 logs.

Significance: The results of the risk assessment support a conclusion that raw runner peanuts may be converted to a very low risk food item by application of a validated microbial intervention of less than 5 logs.

P1-07 Quantitative Risk Assessment of *Staphyloccus aureus* through Consumption of Seasoned Dried Fish Products in Korea

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Introduction: Seasoned dried fish (SDF) is a popular food as snack or side-dish in Korea. It is one of the ready-to-eat foods consumed raw or slightly heated before serving. Previous study indicated that SDF could be contaminated by a variety of bacteria including *Staphylococcus aureus* in Korea.

Purpose: This study was performed to predict the growth probabilities and enterotoxin production for the *S. aureus* in SDF and to provide the risk management options using Monte Carlo simulation.

Methods: The risk model deals with the consumer's practice on the storage and cooking before consumption. Data on the prevalence and concentration of *S. aureus*, time/temperature of storage and ratio of cooking before consumption were collected and were used to build risk model using @Risk program. Survivals of *S. aureus* on the SDF were estimated using secondary survival model developed to predict cell number during storage. The correlation between toxin level and cell number was used to quantify the probability of staphylococcus food poisoning.

Results: As a result of Monte Carlo simulation, contamination of *S. aureus* in SDF at consumption step was lower than 1.8 log CFU/g for 99th percentile value. The simulated level was lower than the threshold cell number use in this study for toxin production (5 log CFU/g). The estimated probability for staphylococcal intoxication was very low, using currently available data. Simulation result was also showed that survival of *S. aureus* in SDF during storage was affected by storage temperature.

Significance: The risk model developed in this study based on consumer's practice, survival model and toxin production model for *S. aureus* could be used to predict the risk of staphylococcal intoxication through consumption of ready-to-eat foods as well as SDF in Korea.

P1-08 Predictive Model for Survival and Growth of *Salmonella* on Chicken during Cold Storage

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Introduction: *Salmonella* are a leading cause of foodborne illness. Predictive models are useful tools for assessing and managing the risk of foodborne illness from human pathogens like *Salmonella*. During cold storage the number of *Salmonella* on chicken may stay the same, increase, or decrease depending on time and temperature of storage and type of chicken meat.

Purpose: Consequently, the objective of the current study was to model the behavior of *Salmonella* on different types of chicken meat during frozen and refrigerated storage.

Methods: Portions (0.76 g) of chicken meat (skin, breast, thigh) were inoculated with *Salmonella* Typhimurium DT104 (2.8 log) followed by storage for 0 to 8 days at -8, -4, 0, 4, 8, 10, 12, 14 or 16°C. A general regression neural network (GRNN) model was developed using NeuralTools and a dataset of 717 observations. Performance of the model was considered acceptable when the proportion of residuals (observed – predicted) in an acceptable prediction zone (pAPZ) from -1 (fail-safe) to 0.5 log (fail-dangerous) was ≥ 0.7.

Results: Growth of *Salmonella* on chicken was only observed at 12, 14 and 16°C and differed ($P < 0.05$) among types of chicken meat. Growth was highest on thigh, intermediate on skin, and lowest on breast. At lower temperatures (-8, -4, 0, 4, 8 and 10°C), the number of *Salmonella* remained at initial levels throughout 8 days of storage. The GRNN model had acceptable performance for all survival and growth curves with pAPZ that ranged from 0.81 to 1.00.

Significance: Results of this study indicate that it is important to include type of chicken meat as an independent variable in the model and that the model can be used with confidence to assess and manage effects of cold storage deviations on the risk of salmonellosis from chicken.

P1-09 Norovirus Transfer between Foods and Food Contact Materials

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Introduction: Noroviruses (NoV) are a major cause of food borne gastroenteritis worldwide and are often transmitted via infected food handlers.

Purpose: The current study aimed to provide more detailed data regarding the transfer of MNV-1 between food preparation related surfaces and various food products.

Methods: Transfer of MNV-1 between the different surfaces was performed by pressing an inoculated donor surface against an acceptor surface at a pressure of 0.2-0.4 kg/cm² for 10 seconds while performing a 90° twist. All transfers were tested in threefold (n = 3). To evaluate the effect of subsequent contact moments, donor surfaces were tested a second time for transfer to an identical acceptor surface using the same transfer protocol.

Results: Transfer of MNV-1 to stainless steel was inefficient (transfer efficiency < 2 %) regardless of the donor surface. The low affinity of this surface for MNV-1 supports the use of stainless steel in food preparation areas. Regarding the use of gloves as acceptor surface, 9.7 % of the MNV-1 inoculum could on average be transferred from foods and the stainless steel discs to the gloves. Ham and the crust of a deli sandwich (mean transfer efficiencies of 21.2 % and 26.2 %, respectively) were significant better acceptor surfaces compared to lettuce (mean transfer efficiency of 2.4 %), most likely due to the roughness of these surfaces. Finally, it was shown that the MNV-1 inoculum was significantly less efficient transferred to an acceptor surface at the second contact moment in most cases.

Significance: This finding suggests that gloves should be replaced frequently during preparation and handling of foods. Furthermore, significant differences were observed for the transfer efficiency of MNV-1 from gloves and the stainless steel discs to different food products. Overall, data obtained from the current study can be included in risk assessment models describing the transmission of noroviruses related to preparation of food products and may aid the correct use of intervention measures to prevent NoV transmission during the preparation of food products such as deli sandwiches.

P1-10 Development of Predictive Mathematical Models to Predict *Staphylococcus aureus* Growth in Ready-to-Eat Salads at Constant and Dynamic Temperatures

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Introduction: *Staphylococcus aureus* is enterotoxigenic foodborne bacteria, and the pathogen has been isolated from various ready-to-eat salads. To predict the fates of foodborne pathogens in foods, predictive models have been developed.

Purpose: This study developed kinetic mathematical models to predict *S. aureus* growth in ready-to-eat salads under constant and dynamic temperatures.

Methods: A five-strain mixture of *S. aureus* was inoculated in 5 g potato and sweet potato salads at 5 log CFU/g. The samples were stored at 10 (216 h), 15 (216 h), 20 (120 h), 25 (48 h), and 30°C (48 h). Cell counts of total bacteria (tryptic soy agar) and *S. aureus* (mannitol salt agar) were enumerated. The growth data were fitted to the modified Gompertz model and the Baranyi model to calculate lag phase duration (LPD; h), maximum specific growth rate (μ_{max}; log CFU/g/h), lower asymptote (log CFU/g), and upper asymptote (log CFU/g). The parameters were fitted to the Davey model, the square root model, and a polynomial equation. Accordingly, *S. aureus* growth was simulated under constant (20 and 27°C) and dynamic temperatures, and the data was compared with observed data. To evaluate the model performance, root mean square error (RMSE) was calculated.

Results: Growth of total bacteria and *S. aureus* was observed in potato salad and sweet potato salad at 15-30°C. For both primary models, μ_{max} values were increased, but LPD values were decreased as temperature increased. R^2 values (0.789-0.976) of the developed secondary models were acceptable. RMSE values were 0.67-0.83 and 0.67-0.7 for 20 and 27°C, respectively, indicating that the model performance was acceptable. Moreover, the predicted *S. aureus* cell counts calculated by the model simulation under dynamic storage temperature were close to the observed *S. aureus* cell counts.

Significance: The results indicate that the developed models for potato and sweet potato salad should be useful in predicting *S. aureus* growth.

P1-11 Differences in Survival of Acid-stress Resistant Phenotype of *Listeria monocytogenes* in Quaternary Ammonium Compounds, Ethanol, NaOCl, and H_2O_2

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Introduction: *Listeria monocytogenes* (*Lm*) has sophisticated adaptive mechanisms to various physiological and environmental stresses. There is a limited knowledge on the survival of acid-stress resistant phenotype of *Lm* in the presence of commonly used disinfectants.

Purpose: Conditions inducing acid-stress adaptation in *Lm* were investigated and the difference in survival of acid-stress resistant phenotype of *Lm* was determined under various lethal stresses.

Methods: Acid-stress adapted cells of *Lm* were prepared by pre-exposing to pH 5 for 1 h in TSB-YE. The survival of acid-stress adapted and non-adapted cells was determined under six lethal stress conditions including, pH 3.5 lactic acid, 5-7 ppm quaternary ammonium compounds (QAC-1, QAC-2), 20% ethanol, 1000 ppm NaOCl and 1000 ppm H_2O_2 in TSB-YE.

Results: Acid-stress resistant phenotype of *Lm* was induced when cells were pre-exposed to pH 5.0 for 15 min for Bug 600 (serotype 1/2a) or in 30 min for Scott A (serotype 4b). Also, acid-stress resistant phenotype of *Lm* was readily observed in acidic whey. Such acid-stress adaptation in *Lm* was reversible when cells were transferred to pH 7 for 1 h at 37°C. Acid-stress resistant phenotype of *Lm* exhibited about 1.5-3.5 log CFU/ml greater survival in 7 ppm QAC-1, 5 ppm QAC-2 or in 20% ethanol compared to that of non-adapted cells. However, acid-stress resistant phenotype of *Lm* did not exhibit any greater survival in 1000 ppm NaOCl and was sensitive to 1000 ppm H_2O_2 compared to that of non-adapted cells.

Significance: Acid-stress resistant phenotype of *Lm* exhibits cross-resistance to QAC and ethanol but not to NaOCl or H_2O_2.

P1-12 Oxidative-stress Resistance Response of *Listeria monocytogenes* and Its Cross Functionality against Lethal Processing Treatments

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Introduction: *Listeria monocytogenes* (*Lm*) is capable of oxidative stress response upon pre-exposure to a sublethal H_2O_2 which allows it to survive subsequent lethal H_2O_2 stress. There is no knowledge on the effect of series of other sublethal stresses in combination with oxidative stress on the survival of *Lm* under lethal inactivation treatments.

Purpose: Determine the oxidative stress adaptive response in *Lm* serotypes 1/2a and 4b when cells were pre-exposed to a single or a series of sublethal stresses and their subsequent resistance to lethal H_2O_2, acid, alkaline and antimicrobials.

Methods: Concentrations of H_2O_2 and pre-exposure times were determined for the formation of oxidative-stress resistant phenotype of *Lm* which was detected by its survival in lethal 1000 ppm H_2O_2 at 37°C or 25°C. *Lm* cells pre-exposed to a sequential sublethal stresses (alkali pH of 9 followed by 50 ppm H_2O_2 for 30 min in TSBYE) were compared with that cells pre-exposed to a single sublethal stress for their subsequent cross resistance to lethal stresses (pH 3.5, pH 11.75, 60 °C, and QAC 2.5 or 5ppm).

Results: The oxidative stress resistant phenotype of *Lm* was induced when *Lm* cells were exposed to 50 ppm H_2O_2 for 30 min at 25°C and 37°C in TSBYE. When *Lm* cells were pre-exposed to sublethal alkali (pH 9) followed by sublethal H_2O_2 (50 ppm), it resulted in a phenotype that exhibited a greater survival in 1000 ppm of H_2O_2 by more than 4 log CFU/ml than the cells that were pre-exposed to sublethal H_2O_2 (50 ppm) alone. Also, sequential alkali- oxidative sublethally stressed cells of *Lm* exhibited greater resistance against both lethal alkali (pH 11.75) and QAC (5 ppm).

Significance: Highly stable oxidative-stress and alkali-stress resistant phenotype of *Lm* is formed when cells were pre-exposed to mild alkaline stress which was not known previously.

P1-13 Development of a Predictive Model Describing the Growth of *Staphylococcus aureus* in Salad Dressing Sauce

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Introduction: Although sauce outbreaks related to *Staphylococcus aureus* have occurred, the predictive models of *S. aureus* in salad dressing sauce were not developed for microbial risk assessment.

Purpose: The objective of this study was to develop mathematical models for prediction of the growth of *S. aureus* in salad dressing sauce, leading to the development of effective control methods for *S. aureus*.

Methods: A five-strain mixture (*S. aureus* ATCC14458, 27664, 23235, 13565 and 19095) was inoculated in 250 g of salad dressing sauce at 2 log CFU/g. The inoculated samples were then exposed to 10, 20, 25 and 35°C, and growth of *S. aureus* were enumerated on Baird Parker agar for 720 h. Moreover, growth data of *S. aureus* were fitted to the Gompertz and Polynomial model to calculate specific growth rates (SGR) and lag time (LT).

Results: The growth rate data fit well with the Gompertz and Polynomial equation model, evidencing a high degree of goodness of fit (R^2 = 0.9472 to 0.9945) for all temperatures. Temperature also exerted a significant effect on both the SGR and LT. As storage temperature increased, the SGR increased markedly and the LT decreased. The results of R^2, B_f, and A_f which were different statistical indices for the SGR and LT of *S. aureus* in salad dressing sauce were 0.9825, 1.17, 1.27 and 0.9765, 0.94, 1.16, respectively. The higher r^2 ($0<r^2<1$) values resulted in more accurate model predictions.

Significance: The predictive model of *S. aureus* in salad dressing sauce is expected to provide risk managers and consumers with important risk information regarding the shelf life and appropriate storage condition.

P1-14 Mathematical Models to Describe the Kinetic Behavior of *Staphylococcus aureus* on Processed Cheeses

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Introduction: *Staphylococcus aureus*is one of major foodborne pathogens related to foodborne disease in many cheeses. Mathematical models could be used to predict bacterial growth on cheese under various growth conditions.

Purpose: The objective of this study was to develop a kinetic model to describe the kinetic behavior of *S. aureus*on processed cheeses.

Methods: Mozzarella and cheddar slice cheeses from Company A and B, and gouda slice cheese from Company A were inoculated with a five-strain mixture of *S. aureus* at 3 log CFU/g. The samples were stored at 4 (1440 h), 15 (288 h), 25 (72 h) and 30°C (48 h). Total bacterial and *S. aureus* cell counts were enumerated on tryptic soy agar and mannitol salt agar, respectively. The growth data of *S. aureus* were fitted to the Baranyi model to calculate maximum specific growth rate (μ_{max}; log CFU/g/h) and lag phase duration (LPD; h). The kinetic parameters were further analyzed by the square root model as a function of temperature. The model performance was validated with observed data, and root mean square error (RMSE) was calculated.

Results: At 4 and 15°C, *S. aureus* cell growth was not observed for all processed cheeses. However, the growth of *S. aureus* on mozzarella, cheddar, and gouda slice cheeses was observed at 25 and 30°C. The μ_{max} values increased, but LPDs decreased as storage temperature increased. No differences of the μ_{max} and LPD values were not observed between mozzarella and cheddar slice cheeses, but the *S. aureus* had lower ($P < 0.05$) growth on gouda sliced cheese than those of mozzarella and cheddar sliced cheeses. In addition, the developed models showed acceptable performance (RMSE=0.413-0.750).

Significance: This result indicates that the developed model should be useful in predicting *S. aureus* growth in mozzarella, cheddar and gouda sliced cheeses.

P1-15 A Qualitative Microbiological and Chemical Risk Assessment for Potatoes

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Introduction: Following the direction of the Food Safety Modernization Act that food safety standards for fresh produce use risk-based approaches, a qualitative risk assessment (QRA) was conducted as a foundation for developing food safety guidelines for the production, harvest, storage and packing of potatoes.

Purpose: The objective of this QRA was to provide a systematic evaluation of potential chemical and microbiological food safety risks that could arise during primary production and packing operations and the adverse human health effects that could result if adulterated potatoes are consumed.

Methods: The QRA was conducted using the framework developed by National Research Council for assessing exposure risks that may cause adverse human health effects, which includes four elements: hazard identification, exposure assessment, hazard characterization/dose-response assessment, and risk characterization. As this assessment was qualitative, a rating system was used to characterize the overall risk. Risk was characterized as negligible (the probability is extremely low), low (the probability is low but clearly possible), medium (the probability is likely), or high (the probability is very likely or certain) or a combination thereof. An overall risk rating was derived by combining individual risk ratings for potential adverse health effects resulting from exposure to each microbiological or chemical hazard.

Results: We assessed the risk of select human pathogens, cadmium, glycoalkaloids, and pesticides to human health. Based on the results of this analysis, it was concluded that the overall potential risk of adverse health effects due to: 1) human pathogens, 2) cadmium, 3) glycoalkaloids, and 4) pesticide residues obtained through potato consumption were determined to be low.

Significance: The results help focus primary production and packing food safety efforts on areas demonstrating greatest potential risk to human health. Examining where a pathogen may potentially emerge in the potato supply chain or determining if chemicals in soil are present in tubers, assists the potato industry with the identification of mitigation measures.

P1-16 Assessment of the Contamination Potentials of Biofilms for Food Products

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Introduction: Biofilm is known as an important source of food contamination and has become a concern of the food processing industry worldwide.

Purpose: This study measured the biofilms formed by different bacterial strains on glass slides and quantified changes in biofilm mass and biofilm-associated cell populations after brief contacts between biofilms and either media agar or food products.

Methods: Two *Listeria monocytogenes* and *Escherichia coli* strains and a single *Staphylococcus aureus* strain were inoculated separately in tryptic soy broth containing a 1 x 2 cm^2 glass slide and incubated for 24, 48 or 72 h at 37°C. Biofilms formed by individual bacterial strains and biofilm-associated cell populations were determined. The biofilms were subsequently allowed to have brief contacts (1 - 3 times), through gentle touching, with either agar or food products including meat and soft white cheese (2 cm^2). Changes in biofilm mass on glass slides and cell populations embedded in biofilms were quantified. Biofilms that were not in contact with the agar or foods served as controls.

Results: A nonpathogenic *E. coli* formed more biofilms than an *E. coli* O157:H7 strain. Biofilms formed by *S. aureus* and *L. monocytogenes* were essentially similar. Biofilm mass increased as incubation time increased within 48 h of the incubation. Biofilm mass at 48 and 72 h of the incubation was not significantly different. More frequent contacts with agar or foods did not remove more biofilms or biofilm-associated cells from glass slides. More *S. aureus* biofilms were removed followed by *Listeria* and *E. coli* biofilms. The populations of bacteria embedded in biofilms after brief contacts with agar or food decreased 0.00 to 0.54 log CFU/cm^2. Greater reductions in cell populations were observed with *S. aureus* and *Listeria* biofilms.

Significance: Results suggest that biofilms could serve as a source of contamination for foods that come in contact with them.

P1-17 Microbiological Safety Assessment for Cultivation Farms of Balloon Flower to Establish a Good Agricultural Practices (GAP) Model

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Introduction: Balloon flower is a perennial herb widely spread in Northeast Asia and generally used as a common food and folk remedy. Since the plant has been usually cultivated in soil, significant microbial contamination which causes food poisoning on balloon flowers is predicted.

Purpose: Objectives of this study were to assess microbial risks of balloon flower farm at the cultivation stage and to provide basic data for the establishment of a good agricultural practices (GAP) model.

Methods: Samples (96 samples) were collected from cultivation environments (soil, irrigation water, and atmosphere), plants (balloon flower and leaf), and personal hygiene (hand, glove, cloth) of 3 balloon flower farms during cultivation. The collected samples were subjected to determine sanitary indicators (aerobic plate count, coliforms and *Escherichia coli*), major foodborne pathogens (*Bacillus cereus*, *Staphylococcus aureus*, *E. coli* O157, *Listeria monocytogenes,* and *Salmonella* spp.), and fungi.

Results: Aerobic plate count and coliform bacterial populations in the all samples were detected at levels of 2.7~7.0 and 1.0~6.4 log CFU/(g, leaf, ml, hand, 100 cm^2), but *E. coli* was not detected in all samples. The levels of fungi in the all samples were 1.0~5.3 log CFU/(g, leaf, ml, hand, 100 cm^2). In case of pathogens, *B. cereus* was only determined in the range of 4.0~5.7 log CFU/g in soil samples, and *S. aureus* (1.8 log CFU/ hand or 100 cm^2) was also detected in the workers. However, other pathogenic bacteria such as *E. coli*, *E. coli* O157, *L. monocytogenes* and *Salmonella* were not detected anywhere.

Significance: According to the results, the microbial population on the balloon flowers was lower than the regulation set by Korean government. However, since cross-contamination of microorganism from cultivation environments and worker has been generally reported, an efficient GAP model is necessary to improve the safety of balloon flower for microbiological hazards. Therefore, the results could be used as basic data to establish GAP model for the production of safe balloon flowers.

P1-18 Microbiological Hazard Analysis of Ginseng Farms at the Cultivation Stage to Develop a Good Agricultural Practices (GAP) Model

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Introduction: The interest for and consumption of ginseng has increased due to its reported health benefits. As most of ginsengs are grown in soil, there is great potential to be contaminated with bacteria from the soil.

Purpose: This study validated microbiological hazards of ginseng farms at the cultivation stage and suggested recommendations to develop a good agricultural practices (GAP) model.

Methods: A total of 96 samples were collected from cultivation environments (soil, irrigation water, and atmosphere), plants (ginseng and its leaf), personnel hygiene (gloves, clothes, and hands) of 3 ginseng farms (A, B, and C) located in Chungnam, Korea, and were tested to analyze sanitary indicator bacteria (aerobic plate count, coliforms and *Escherichia coli*), major foodborne pathogens (*E. coli O157:H7, Listeria monocytogenes, Salmonella spp., Staphylococcus aureus*, and *Bacillus cereus*), and fungi.

Results: Total bacteria, coliform, and fungi in the 3 ginseng farms were detected at the level of 1.3-6.0, 0.1-5.1, and 0.5-4.9 log CFU/(g, leaf, mL, hand, 100cm^2), respectively. Only irrigation water collected from one ginseng farm was confirmed to be *E. coli* positive. In case of pathogenic bacteria, *B. cereus* was detected at levels of 0.7 - 5.0 log CFU/g (or ml, hand, and 100 cm^2) in all samples, but other pathogens including *S. aureus*, *E. coli O157*, *L. monocytogenes*, and *Salmonella* spp. were not detected in any samples from all farms. Although *E. coli* were detected in irrigation water, populations for the three farms were lower than the regulation limit.

Significance: According to the results, ginseng produced from the 3 farms were comparatively safe with respect to microbial contamination. However, cross-contamination of bacteria from environments and workers to ginseng has been considered as potential risks. Therefore, to minimize microbial contamination in ginseng, GAP model should be applied for ensuring the safety of ginseng.

P1-19 Consumer Storage Practices and Their Impacts on Microbial Safety of Home Refrigerated Foods

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Introduction: Studies have shown that consumers often failed to follow the recommended guidelines for proper storage of refrigerated foods at home. However, the relation of improper refrigeration practices and microbiological contamination of refrigerated foods has not been fully assessed.

Purpose: The objective of this study was to identify risk factors for microbial contamination related to storage practices of refrigerated foods.

Methods: In-home interviews and observations were conducted in seventy homes in Nashville, Tennessee. A total of three hundred and twenty samples were collected from participants' refrigerators during home visits. Swab samples were taken from shelf surfaces in the refrigerators and food samples (including home prepared foods, leftovers, and opened packages of ready-to-eat foods) were collected from the participants' refrigerators. Microbiological analyses were performed and molecular fingerprints of *Staphylococcus aureus* (SA), and *Bacillus cereus* (BC) were studied using Pulsed Field Gel Electrophoresis (PFGE).

Results: Eighty-four percent of the observed leftover containers were covered, but only 3.3% of the leftovers had a date labeled. When asked about how long the leftovers were in the refrigerator, 20.5% answered longer than two weeks and 38.5% did not know. Microbiological analyses indicated refrigerator shelf surfaces were often contaminated with high level (more than 10^3 CFU/100cm^2) of SA (15.7%) and BC (22.1 %). Significant numbers of food samples contained high level (more than 10^3 CFU/g) of SA (13.9%) and BC (16.7%). Of the 180 food samples, two of SA and five of BC contamination in refrigerated foods belong to the same PFGE type from the refrigerator shelf surfaces where the foods were stored.

Significance: The results suggested poor sanitation condition and prolonged storage were the most significant factors that affect microbiological safety of home refrigerated foods.

P1-20 Isolation and Identification of Zoonotic Species of Genus *Arcobacter* from Chicken Viscera Obtained from Retail Distributors of the Metropolitan Area of San Jose, Costa Rica

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Introduction: *Arcobacter* is a genus of growing importance worldwide. Some of its species have been considered as emerging enteropathogens and potentially zoonotic agents.

Purpose: In Costa Rica, as well as in other countries, its isolation has been reported, so the objective of this project was to evaluate and identify the presence of *Arcobacter* in chicken visceras sold in the Metropolitan Area of San José, Costa Rica, as well as to determine the antimicrobial resistance patterns associated to it.

Methods: 150 samples of chicken viscera were purchased from various local retailers. 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*. Susceptibility to ampicillin, ciprofloxacin, chloramphenicol, erythromycin, gentamicin and tetracycline was done using E test method.

Results: The isolation frequency of *Arcobacter* genus obtained in this study was of 17.3%. A total of 33 isolates were obtained from the poultry samples, and according to the multiplex PCR methodology, 22 isolates were identified as *A. butzleri* (66.7%), 8 as *A. cryoaerophilus* (24.2%) and 1 as *A. skirrowii* (3.1%). Two strains were not identified. Resistance towards chloranphenicol was of 87.5%, followed by ampicillin (43.75%) and ciprofloxacine (18.75%); all strains were susceptible to tetracycline.

Significance: The potential health risk associated with the presence of *Arcobacter* sp. in chicken viscera products sold in Costa Rica is demonstrated.

P1-21 Food Safety Challenges and Training Needs at Korean Restaurants in the U.S.: A Review of Health Inspection Reports

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Introduction: Ethnic cuisines appeal to the U.S. market and rely on preparation and service techniques that closely resemble their unique culinary traditions. These same practices may impose specific food safety risks, as evidenced by more frequent food code violations in ethnic restaurants than in non-ethnic restaurants.

Purpose: This study was conducted to identify specific food safety challenges and training needs in Korean restaurants in the U.S. through health inspection reports.

Methods: A list of Korean restaurants (n= 1,120) was collected from an online resource (http://www.ussaram.com/), which included various Korean American-owned businesses. Food code violations of 230 randomly selected establishments across 54 cities in 20 states were recorded from the most recent online state or local health inspection reports. All violations were re-categorized using the state of NY inspection form. Descriptive statistics for total, critical, and behavior-related violations and frequencies for individual violations were used to target food safety challenges in Korean restaurants.

Results: The distribution of selected establishments emulated that of Korean American population in the U.S. The number of total violations per inspection ranged from 0 to 20 (Mean ± SD = 5.8 ± 3.5) with 2.4 ± 1.9 critical, 3.8 ± 2.5 behavioral, and 1.1 ± 1.3 critical-behavioral violations. Most Korean restaurants had one or more critical (89.7%) and behavioral (97.0%) violations. The three most frequently-cited behavioral violations were: 1) food not being protected through the food flow (n = 109, 47.4%), 2) floor and facility maintenance (n = 82, 35.7%), and 3) non-food contact surface maintenance (n = 79, 34.3%). The most frequently-cited critical violations were improper food temperatures during cold-holding (27.8%), hot-holding (18.3%), and storage (11.8%).

Significance: The types of violations in Korean restaurants were similar to those previously published for other ethnic restaurants. However, Korean restaurants showed greater food safety training needs for properly labeling and covering food during storage as well as in maintaining floor, facility, and non-food contact surfaces than other ethnic restaurants.

P1-22 Is Antibiotic Resistance a Selective Advantage to Environment Stresses?

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Introduction : The presence of antibiotic resistant bacteria in the food supply chain is a major concern. Different intervention strategies are typically used to eliminate pathogenic organisms from food processing environments. Lactic acid is one of the organic acids that have been used most extensively as antimicrobial. Recurring outbreaks pose a great need to revalidate the effectiveness of these decontamination procedures for multidrug-resistant strains.

Purpose : The purpose of this study was to determine the survival kinetics of antibiotic-resistant non-O157 Shiga-toxin producing *Escherichia coli* (STEC) strains to environment stresses.

Methods : A total of 6 non-O157 STEC strains (serotypes O26 and O103) with three different antibiotic resistant profiles (susceptible, medium and high resistance) were chosen for intervention studies. Tryptic soy broth (pH 6.5) samples were inoculated (10^7CFU/ml) and treated with lactic acid (2.5, 3.5, 5%). All samples were serially diluted into buffered peptone water (pH = 7.4) at 0, 1, 2.5, 5, 7.5 and 10 min; and plated on tryptic soy agar (TSA). All plates were incubated at 37°C for 24 h and bacterial counts were determined.

Results : *E. coli* strains had similar acid survival characteristics. When treated with 5% lactic acid (LA), bacterial counts were reduced by approx. 2 logs CFU/mL within 10 min of the experimental period. Bacterial counts were reduced to approx. 1 log CFU/ml with 3.5% LA for O26 and O103 strains. No reduction in bacterial counts was observed with 2.5% LA treatment for both serotypes.

Significance : The results reported the effectiveness of lactic acid to inactivate antibiotic-resistant non-O157 STEC. These findings suggest that *E. coli* strains showed high acid tolerance and antibiotic resistance had minimal effects on the adaptation to environmental stresses.

P1-23 Stability of Patulin in Apple Juice during Storage as Determined by GC-MS/MS

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Introduction: Patulin is a good quality indicator to use during manufacturing of apple juice. It is a heat stable compound in acidic aqueous solutions and, depending on temperature and storage time, it has considerable stability in apple juice.

Purpose: To determine the stability of patulin during pasteurization and storage of apple juice.

Methods: Sample preparation was based on the QuEChERS procedure, involving an initial extraction step with water and acetronitrile, followed by a partitioning step after the addition of magnesium sulfate and sodium chloride. Commercially sterile apple juice was fortified with different levels of patulin, and three QuEChERS sample extraction methods were compared. The cleanup was performed by using dispersive solid-phase extraction with a mixture of magnesium sulfate, primary secondary amine sorbent, and graphitized carbon black. The filtrate was derived with an equal amount of N,O-bis-trimethylsilyl-triflouroacetamide at 60°C for 20 min. GC/MS/MS analyses were optimized on an Agilent 7000A triple quadruple chromatograph. Mass spectrometric data was collected in multiple ion monitoring mode. For patulin stability, apple juice was fortified with either 25, 50 or 100 ppb patulin and stored at three different temperatures for 28 days.

Results: Stability of fortified patulin in clear apple juice was affected by storage time, with the recovery of patulin gradually decreasing as the storage period progressed. Patulin was much more stable at 4°C than at ambient or accelerated shelf-life temperatures. Our results indicate that patulin reduction was time and temperature dependent. Reduction in 50 and 100 ppb fortified patulin levels were more pronounced after 28 d of storage than that of the 25 ppb fortified samples, indicating a concentration effect.

Significance: Although storage for extended periods and heat treatments reduce the level of patulin in apple juice, the reduction is not sufficient to totally eliminate the toxin. This indicates that the selection of raw materials for apple juice manufacturing should be considered as critical for production of apple juice concentrates with low patulin levels.

P1-24 Distribution of *Fusarium* spp. and Mycotoxins Nivalenol and Zearalenone in Rice (*Oryza sativa*) Harvested from Korea

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Introduction: *Fusarium* species can produce highly toxic metabolites, such as trichothecene (mainly nivalenol (NIV) and zearalenone (ZEN)). NIV and ZEN were evaluated by the international Agency for Research on Cancer (IARC) and classified in the group 3. Rice is a staple food in Korea and used in the form of several food through processing steps. NIV and ZEN contamination study in rice milling factories have unusually been reported.

Purpose: *Fusarium* species and the contamination level of NIV and ZEN in rice from Korea were assessed.

Methods: Eighty rice samples were freshly harvested from 12 farm fields in Korea. After isolation of *Fusarium* species from the paddy rice, NIV and ZEN-producing strains were determined and characterized with multiplex PCR. We extracted DNA from 480 of Fusarium isolates, and amplified four genes in the NIV biosynthetic pathway (tri4, tri5-6 intergenic region, tri7 and tri13), and pks4 gene for ZEN biosynthesis. Rice was milled using a laboratory-scale test mill to produce four fractions: white rice, bran, brown rice and hulls. The four milling fractions were analyzed for NIV and ZEN with validated analytical method using high-performance liquid chromatography (HPLC) with UV absorbance and fluorescence detection.

Results: *F. graminearum* was the most frequent isolate in rice samples (92.5%), followed by *F. proliferatum* (33.8%), *F. equiseti* (29.4%), *F. asiaticum* (24.4%) and *F. culmorum* (8.1%). PCR assay results showed that NIV/ZEN genotype was the most predominant (75.4%) followed by ZEN genotype (10.2%) and NIV genotype (1.0%). Recoveries of NIV and ZEN in milling fractions with HPLC were 86.5 – 123.5% and 79.5 – 92.0%, respectively. The contamination levels in hulls, brown rice, bran and white rice were 180.4, 36.1, 108.5 and 12.4 µg/kg for NIV, and 537.8, 18.1, 93.9 and 0.6 µg/kg for ZEN, respectively. The contaminations of NIV and ZEN in rice have a good correlation with the frequency of toxigenic *Fusarium* isolates.

Significance: We confirmed that *Fusarium* species in Korean paddy rice was generally NIV and ZEN genotype and the contamination level of NIV and ZEN was reduced through milling process. This information is necessary of high priority in order to product the consumer's health from the risk of exposure to these toxins.

P1-25 Withdrawn

P1-26 Thermal Inactivation of Acid Adapted and Non-adapted Stationary Phase *Salmonella* spp. and *Listeria monocytogenes* in Orange Juice

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Developing Scientist Competitor

Introduction: Thermal inactivation parameters of stationary phase, acid and non-acid adapted pathogens, primarily as cocktails of multiple strains, have been studied in various juice products.

Purpose: The objective of this study was to evaluate the heat resistance of acid adapted and non-adapted stationary phase *Salmonella* spp. and *Listeria monocytogenes* in single strength orange juice by evaluating the thermal inactivation response of each strain/serotypes.

Methods: Three *Salmonella* and *L. monocytogenes* strains/serotypes were evaluated. *Salmonella* isolates were grown in TSB and *L. monocytogenes* strains were grown in BHI, supplemented with 1% glucose for acid adaption, and inoculated into single-strength pasteurized orange juice without pulp. Sealed microcapillary tubes with inoculated juice were immersed into water baths at 55, 58, and 60°C for *Salmonella* serotypes and 56, 58, and 60°C for *L. monocytogenes* strains, removed at predetermined time intervals, and placed immediately onto ice. Populations of *Salmonella* were enumerated on TSA supplemented with 0.1% sodium pyruvate; BHI agar supplemented with 0.1% sodium pyruvate was used for *L. monocytogenes* strains.

Results: Different strains in the same species responded to heat differently. Thermal tolerance did not increase significantly ($P < 0.05$) for acid adapted *Salmonella* spp. and *L. monocytogenes* strains at most temperatures tested. *Salmonella* serotypes are less heat resistant at all temperatures than *L. monocytogenes* strains. Combining individual results of *Salmonella* spp. and *L. monocytogenes* strains and three strains of Shiga toxin-producing *E. coli* previously tested, the formula of log D = 8.2 – 0.14*T* (°C) was used to calculate a general process for orange juice at 71.1°C. Using this equation, a 5-log reduction of all three pathogens in single strength orange juice requires 5.29 s at 71.1°C, with a *z*-value of 7.1°C.

Significance: All available *D*-values for pathogens in orange juice were obtained using strain cocktails. Evaluation of individual strains using the microcapillary tubes allows understanding of strain to strain variability that may impact public health.

P1-27 Comparison of Propidium Monoazide Real-time PCR and a Conventional Culture-based Method (EPA Method 1603) for Detection of Viable *Escherichia coli* in Water

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Introduction: *Escherichia coli* is a fecal indicator bacterium that is monitored to assess water quality by the U.S. Environmental Protection Agency (EPA). Real-time PCR (qPCR) assays enable rapid and sensitive detection of *E. coli*. However, qPCR may overestimate target numbers because it quantifies DNA from both viable and dead cells.

Purpose: The objective of this study was to compare numbers of viable *E. coli* in water as measured by the EPA culture-based method with those measured by propidium monoazide (PMA)-qPCR. PMA is a dye that can penetrate dead cells and bind to cellular DNA, preventing its amplification via a subsequent PCR.

Methods: *E. coli* ATCC 25922 was serially diluted to generate cell suspensions ranging from 10 to 10^7 CFU ml^{-1}. Dead cells were obtained by heating the suspensions at 85°C for 15 min. Suspensions were inoculated into sterile distilled water. Numbers of *E. coli* in water samples were measured by EPA Method 1603. Water samples were treated with PMA and DNA was extracted and amplified by TaqMan® real-time PCR targeting the *uidA* gene to detect only viable *E. coli* cells.

Results: PMA-qPCR could detect as low as 10^3 CFU ml^{-1} viable *E. coli* in distilled water samples, while completely preventing false-positive PCR results generated by 10^4 CFU ml^{-1} of dead *E. coli* cells. Numbers of viable *E. coli* measured using PMA-qPCR were significantly correlated with those measured using EPA Method 1603. PMA-qPCR could detect viable *E. coli* in water within 3 h whereas EPA Method 1603 required more than 24 h. Studies detecting viable *E. coli* in environmental water samples are ongoing.

Significance: In conclusion, good qualitative agreement was found between EPA Method 1630 and the PMA-qPCR assay in terms of detecting *E. coli* in distilled water. The PMA-qPCR assay could be a promising alternative to detect only viable *E. coli* in waters.

P1-28 Efficient Reduction of *Cryptosporidium parvum* Oocysts from Apple Cider by Combining Microfiltration with Ultraviolet Treatment

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Developing Scientist Competitor

Introduction: Outbreaks of cryptosporidiosis due to the consumption of unpasteurized apple cider have been reported in recent years. Ultraviolet (UV) is a FDA recognized alternative to thermal processing of apple juice and cider and can achieve a 5-log reduction of *Escherichia coli* O157:H7 and *Cryptosporidium parvum*. However, high doses of UV can negatively affect juice color and flavor, while suspended solids in cider can limit the process efficiency. Microfiltration (MF) can physically remove suspended solids and microorganisms from apple cider, which can enhance the effectiveness of UV and allow a lower UV dose to be used.

Purpose: In this study, the efficiency of a combined UV & MF treatment in the reduction of *Cryptosporidium parvum* from apple cider was evaluated.

Methods: Apple cider (pH3.7, 14.1°Brix) was inoculated with *Cryptosporidium parvum* at 10^6 oocysts/ml, and subjected to the individual or combined treatments. MF was performed with 0.8µm and 1.4µm ceramic membranes at 10°C and 155kPa. UV treatments were conducted using CiderSure 3500, at a low UV dose of 1.75 mJ/cm^2. Oocyst viability before and after processing was assessed by a cell culture infectivity assay using a human ileocecal cell line (HCT-8) and quantification was done by DNA extraction coupled with PCR for the sporozoite heat shock and oocyst wall proteins. The study was performed in triplicate.

Results: After MF of cider using 0.8µm and 1.4µm membranes, no oocysts were detected. No oocysts were detected after the combined MF & UV treatment. MF alone was shown to achieve greater than 5-log reduction of *Cryptosporidium parvum*, and could be combined with UV as a final kill step.

Significance: The developed non-thermal hurdle treatment can significantly reduce this protozoan parasite in apple cider, as well as spores, yeasts, molds and bacterial pathogens. This will help processors improve the safety and quality of apple juice and cider and potentially other beverages.

P1-29 Microbiological Quality of Ice Made and Bagged On-premises in Retail Stores and in Self-serve Vending Machines in Georgia

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Developing Scientist Competitor

Introduction: Contaminated ice could pose a food safety risk. Ice made on-site and manually bagged at retail outlets and in self-service vending machines may be done under varying sanitary conditions. The International Packaged Ice Association (IPIA) has microbiological quality standards for manufactured, packaged ice, but ice produced at retail outlets and in vending machines does not fall under this criteria.

Purpose: This study evaluated the microbiological quality of ice produced at retail outlets and in self-service vending machines in Georgia and compared the quality to industry standards for manufactured ice.

Methods: Packaged ice samples (250 bags) from retail locations throughout Georgia that produced and bagged ice on-premises, from self-service vending machines and packaged ice samples (25 bags) from two manufacturing plants were analyzed for heterotrophic plate counts, total coliforms and *E. coli*, and enterococci. To test for *Salmonella* and *Listeria monocytogenes*, melted ice was membrane filtered (45 µ) and any *Salmonella* or *L. monocytogenes* trapped on the filter was enriched in universal pre-enrichment broth before plating onto selective agar plates. Confirmation tests were done for presumptive positives.

Results: Of the total retail and vending machines samples, 6.4% contained unsatisfactory levels of heterotrophs according to the limits set by the IPIA (<500 CFU/100 ml). Twenty-six percent of all samples contained unsatisfactory levels of coliforms (<2.2 MPN/ml). *E. coli* and enterococci were present in 1.2 and 13.2% of the samples, respectively. One sample tested positive for *Salmonella*, but no *L. monocytogenes* was found. Samples from the manufacturing plants were well within the IPIA microbial limits.

Significance: Ice made and packaged on-site at retail outlets and in self-service vending machines is not subject to the microbial standards established by IPIA, unlike the two ice manufacturing plants. The presence of microorganisms in samples from retail and vending sites at levels exceeding those established indicates the need for greater sanitary oversight for these segments of the ice industry.

P1-30 The Use of the BacT/ALERT and a New Neutralizing Medium for the Improved Recovery of Microbial Contamination in a Variety of Aseptic Chocolate Product

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Introduction: Cacao, a major component in chocolate, contains flavonoids which are known to provide natural antibacterial properties. The BacT/ALERT® Microbial Detection system is an automated detection method used in aseptic beverage and food testing for the presence of microbial contamination based on CO_2 production. The BacT/ALERT system has addressed antibacterial requirements of chocolate by the use of the iFA BacT/ALERT cultural bottle containing charcoal for neutralization. A newly developed iFA Plus medium containing adsorbent polymeric beads for neutralization was compared to the charcoal media for improved recovery of bacterial contamination in a variety of chocolate product.

Purpose: A study was conducted comparing the growth and recovery of *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in the two different neutralizing media using aseptic milk chocolate beverages and puddings compared to the same inoculum in a dark chocolate version of the same brand.

Methods: Challenge studies were performed by inoculating 10 ml of the milk or dark chocolate product. The study included two brands each of a low acid aseptic beverage drink and two brand types of aseptic pudding. Samples were inoculated at target levels of 10 CFU per 10 ml product volume. Challenge studies were performed in triplicate to establish reproducibility. Culture confirmation and biochemical identification were performed on 1 sample of each triplicate set.

Results: Inoculated studies with the different brand and product types of milk chocolate did not display any significant delay in growth or detection with all organisms detected within 24 + 2 hours regardless of the bottle type. However, depending on the specific product type, inoculated studies with the dark chocolate did demonstrate delayed growth for *Staphylococcus aureus* in the charcoal bottle often requiring 33 - 41 hours for detection. The same *Staphylococcus aureus* inoculated sample could be detected within 24 + 2 hours with the use of BacT/ALERT iFA Plus medium containing adsorbent polymeric beads for added neutralization.

Significance: *Staphylococcus aureus* was the most effected by the dark chocolate product with delayed growth and detection. The detection of *Staphylococcus* was improved by as much as 16 hours by the use of the new iFA Plus neutralizing medium.

P1-31 Prevalence and Quantification of *Campylobacter* in Chicken Carcasses of Slaughtering Process

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Introduction: Food poisoning cases related to *Campylobacter* spp. are increasing worldwide. Some countries are adding this pathogen as another criterion of poultry slaughterhouse. In Korea, there is no regulation regarding this pathogen in poultry slaughterhouse, and research data about the prevalence and concentration of *C. jejuni, C. lari,* and *C. coli* is not enough.

Purpose: In this study, we intended to apprehend the *Campylobacter* contamination level in chicken carcasses from slaughterhouses and to discern which step of slaughtering process is mainly causing the contamination, in order to develop a plan for control of this pathogen.

Methods: Samples were taken from eight chicken slaughterhouses in 2012. Among the total 1150 samples, 1090 samples were collected from two chicken slaughterhouses continually for five months from four sites of slaughtering process. Samples were collected from; 1) anus swab in live bird that was waiting to enter the slaughter line; 2) carcass after removal of intestine; 3) carcass after washing the interior and exterior, and 4) final carcass. Detection and quantification tests were done according to USDA microbial laboratory guidelines. In addition, multiplex PCR was used to

identify the colonies as *Campylobacter jejuni, C. coli* and *C. lari.* To quantify the colonies, samples were directly spread onto campy-cefex agar. The genetic relationships between the isolates were analyzed by Rep-PCR.

Results: 118 samples were positive for *Campylobacter* spp. (10.3%). The prevalence of *C. jejuni, C. lari and C. coli* was 6.1%, 2.4% and 3.0%, respectively. Forty-one percent of total isolates were from the carcass after removal of intestine, and 32.2% of those isolates were more than 10^3 CFU/ml. The genetic homology of the isolates from the same slaughterhouse was approximately 80%.

Significance: Contamination rate of *Campylobacter* spp. in final carcasses shows low levels compared to that of other countries. Among the samples, carcasses after removal of intestines had the highest contamination rate. To lower the contamination rate further, it is suggested that the process of removal of intestine needs to be performed without rupture and sufficient washing step must be maintained.

P1-32 Program Development to Determine Internalized *Salmonella* Prevalence Rate in Turkey Flocks by Testing Spleens

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Introduction: Food safety is an important issue for everyone in the food supply chain, farm to fork. *Salmonella* has been known to be internalized in poultry. Understanding the flock quantitative and qualitative levels of *Salmonella* infection/prevalence rate would provide an accurate assessment of pre-harvest contamination.

Purpose: To develop a spleen testing protocol to determine flock prevalence and quantitative levels of *Salmonella*.

Methods: *Salmonella* spleen flock prevalence rate was determined by sampling 1% of each flock at a commercial slaughter operation during evisceration over a four month testing period. The external surface of spleens was sterilized prior to *Salmonella* analysis utilizing Dupont BAX. Flock prevalence rate was calculated by (number positive samples per flock/total number of samples per flock) multiplied by 100. *Salmonella* enumeration was completed on all *Salmonella*-positive samples utilizing FSIS MPN methodology.

Results: During the four month test, 104 flocks were sampled. Forty-four flocks yielded negative *Salmonella* spleen samples. There were fifty flocks with less than 50% *Salmonella* spleen incidence. Only ten flocks had greater than 50% *Salmonella* spleen incidence. This study demonstrated that *Salmonella* presence in spleens varied widely by flock, indicating a qualitative testing approach would generate value. The enumeration level of *Salmonella* did not trend with the *Salmonella* % flock positive rate.

Significance: The *Salmonella* spleen incidence data could be used to help improve bird health and may help explain the variation in ground turkey incidence rate. A flock spleen qualitative *Salmonella* testing can advance *Salmonella* control through production scheduling based on prevalence.

P1-33 Identification and Prevalence of *Escherichia fergusonii* in Broiler Chickens

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Introduction: *Escherichia fergusonii* has been found to be involved in infections causing abortion, diarrhea, and mastitis in animals. This bacterium has also been implicated in human sepsis, urinary tract infections, wound infections and enteric diseases demonstrating its zoonotic potential. However, little is known about the prevalence of *E. fergusonii* in chickens.

Purpose: To investigate the prevalence of *E. fergusonii* in broiler chicken farms located in the Fraser Valley of British Columbia, and to develop a simple and accurate molecular identification method of this bacterium.

Methods: Five hundred eighty broiler chickens aged from 28 to 36 days were sampled from 32 farms (up to 20/farm in one or two visits). Cecal and cloacal samples from each bird were aseptically collected and cultivated on a selective Simmons-citrate-adonitol agar medium and presumptive *E. fergusonii* colonies were then purified on MacConkey-sorbitol agar. Isolates were identified by API® 20E and by a duplex PCR method using *E. fergusonii*-specific primers.

Results: Of 207 presumptive isolates screened, 95 (45.9%) from 21 of the 32 screened farms (65.6%) were confirmed as *E. fergusonii* by API® 20E. Of these 95 *E. Fergusonii* isolates, 62 (68.1%) and 33 (36.3%) were from ceca and cloacae, respectively. *E. fergusonii* was found in 60.0% (6/10), 72.7% (8/11) and 63.6% (7/11) of farms in the east, north, and south Fraser Valley regions, respectively. The duplex PCR identified 182 *E. fergusonii* of 207 presumptive isolates (87.9%) from 29 farms, with 101 (60.1%) and 71 (42.3%) being from ceca and cloacae, respectively. Results showed that more *E. fergusonii* were detected by duplex PCR than by API® 20E and suggest that further investigations on *E. fergusonii* identification are warranted.

Significance: This study showed that in the investigated area, having dense broiler population, *E. fergusonii* is widespread which could have implications for both poultry and public health. The need for an accurate and efficient identification method is imperative.

P1-34 Sponge and Skin Excision Sampling for Recovery of Inoculated *Salmonella* and *Campylobacter* from Defeathered Broiler Carcasses

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Introduction: *Salmonella* and *Campylobacter* contamination of broiler carcass skin increases during feather removal. There are several methods for sampling carcasses including sponging or swabbing of skin surface and skin excision. It is unclear whether sponge sampling is adequate to remove bacteria from the skin or if subsequent skin excision would greatly enhance the likelihood of recovery.

Purpose: The purpose of this study was to test sponge sampling followed by skin excision to recover inoculated *Salmonella* and *Campylobacter* from the breast skin of defeathered broiler carcasses.

Methods: On each of three replicate days, five freshly defeathered broiler carcasses were obtained from a commercial processor. The skin of each breast (2 per carcass) was inoculated with approximately 10^6 cells of an antimicrobial resistant *Salmonella* and *Campylobacter*. Inocula were allowed 60 s contact time before using three passes with a sterile sponge to sample the skin. Subsequently, the breast skin was aseptically excised. Skin and sponge samples were cultured for enumeration of both inoculated organisms. Numbers recovered were compared by Student's T test.

Results: Mean *Salmonella* inoculum was 6.48 log CFU per breast. Sponge sampling recovered a mean of 5.12 log CFU representing 5 % of the inoculum; subsequent skin excision recovered an additional 0.8% of the inoculum resulting in a total of 5.20 log CFU per breast. *Campylobacter*

results were similar with a mean inoculum of 6.65 log CFU, recovery by sponge of 5.58 log CFU (10% of the inoculum); additional *Campylobacter* recovered by skin excision resulted in a total of 5.62 log CFU recovered per breast.

Significance: Sampling broiler skin by sponge wipe allows recovery of 5 to 10% of inoculated bacteria, subsequent skin excision does not significantly ($P > 0.05$) increase recovery beyond what is possible by sponge sampling.

P1-35 Evaluating Post-evisceration Processing Steps and In-plant Antimicrobial Treatments against *Campylobacter*, *Escherichia coli*, and Aerobic Bacteria on Poultry Carcasses

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Developing Scientist Competitor

Introduction: *Campylobacter* is a pathogenic bacterium commonly found in the intestinal tract of poultry, and widely associated with poultry-borne human illnesses. Interventions such as chlorine and peracetic acid are commonly used to control this pathogen from poultry products, reducing the risk of poultry-related illness. These antimicrobials also assist in enhancing the shelf life of poultry products by reducing the bacterial load on carcasses.

Purpose: The purpose of this study was to evaluate the effectiveness of processing steps and different antimicrobial treatments in poultry processing plants; focusing on pathogen elimination and non-pathogenic microbial load reduction.

Methods: Post-evisceration whole bird rinse samples were taken off the processing line before entering the inside-outside bird washer (IOBW), post-IOBW treatment, after pre-chilling, after main chiller treatment, and after post-chiller treatment from five processing plants. Rinse samples were then plated on campy-cefex agar, violet red bile agar, and plate count agar to determine the level (log CFU/ml) or prevalence of *Campylobacter*, *Escherichia coli*, and aerobic plate counts (APC), respectively. Average log CFU/ml bacterial growth was recorded for each sampling point and compared to determine the effectiveness of antimicrobial treatments.

Results: Carcasses treated with chlorine-based antimicrobials including sodium hypochlorite showed significant ($P < 0.05$) reductions in *Campylobacter* and APC after the IOBW, while a significant ($P < 0.05$) reduction in *E. coli* was observed after exiting the pre-chiller; however, peracetic acid treatment resulted only in the significant ($P < 0.05$) reduction of *Campylobacter* after the IOBW, while significant ($P < 0.05$) reductions in *E. coli* and APC were observed after exiting the pre-chiller.

Significance: Results suggest that chlorine-based antimicrobials and peracetic acid are all effective in inhibiting foodborne pathogens such as *Campylobacter*, *E. coli*, and APC on poultry carcasses. However, exposure times and the point of application of antimicrobials may be critical in significantly reducing/eliminating bacteria from poultry carcasses.

P1-36 Prevalence of *Campylobacter* spp. on Poultry Carcasses during Processing and in Slaughtering Environment

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Introduction: As the major cause of food-poisoning worldwide, most cases of campylobacteriosis are attributable to the handling and consumption of poultry meat, and control throughout the food chain has been recommended.

Purpose: This study was to detect the level of *Campylobacter* contamination during poultry processing and compare the sensitivity of traditional biochemical method with real-time PCR assay for *Campylobacter* confirmation.

Methods: A survey was conducted in 2012 to estimate *Campylobacter* prevalence on broiler carcasses and processing environment during two visits to a poultry plant. A total of 73 samples collected at different sites along the processing line were examined by direct plating onto modified Charcoal Cefoperazone Deoxycholate agar (mCCDA) and enrichment in Bolton broth followed by plating onto mCCDA. Isolates were confirmed as members of *Campylobacter* genus using oxidase biochemical method and a real-time PCR reaction based on 16S rRNA gene.

Results: Our results revealed that 54.8% (40/73) of the analyzed samples were positive for *Campylobacter* spp. by real-time PCR but all of them were negative when tested by oxidase phenotypic method. All except two samples positive for *Campylobacter* spp. were *C. jejuni*. There was a significant difference in *Campylobacter* contamination between sampling days A and B, with positive rates of 72.7% and 27.6%, respectively. Water and swab samples, pre-scald and pre-evisceration sites on day A, were 100% positive, whereas no campylobacters were isolated from machinery on sampling day B. Processing reduced ($P < 0.05$) the average concentration of *C. jejuni*for 1.5 ± 0.4 and <1.0 log CFU/ml on the broiler carcasses post-chilling for days A and B, respectively.

Significance: Our survey revealed the widespread of *C. jejuni* on poultry carcasses and in poultry processing environment and confirmed that the newly optimized real-time PCR assay was more sensitive than oxidase phenotypic method for *Campylobacter* isolate confirmation.

P1-37 Validating an Inside-Outside Bird Washer as an Effective On-line Reprocessing System

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Introduction: On-line reprocessing (OLR) systems and inside-outside bird washers (IOBW) are two critical steps in most poultry slaughter processes that are responsible for helping clean the carcasses by removing visible contaminants such as ingesta and feces. By adding an antimicrobial to the IOBW it can replace the existing OLR system.

Purpose: The purpose of this study was to validate the utilization of an IOBW as an OLR system by applying a single use or recycled antimicrobial solution through the IOBW.

Methods: Poultry carcasses (10 per day, 8 days) from three poultry slaughter plants (A, B and C) were categorized as visually clean or contaminated with digestive tract material and in need of reprocessing. For reprocessing, carcasses were passed through an IOBW applying a peroxyoctanoic and peroxyacetic acid mixture (POAA; 30 - 50 ppm, 480 – 620 kPa, 10 – 15 s) as an antimicrobial treatment. Plant A applied a single use antimicrobial solution through the IOBW, while Plants B and C applied a filtered and recycled solution. Bacteria were rinsed (1 min, 400 ml buffered peptone water solution) from clean carcasses collected prior to the IOBW and contaminated carcasses after being reprocessed through the IOBW. Rinsates were analyzed for *Escherichia coli, Salmonella,* and *Campylobacter* at Plant A and for *E. coli* at plants B and C. Data were analyzed using a 2-sample *t*-test (counts) or chi-square test (incidence) in Minitab with a significance level of α=0.05.

Results: No fecal material was observed on any of the carcasses sampled after the IOBW. Recovered *E. coli*, *Salmonella*, and *Campylobacter* on reprocessed carcasses were not different ($P \geq 0.05$) from those from visually clean carcasses sampled prior to the IOBW applying a single use POAA solution (2.5 ± 0.9 and 2.5 ± 0.9, 0.1 ± 0.5 and 0.2 ± 0.5, and 1.0 ± 1.2 and 1.3 ± 1.1 log CFU/ml, respectively); and the incidence rates were similar ($P \geq 0.05$) for *Salmonella* (96.3 and 98.8%, respectively) and lower ($P < 0.05$) for *Campylobacter* (92.5 and 71.3%, respectively). When a recycled solution of POAA was applied through the IOBW, *E. coli* populations of clean and reprocessed carcasses were different ($P < 0.05$) but not biologically meaningful (1.9 ± 0.6 log and 2.1 ± 0.7 CFU/ml, respectively).

Significance: These data indicate that an IOBW that applies a single use or recycled solution of POAA can be used for OLR and can eliminate the need for a stand-alone OLR system.

P1-38 Effects of Commercial Seasoning and Cultured Sugar/Vinegar Blend on the Behavior of *Campylobacter jejuni* and *Salmonella* Typhimurium of Precooked Chicken Breast during Refrigerated Storage

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Undergraduate Student Award Competitor

Introduction: *Campylobacter jejuni* is major bacterial contaminant in poultry product along with *Salmonella.* Precooked and refrigerated ready-to-eat poultry product is gaining popularity due to convenience of use, but partially heated process can be risky to the consumer. Various techniques including marinating have been studied to improve microbial safety and meat quality of chicken during storage.

Purpose: The object of this study was to investigate the effects of commercial marinade seasoning and cultured sugar/vinegar blend on *C. jejuni* and *S.* Typhimurium populations as well as on tenderness of precooked chicken breast during frozen and refrigerated storage at retail market.

Methods: Precooked chicken breasts were inoculated with *C. jejuni* and *S.* Typhimurium, which were treated with 3% cultured sugar/vinegar blend with and without 0.6% polishrub seasoning containing herb 32%, vacuum packaged and stored at 4 and 10°C. In addition, the shear force of precooked chicken breast treated with different antimicrobial treatments was measured after samples were subjected to 3 freeze-thaw cycles and refrigerated storage.

Results: Survival or growth curves of *C. jejuni* and *S.* Typhimurium were fitted well to Baranyi model. The greatest survival of *C. jejuni* was observed in nontreated precooked chicken breast storage at 4°C. At 10°C, the survival of *C. jejuni* and growth of *S.* Typhimurium on precooked chicken breast treated with 3% cultured sugar/vinegar blend, and 0.6% seasoning plus 3% cultured sugar/vinegar blend was more significantly inhibited than 0.6% seasoning alone. In addition, the 3% cultured sugar/vinegar blend improved tenderness of precooked chicken breasts during frozen and refrigerated storage.

Significance: The mixture of 0.6% seasoning 3% cultured sugar/vinegar blend can be used as an extra hurdle technique to control the survive or growth of *C. jejuni* and *S.* Typhimurium. The cultured sugar and vinegar can be applied in manufacturing of natural and organic precooked chicken products to improve the microbial safety and quality of precooked chicken products.

P1-39 Bromine-based Biocides for the Control of Pathogens in Simulated Chill Tanks in Poultry Processing

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Introduction: *Salmonella* and *Campylobacter* are pathogens of major concern in the poultry industry. Chlorine-based antimicrobials have been widely used in the poultry 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: they are effective at a wider range of pH values than chlorine, they have low odor and their bromamines by-products are more efficacious than chloramines.

Purpose: To evaluate AviBrom® (1,3-Dibromo-5,5-Dimethyl hydantoin), a bromine-based biocide as means to control *Salmonella* and *Campylobacter* on chicken legs.

Methods: *Salmonella enterica* and *Campylobacter jejuni* were grown overnight at 37°C in Luria-Bertani broth. Chicken drumsticks purchased from local stores were inoculated with 8-log CFU of either *Salmonella* or *Campylobacter* and allowed to attach for 30 minutes at room temperature. Simulating a poultry chill tank, drumsticks were submerged for one hour in the pre-chilled biocide solution at 4°C; the treatments included bromine-base biocide at 100 and 400 ppm (as bromine), sodium hypochlorite at 50 ppm (as chlorine) and peroxyacetic acid at 25 and 100 ppm. Refreshing solution was added at 30 minutes for each treatment. Tap water at 4°C was used as control. After treatment, drumsticks were drained for 15 seconds and a whole-bird rinse was performed following FSIS protocol. The rinsate was analyzed using standardized methods to detect and quantify *Salmonella* and *Campylobacter*.

Results: The bromine-based biocide at levels lower than FDA's approved limits, was more efficacious controlling *Salmonella* and *Campylobacter* than bleach at all concentrations tested ($P < 0.001$) and performed similarly to peroxyacetic acid. Microbial reductions ranged from 2.0 to 3.6 log CFU/g for *Salmonella* and 2.3 log CFU/g to complete kill for *Campylobacter*.

Significance: Bromine-based antimicrobials are an effective intervention to reduce *Salmonella* and *Campylobacter* in the poultry industry.

P1-40 Inhibition of *Clostridium perfringens* by Innostatin 007B and Mostatin V in Uncured and Naturally Cured Turkey during Abusive Cooling

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Introduction: Reduction or elimination of sodium nitrite from meat formulations to meet consumer demand may present the risk of *C. perfringens* spore germination and outgrowth during abusive cooling after cooking of RTE meat products.

Purpose: Evaluate inhibition of *C. perfringens* spore germination and outgrowth by INNOstatin 007B and MOstatin V in uncured and naturally cured turkey breast.

Methods: Turkey breast meat was injected with brine solution containing salt, cane sugar and sodium phosphate to final concentrations (w/w) of 1.75%, 1.50% and 0.35%, respectively, to prepare the uncured product. Celery powder (0.35%) was added to the uncured product to prepare naturally cured product. INNOstatin 007B and MOstatin V (2%) were added to the uncured and naturally cured (control) products and mixed. The ground product (10 g) was placed in vacuum bags, inoculated with a 3-strain *C. perfringens* spore cocktail. The inoculated products were heat

treated (75 °C for 20 min) and cooled exponentially from 54.4 to 4.4 °C within 6.5, 9, 12, 15, 18, or 21 h. *C. perfringens* populations were determined after the heat treatment and after cooling by plating on tryptose sulfite cycloserine agar.

Results: Cooling of uncured turkey product from 54.4 to 4°C within 6.5, 9, 12, 15, 18, or 21 h resulted in *C. perfringens* spore germination and outgrowth of 0.53, 1.49, 3.08, 4.34, 3.67 and 6.05 log CFU/g, respectively. Incorporation of natural cure (as celery powder) inhibited *C. perfringens* growth, with final population increases of 0.03, 0.07, 0.27, 0.45, 1.11 and 3.78 log CFU/g, respectively, for the same cooling times. Incorporation of Innostatin 007B resulted in inhibition (<1.00 log CFU/g) of *C. perfringens* to up to 9 h and 18 h for uncured and cured products, respectively. Incorporation of MOstatin V into the product resulted in *C. perfringens* inhibition up to 12 and 18 h cooling times for uncured and cured products, respectively.

Significance: Incorporation of natural cure (celery powder), and antimicrobials INNOstatin or MOstatin can inhibit *C. perfringens* spore germination and outgrowth in turkey product during abusive cooling.

P1-41 Inhibition of *Listeria monocytogenes* on Non-cured Turkey Breast by MOstatin V and INNOstatin 007B during Refrigerated Storage

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Introduction: The USDA-FSIS requires the ready-to-eat (RTE) meat and poultry processors to reduce and/or control *L. monocytogenes*on the product through sanitation, use of antimicrobial agents and/or processes or application of post-lethality treatments.

Purpose: Evaluate inhibition of *L. monocytogenes*on uncured turkey breast by MOstatin V and INNOstatin 007B and extension of microbial shelf life during refrigerated storage

Methods: Product was prepared by mixing ground, skinless boneless turkey breast meat (76.92% w/w), salt (1.75% w/w), cane sugar (1.50%), sodium phosphate (0.35%) and water. MOstatin V or INNOstatin 007B were added (2%) to treatments requiring the antimicrobials. The product was cooked, cooled and sliced to 1/8". A slice of the product was inoculated with a 5-strain *L. monocytogenes* cocktail (*ca*. 3.0 log CFU/cm^2), placed between non-inoculated slices and vacuum packaged. The inoculated product was stored at 4 or 10°C for 90 or 56 days, respectively. Non-inoculated product was used to evaluate the microbial shelf life of the product.

Results: *L. monocytogenes* growth of > 2.0 log CFU/cm^2 was observed on control product within 7 and 14 days of storage at 10 and 4°C, respectively. Incorporation of MOstatin and INNOstatin in the product inhibited *L. monocytogenes* growth, with > 2.0 log CFU/cm^2 growth observed by 28 and 35 days, respectively in product stored at 10°C and by 70 and > 84 days, respectively in product stored at 4°C. TPC of product reached > 5.0 log CFU/cm^2 by 28 days in control product stored at either 10 or 4°C; while the final populations were < 4.5 log CFU/cm^{2}by 56 and 84 days in products containing the antimicrobials. Similar observations were made for PSY and Y&M counts.

Significance: MOstatin V and INNOstatin were effective in inhibiting *L. monocytogenes* growth during refrigerated storage of non-cured turkey breast extended the microbial shelf life of the product.

P1-42 Occurrence of *Listeria* spp. in Bovine Carcasses Processing Plants and Characterization of *L. monocytogenes* Isolates

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Introduction: *Listeria monocytogenes* is a foodborne pathogen usually associated with beef and meat products. Bovine carcasses are often described as common sources of *Listeria*spp. in slaughterhouses, being mandatory the identification of the key points of contamination during their processing. Based on this, it is possible to avoid the contamination in end products.

Purpose: To determine the occurrence of *Listeria* spp. and *L. monocytogenes*in distinct steps of bovine carcasses processing, and characterize the obtained isolates by phenotypic and genotypic methods.

Methods: Two hundred and nine (n = 209) bovine carcasses from three selected slaughterhouses from Minas Gerais state, Brazil, were surface sampled (400 cm^2) in the following processing steps: A) after bleeding; B) after skinning; C) after evisceration, and D) after end washing. All samples were subjected to *Listeria* spp. detection following ISO 11.290-2, and suspect isolates were subjected to biochemical and serological identification by phenotypic tests. *L. monocytogenes* isolates were subjected to PCR reactions to identify their serological groups and virulence genes (*inlA, inlB, inlC, inlJ, plcA, hlyA, actA* and *iap*).

Results: Considering the bovine carcass processing steps, the following frequencies of positive results for *Listeria* spp. were recorded: A) 3/209; B) 1/209; C) 4/209, and D) 4/209; *L. monocytogenes* was recorded only in steps A (1/209) and D (1/209). Thirty isolates were identified as *Listeria* spp., being *L. innocua* (23), *L. monocytogenes* (5), and *L. welshmeri* (2). *L. monocytogenes* isolates presented positive results for all tested virulence genes; all isolates were serotyped as *1/2b* by phenotypic test, while being identified by PCR as belonging to group "*1/2c or 3c*".

Significance: The bovine carcass processing can be characterized as a potential source of *L. monocytogenes* contamination in slaughterhouses, despite the low frequency of occurrence of this foodborne pathogen. *Acknowledgments:* CNPq, CAPES, FAPEMIG, and Fundação Oswaldo Cruz.

P1-43 Evaluation of Beef Trim Sampling Methods for Detection of Shiga Toxin-producing *Escherichia coli* (STEC)

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Introduction: Presence of Shiga toxin-producing *Escherichia coli* (STEC) is a major concern in ground beef. The methods for sampling commercial beef trim prior to grinding currently includes excision and core sampling, with some variations noted (e.g., N-value and number of combo units making up a lot).

Purpose: The purpose of this study was to determine the efficacy of five different sampling methods for detecting randomized STEC contamination in beef trim contained in single combo units.

Methods: Standard combo bins (1.1 x 0.9 x 1.1 m) were divided into 27 sectors (9 on the bottom, middle and top layers each). A STEC strain (O103, O145 or O157) was inoculated onto a 908 g piece of beef trim (ca. 3 log CFU/g) and placed in a randomized bin sector. The combo was then filled with non-inoculated beef trim. Sampling methods were N-60 surface excision, N-90 surface excision, N-60 core drill shaving, Cozzini

core sampler, and purge. Meat samples (375 g composites) were combined with 1.5 L of mEHEC and enriched at 42°C for 18 h prior to PCR to determine STEC presence.

Results: Purge sampling was the most effective method, with 13/14 samples positive. Cozzini corer, N-60 core drill shaving, N-60 excision and N-90 excision methods resulted in 11/14, 9/14, 7/14 and 7/14 samples being positive, respectively. The purge sampling and Cozzini corer were superior combo sampling methods, because they were able to detect contamination in the lower combo sectors. The N-60 and N-90 excision sampling methods were effective if contamination was placed into one of the top or middle sectors, but only 3/12 samples were positive for bottom sector inoculated samples.

Significance: Sampling of beef trim is an important component of ground beef safety programs. Excision sampling of combos utilizing N-60 or greater is commonly utilized. Selection of a sampling method (purge sampling or deep coring) capable of contacting trim in the bottom half of combos will yield a greater probability of identifying STEC contaminated combos.

P1-44 Distribution and Detection of Shiga Toxin-producing *Escherichia coli* (STEC) during Large-scale Grinding of Beef Trim

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Introduction: It is important to understand Shiga toxin-producing *Escherichia coli*(STEC) distribution and our ability to detect contamination during ground beef manufacturing if a portion of contaminated beef trim enters the process.

Purpose: Utilize a high-level biocontainment processing facility containing industry-scale beef grinding equipment to conduct inoculated studies to characterize distribution of contamination, and our ability to detect the contamination, during ground beef manufacturing.

Methods: A cocktail of STEC serotypes (O26, O111, O45, O103, O121 and O145) was inoculated onto a 908 g piece of beef trim (yielding 8.9 and 5.8 log CFU/g of the inoculated trim for runs 1 and 2, respectively). This inoculated portion was placed into a commercial grinder followed by grinding two combos of beef trim. The meat was ground, blended, re-ground and chub packaged. Samples (375 g) were collected sequentially after each 45 kg portion of first grind, and every 20thfive-pound chub. Samples were enriched and STEC presence was determined using PCR.

Results: Run 1 (high inoculation level) samples were STEC positive at all sampling points. For run 2 (low level), 16 of the first 17 samples from first grind were positive and only one of the remaining 15 samples was positive. Run 2 chub samples were mostly negative (30/34).

Significance: Initial level of STEC contamination of isolated portions of beef trim entering a grinder greatly impacts the distribution levels and our ability to detect contamination in final chub packages. When a portion of trim is heavily contaminated and ground/blended/packaged, as in run 1, the entire system becomes contaminated and all samples are positive by PCR. At a lower contamination level, contamination in the grinder appears to dilute STEC to below detection after approximately one combo being ground; however, the blending and packaging process results in further dilution and yields only sporadic positives in finished chubs.

P1-45 Prevalence of *Salmonella* on Beef Hides and Carcasses at an Abattoir in Merida, Mexico

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Introduction: *Salmonella* is a leading foodborne pathogen associated with diarrhea in Mexico, where diarrheal diseases constitute a major health problem, especially among children and the elderly. In Mexico, there is limited scientific information regarding *Salmonella*prevalence of pre and post-harvest beef cattle.

Purpose: The purpose of this study was to determine *Salmonella* prevalence on beef hides and subsequent contamination of carcasses at a municipal abattoir in Merida, Mexico.

Methods: Hide and carcass (pre- and post-evisceration on the foreshank) samples were collected using a spongescicle hydrated with buffered peptone water (BPW) and transported back to the U.S. *Salmonella* was detected using standard protocols for the Dupont Qualicon BAX®system. Positive samples were isolated using traditional culture methods and confirmed via agglutination.

Results: A total of 687 samples were collected from the 3 sample locations on the beef carcass over a 3-year span. *Salmonella* was identified on 64.3% of these samples, with the highest prevalence found on hides (n = 231) at 8.4% (95% CI = 7.9, 8.9), followed by pre-evisceration (n = 231) at 6.0% (95% CI = 5.3, 6.6), and post-evisceration (n = 227) at 4.9% (95% CI = 4.2, 5.5). *Salmonella* was recovered in all seasons; however, the spring season represented the highest *Salmonella* prevalence at 8.2% (95% CI = 7.5, 8.7).

Significance: High prevalence of *Salmonella* contamination was observed in the abattoir environment, indicating a serious risk of *Salmonella* entry into the food chain. This study demonstrates the necessity of implementing food safety programs for Mexico's beef industry and, more specifically, the need for microbiological interventions in the abattoir environment.

P1-46 Enrichment Temperature Variation Effects on *Shigella* qPCR in High Background Food Matrices

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Introduction: *Shigella* is the third most common foodborne related pathogen that causes illness. Current approved methods incorporate varying enrichment temperatures for *Shigella* detection. An effective enrichment temperature for high background matrices is necessary to produce reliable and repeatable results in low level *Shigella* detection using qPCR.

Purpose: This study compares 35°C to 42°C enrichment temperatures for its effect on *Shigella* and high background competitive growth used in conjunction with a qPCR endpoint for detection.

Methods: To determine the effect of temperature on the flora of high background matrices as well as the enumeration of *Shigella* species in *Shigella* broth, raw ground beef samples were enriched with *Shigella* broth. Duplicates were run in parallel at 35°C and 42°C overnight. Enrichment broths (non-matrix) were also fortified with a fresh inoculum of approximately 20 CFU of *Shigella flexneri* 2457M.

Two sample sets were analyzed on consecutive days with raw ground beef samples that contained varying fat contents and bacterial loads. Samples were fortified at levels of 2.0 – 0.2 CFU/g at intervals of 0.2. Parallel samples were enriched at 35°C and 42°C incubations. Samples were analyzed following the FERN-MIC.0013.01 qPCR method in duplicate using the Smart Cycler II with ipaH and IC gene targets.

Results: Overall the mean ipaH target threshold values for 42°C were 5.93 (27.01vs.32.94) cycles shorter than those at 35°C and were consistent throughout each inoculum level. Data generated indicated that *Shigella* spp. tend to grow slower in enrichment at 42°C vs. 35°C by nearly a half log ($1.0x10^9$ vs. $1.5x10^9$) but the 42°C enrichment reduced the amount of background competitors by an entire log ($4.0x10^8$ vs. $1.6x10^9$ CFU/g).

Significance: The results presented here indicate a 42°C enrichment temperature for *Shigella* broth is more effective than 35°C for inhibiting the growth of background competitors in high background matrices and thus demonstrated a lower limit of detection for endpoint qPCR.

P1-47 Evaluation of Process Control to Prevent Contamination of Beef with Non-O157 Shiga Toxin-producing *Escherichia coli* (STEC) in U.S. Export Abattoirs in Costa Rica

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Introduction: A recent increase in the number of non-O157 Shiga toxin-producing *Escherichia coli*(STEC) cases has led government and health agencies to focus their attention on these serotypes as pathogens of concern. In 2012, the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture (USDA) declared six new STEC serotypes as adulterants of non-intact raw beef products and product components.

Purpose: The purpose of this project was to determine 1) the prevalence of non-O157 STEC on beef hides and carcasses in U.S. export abattoirs in Costa Rica and 2) if current practices and interventions controlled final carcass contamination.

Methods: Three abattoirs (A, B, C) were visited during November of 2012. Carcasses were followed and the foreshank swabbed at three stages: hides, prior to evisceration, and after application of the antimicrobial intervention (carcass spray with a 200-ppm peroxyacetic acid solution). Thirty swabs were collected at each point in all plants. An FSIS-approved real-time PCR protocol was used to assess the presence of non-O157 STEC serogroups O26, O45, O103, O111, O121, and O145 using the DuPont Qualicon BAX® System.

Results: Non-O157 STEC prevalence in Plant A was 86.7% (hides; n = 26/30), 6.75 (pre-evisceration; n = 2/30) and 0% (post-intervention; n = 0/30); Plant B prevalence was 96.7% (hides; n = 29/30), 33.3% (pre-evisceration; n = 10/30) and 6.7% (post-intervention; n = 2/30); prevalence in Plant C was 96.7% (hides; n = 29/30), 3.3% (pre-evisceration; n = 1/30) and 0% (post-intervention; n = 0/30). Serogroups O103 and O45 were the most prevalent in Plant A with corresponding frequencies of 17/35 (48.6%) and 15/35 (42.9%). In Plant B, O121 and O103 were predominant with 37/129 (28.7%) and 35/129 (27.1%), respectively, whereas in Plant C, O145 and O26 predominated with 27/109 (24.8%) and 23/109 (21.1%), respectively.

Significance: These results highlight the significance of hides as a major source of STEC on carcasses. Additionally, proper dressing procedures along with effective antimicrobial interventions reduce contamination with STEC on final carcasses. All of the visited abattoirs exporting to the US are controlling contamination and should continue with current interventions and on-going testing for verification.

P1-48 Biofilm Formation and Sanitizer Resistance Contributes to "High Event" Meat Contamination by *Escherichia coli* O157:H7

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Introduction: *Escherichia coli* O157:H7 is an important foodborne pathogen. A "High Event" is defined as a time period in which commercial meat plants experience a higher than usual rate of *E. coli* O157:H7 contamination. Genetic analysis indicated that within a "High Event", most of the contamination strains belong to a singular dominant O157:H7 strain type. This was in disagreement with the current beef contamination model stating that product contamination occurs when the incoming pathogen load on animal hides, which consists of a diverse strain type, exceeds the intervention capacity. Thus, we hypothesize that the "High Event" contamination is due to certain in-plant colonized *E. coli* O157:H7 strains that are better able to survive sanitization through biofilm formation.

Purpose: To determine if biofilm formation and sanitizer resistance contributes to "High Event" beef contamination by *E. coli* O157:H7.

Methods: A subset of 47 *E. coli* O157:H7 strains obtained from "High Event" beef contamination and a group of 47 *E. coli*O157:H7 "Diversity Control Panel" strains were tested for biofilm formation and sanitizer resistance. Biofilm formation was tested on 96-well polystyrene plates for 1 – 6 days. Biofilm cell survival after sanitization was compared between the two strain sets using common sanitizers, including chlorine, Vanquish, and ProOxine.

Results: No difference in "early stage" biofilms was observed between the two strain sets after incubation at room temperature for 1 or 2 days. However, the "High Event" strains demonstrated significantly higher potency ($P < 0.05$) of "mature" biofilm formation after incubation for 3 – 6 days. The "High Event" strains also exhibited significantly stronger resistance ($P < 0.05$) to sanitizer treatments.

Significance: These data suggest that biofilm formation and sanitizer resistance play critical roles in "High Event" beef contamination by *E. coli* O157:H7, which highlights the importance of proper sanitization in commercial meat plants.

P1-49 Optimization of the Elution Buffer and Concentration Methods to Detect Hepatitis E Virus in Meat Using Nested Reverse Transcription-polymerase Chain Reaction and Real-time Reverse Transcription-polymerase Chain Reaction

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Introduction: Hepatitis E virus (HEV) causing acute hepatitis through contaminated water or food is a major concern of public health in developing countries. The consumption of meat contaminated with HEV is an important transmission route.

Purpose: The purpose of this study was to optimize the elution and concentration methods for HEV in pork liver and to perform the comparative detection of nested RT-PCR and real-time RT-PCR for HEV in pork meat.

Methods: One gram and 10 g of swine liver was eluted using phosphate-buffered saline, threonine buffer, and glycine buffer. Polyethylene glycol (PEG) precipitation and ultrafiltration (UF) were used to concentrate HEV. Nested RT-PCR and real-time RT-PCR were compared for the detection of HEV in liver samples.

Results: When PBS elutes of 10 g liver samples were concentrated with UF, 6 (23.1%) out of 26 were HEV positive by real-time RT-PCR but all were negative for HEV by nested RT-PCR. PBS was the optimal buffer to elute HEV from 10 g liver sample. The combination of UF and real-time RT-PCR is the most sensitive detection method for HEV.

Significance: Compared with previous studies, this study developed the optimal protocol for the elution, concentration, and detection method for HEV in pork liver.

P1-50 Prevalence and Characterization of Methicillin-resistant *Staphylococcus aureus* Isolated from Commercial Pork Processing Plants in Canada

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Introduction: Among livestock, pigs represent the highest prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) colonization, with health consequences in pig farmers/workers and potential for broader community-associated MRSA infections in humans. While epidemiology of MRSA in pigs on farms has been extensively studied, little information is available about the sources of contamination and prevalence of MRSA in slaughter plants.

Purpose: The aim of this study was to determine the MRSA prevalence at several points during the swine slaughter process and in retail pork from these slaughter plants.

Methods: Three plants (A, B and C) were selected in Alberta, Canada and approximately 220 samples were collected from four points during slaughter and processing in each plant for a total of 2,640 samples. Samples sources were: nasal swabs after bleeding (NSAB), nasal swabs after scalding (plant B) or skinning (plant A, C) (NSAS/S), carcass swabs after pasteurization (plant B) or washing (plant A, C; CSAP/W) and retail pork products (RP). MRSA was isolated and confirmed using standard cultural and molecular methods. Randomly selected MRSA isolates were *spa* typed (539 isolates) and antimicrobial susceptibility (246 isolates) was tested for 21 antimicrobials.

Results: Overall MRSA prevalence was 37.5% (330/880), 12.7% (112/880) and 24.2% (213/880) in plant A, B and C, respectively. NSAB samples showed the highest MRSA prevalence (plant A: 77.3%, 170/220; plant B: 34.7%, 77/220 and plant C: 74.1%, 163/220), followed by NSAS/S samples (plant A: 48.9%, 107/219; plant B: 14.1%, 31/220 and plant C: 22.3%, 49/220). The MRSA prevalence in CSAP/W samples was 20.9%, 1.8% and 0% in plant A, B and C, respectively. There was no MRSA detected in RP from plant B, whereas MRSA prevalence was about 3.2% (7/220) and 0.5% (1/220) in plants A and C, respectively. The majority (400/539) of MRSA isolates from the three plants belonged to the livestock-associated MRSA *spa* type t034 (ST398; 74.2%) followed by t002 (15%), and t011 (3.8%). The *spa* types t2971, t4030, t6408, t067, t1184, t808 and t777 were also found in < 1% of isolates. Furthermore, in addition to β-lactams (> 98%), MRSA isolates were often resistant to tetracycline (97%) with low rates of resistance to erythromycin (0.8%), clindamycin (0.8), gentamicin (0.8%), levofloxacin (0.5%), quinupristin/dalfopristin (1.6%).

Significance: This study suggests a reduction in the MRSA prevalence through the slaughter process. The predominant *spa*-type was t034 (ST398) followed by t002 and t011. It appears that standard intervention strategies applied in the pork plants help to reduce MRSA contamination of retail pork.

P1-51 Effects of Rooibos on the Behavior of *Clostridium perfringens* in Jokbal (Pig Trotters)

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Developing Scientist Competitor

Introduction: The foods involved in *Clostridium perfringens* outbreaks are often meat dishes prepared one day and eaten the next. The heat preparation of such foods is presumably inadequate to destroy the heat-resistant endospores and when food is cooled and rewarmed, the endospores germinate and grow.

Purpose: This study estimated the effects of rooibos and potassium lactate and sodium diacetate mixture (PL plus SDA) on growth kinetics of *C. perfringens* in ready-to-eat (RTE) Jokbal, consisting of pigs' feet cooked with soy sauce and spices, which is a very popular, widely sold Korean dish at the retail.

Methods: Pork forelegs were cooked in boiling water for 1.5 h with and without 10% rooibos extract and 4% PL plus SDA mixture was uniformly spread over the surface of cooked pork named Jokbal, which was sliced. One hundred µl of *C. perfringens* vegetative cells or spores were inoculated onto 10 g of sliced Jokbal, which was then vacuum packed or packed aerobically. Growth and survival kinetics of *C. perfringens* were measured as a function of time and temperature (10, 24, and 36°C).

Results: Growth of *C. perfringens* vegetative cells in aerobic packaged Jokbal were significantly delayed than that in anaerobic packaged. At 10°C, significantly shorter delta parameter of Weibull survival model of *C. perfringens* vegetative cell in Jokbal treated with a combination of 10% rooibos and 4% PL plus SDA mixture was measured than that of the control. At 24 and 36°C, addition of 10% rooibos and 4% PL plus SDA mixture significantly reduced vegetative cell growth and spore germination and growth of *C. perfringens* in Jokbal throughout the storage periods, regardless of packaging method.

Significance: The potential of rooibos to inhibit the growth of *C. perfringens* and spore germination in Jokbal may extend a rooibos as a "natural" preservative, which can be a very useful tool in the food industry responding to the current consumers' needs.

P1-52 Development of Multiplex PCR Assay for Species Identification of Cattle, Hog, Chicken and Duck from Raw Meats

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Introduction: Species identification of animal tissues in meat products is an important issue to protect the consumer from illegal and/or undesirable adulteration; for economic, religious and health reasons. In this reason, accurate analytical methods are needed for the labeling of meat products with requiring simple and fast procedure

Purpose: In this study, a multiplex polymerase chain reaction (PCR) assay was developed for the simultaneous identification of four species of cattle, hog, chicken and duck from raw meats

Methods: The primers used in this study were designed in different regions of mitochondrial DNA (16S RNA) after alignment of the available sequences in the Genbank database. The primers generated specific fragments of 94 bp, 192 bp, 279 bp and 477 bp lengths for hog, chicken, cattle and duck, respectively. Thirty-six cycles of amplification were run using a Mastercycler system (Eppendorf) as follows: denaturation at 94°C for 30 sec, annealing at 60 °C for 30 sec, and extension at 72°C for 30 sec.

Results: The detection limit of the multiplex PCR assay was 1 pg of each template DNA extracted from raw meats of cow, pig, chicken and duck. Total 145 samples (cattle 55, hog 30, chicken 30, and duck 30) were tested for PCR assay. The species specificity was 100% in all four species in the multiplex PCR assay. When this PCR test was applied to other animal species of horse, sheep, goat and turkey, no amplification was shown.

Significance: These studies suggest that this multiplex PCR assay can be used for rapid and simultaneous species identification of cattle, hog, chicken and duck from raw meats. Further studies are needed for evaluation of developed multiplex PCR from various samples including processed meat products.

P1-53 Prevalence of *Escherichia coli* Non-O157:H7 STEC in Beef in Mexico

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Introduction: *Escherichia coli* non-O157:H7 STECs are dangerous pathogens that pose a substantial risk to public health. A baseline prevalence must be established in order to effectively validate interventions and modifications to the beef production system in the future.

Purpose: The purpose of this study is to determine the prevalence of *E. coli* non-O157:H7 STECs in beef in Mexico.

Methods: Samples were collected at beef processing plants in three major cities in Mexico. In addition to market samples collected in one city, hide, pre-evisceration, and post-evisceration samples were collected using sponges hydrated with buffered peptone water. All samples were then enriched in Tryptic Soy Broth (TSB), incubated at 37°C for 18 h, and processed in accordance with standard BAX protocols (Dupont Qualicon).

Results: The hide, pre-evisceration, and post-evisceration prevalence varied by city with rates in city one being 96.9% (n = 65), 76.9% (n = 65), and 80.8% (n = 65), respectively; city two 100% (n = 25), 50% (n = 25), and 0% (n = 25); and city three 100% (n = 20), 100% (n = 20), and 75% (n = 20). The prevalence in market samples from city three was 5.7% (n = 105). Serogroups O121, O126, and O103 were the most common, with prevalence rates as high as 96.7% in city two's hide samples, while O111 was the least common serogroup with prevalence rates of 0%-30%.

Significance: Contamination of food animals and food products with *E. coli* non-O157:H7 STECs can pose an important public health risk, especially to populations most susceptible to *E. coli* infections. While *E. coli* O157 has been reported at low prevalence in Mexico, this study effectively establishes that other STECs need to be addressed and controlled in Mexico.

P1-54 Destruction of *Listeria monocytogenes* and Shelf-life Extension of Sliced Roast Beef and Turkey Breast by High Pressure Processing

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Introduction: Ready-to-Eat (RTE) meat and poultry processors follow enhanced sanitation practices, use antimicrobial agent and/or process or apply post-lethality process to minimize the risk *L. monocytogenes*.

Purpose: Evaluate destruction of *L. monocytogenes* by HHP and extension of shelf life of sliced turkey breast and roast beef during refrigerated storage.

Methods: Turkey breast and roast beef (sliced) were obtained from a commercial processor and stored under refrigeration. A slice of either product was inoculated with a 5-strain cocktail of *L. monocytogenes* to attain ca. 3.0 log CFU/cm^2 (low) or 5.0 log CFU/cm^2 (high) on the product. The inoculated slice was placed between non-inoculated product and vacuum packaged. Non-inoculated products were processed and used for evaluation of microbial shelf life. Products were processed at 87,000 psi for 2 (HPP-2 min) or 3 (HPP-3 min) min and stored at 4°C. *L. monocytogenes* survival and microbial shelf life (total plate counts, TPC) was determined after processing (0 day), 30, 60, 90 and 120 days of storage.

Results: HPP for 2 or 3 min resulted in *L. monocytogenes* reductions of 5.29 and 5.52 log CFU/cm^2 on roast beef and 2.85 and 4.48 log CFU/cm^2 on turkey breast, respectively. HPP of roast beef and turkey breast resulted in elimination of *L. monocytogenes* on product inoculated at low level (ca. 2.93 log CFU/cm^2) on both the products. The TPCs of roast beef were 5.24, 4.13 and 4.70 log CFU/cm^2 for control, HPP-2 min and HPP-3 min, respectively, after 90 days of storage. TPCs of turkey breast were 6.23, 2.07 and 2.26 log CFU/cm^{2}for control, HPP-2 min and HPP-3 min, respectively, after 90 days of storage.

Significance: HPP is an effective post-lethality treatment to eliminate *L. monocytogenes* contamination on sliced roast beef and turkey breast and improve microbial shelf life of the products.

P1-55 Multiple Models for Aggregated Foodborne Pathogen Transfer Data between Meat Products and Contact Surfaces

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Developing Scientist Competitor

Introduction: There is a growing body of literature addressing bacterial transfer to/from food products and contact surfaces, but that work is rarely connected to the underlying mechanisms. A quantitative meta-analysis of existing transfer data would be an important step to advance linkages between fundamental research and the observed transfer outcomes.

Purpose: The objective of this work was to compare three candidate models across different transfer scenarios, in order to elucidate phenomenological differences attributable to contact or product type.

Methods: Six studies containing 75 data sets, 253 transfer curves, and 5,838 data points encompassed the major four ready-to-eat (RTE) meat products (ham, turkey, salami, bologna), equipment surfaces (mechanical slicers, kitchen knives, cutting boards, conveyor belts, and countertops), and contact event types (sequential static surface contacts, single knife slicing, and mechanical delicatessen slicing). Three models (linear, Weibull,

and two-phase Weibull-linear with a critical contact value) were fit to each of the transfer curves; the most likely models were determined for each transfer scenario and food type using the Akaike Information Criterion (AIC) and root mean squared error (RMSE).

Results: RMSE ranged from 0.20 to 1.53 log (CFU/cm^2) across all models and data. The aggregate analysis revealed that the Weibull model was the best choice (based on AIC) for 8/8, 14/37, and 27/38 of the knife, slicer, and static contact data sets, respectively. For turkey, ham, and salami slicing data, the two-phase model yielded a mean critical contact value of ~9, ~9, and ~2, indicating fundamental differences among transfer responses. In contrast, sequential static contact data yielded the same shaped response regardless of the meat type or surface.

Significance: Aggregating data from multiple studies revealed underlying transfer characteristics that were not previously evident or reported in the individual studies. There remains a need for standard methods or reporting expectations, in order to maximize the future utility of transfer studies.

P1-56 Development and Validation of Microwave Heating Instructions for Pot Pies to Assure Food Safety

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Introduction: Not-ready-to-eat (NRTE) microwaveable food products have been associated with salmonellosis outbreaks. Microwave heating instructions should be developed based on temperature profiles obtained during heating of the product and subsequently validated using microbial challenge studies.

Purpose: Develop and validate microwave heating instructions for the destruction of *Salmonella* spp. in microwaveable pot-pies.

Methods: Two household microwave ovens of low (700 W) and high (1,350) power were used. For each oven, twenty-four individual temperature profiles were obtained by heating turkey pot-pies. Time to reach 73.8°C was calculated and Final microwave heating times were selected based on the best-fit distributions at 99% upper confidence level (CL) and chi-square value. A five-serovar cocktail of *Salmonella* spp. (ca. 7.0 log CFU/g) was used to inoculate pot pies at the geometric center (filling), crust (center or edges) and the geometrical center with two pot-pies in the oven. Standing time (3 min) was applied for pot pies with *Salmonella* spp. inoculated in the filling and with 2 pot pies placed in the oven to evaluate potential destruction of the pathogen. *Salmonella* spp. populations were enumerated and enrichment method was followed for samples with populations below the detection limit.

Results: *Salmonella* spp. reduction of 5.16 log CFU/g was observed following the heating time of 7.0 and 9.5 min (for high and low power ovens) following 99% CL to attain 73.8°C. However, *Salmonella* spp. survival was observed following the enrichment procedure. Incorporation of a 3 min standing time resulted in elimination of *Salmonella* spp. in the pot-pies. Minimal reduction in *Salmonella* spp. population was observed when two pot-pies were heated in the microwave ovens.

Significance: *Salmonella* spp. destruction in microwaveable pot-pies is affected by the location of the organism in the product as well as the number of samples placed in the oven. Inclusion of standing times subsequent to heating resulted in elimination of *Salmonella* spp. in pot-pies.

P1-57 Development and Validation of Microwave Heating Instructions for Chicken Nuggets

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Introduction: Salmonellosis outbreaks from the consumption of microwave heated chicken nuggets were attributed to non-uniform product temperatures. Microwave heating instructions should be developed using temperature profiles of the product heated in various ovens and subsequent microbial validation studies.

Purpose: Develop science based heating instructions for microwave heating of chicken nuggets and validate the instructions using microbial challenge study.

Methods: Two household microwave ovens of low (700 W) and high (1,350 W) power were used. Temperature profiles were obtained by placing frozen chicken nuggets (4, 6 or 8) at two different positions (edge or center) and heated. Temperature profiles (24) were obtained for each combination of pieces, location and oven, and times to reach 70, 72.2 and 73.8°C were determined. Heating times were selected based on 90, 95 and 99% confidence intervals (CI) and chi-square value. Chicken nuggets were inoculated in the center with a five-strain *Salmonella* spp. cocktail (ca. 7.22 log CFU/g) and heated for the calculated times. *Salmonella* populations were determined by plating and enrichment methods subsequent to heating and after standing time (2 min).

Results: *Salmonella* spp. reductions of 6.56 log CFU/g were observed in chicken nuggets (4 pieces, center) heated for 86 s (99% CI; target temperature, 73.8°C) in the low power oven. Longer times (187 s) resulted in 7.22 log CFU/g *Salmonella* spp. reductions when chicken nuggets were in groups of eight. Use of higher wattage microwave oven resulted in similar *Salmonella* spp. reductions with chicken nuggets at the center, even with shorter heating times. Incorporation of standing time after heating eliminated *Salmonella* spp. regardless of the power of the oven, location and the number of chicken nuggets.

Significance: Position, number of nuggets and the power of microwave oven affected *Salmonella* spp. destruction. Standing times resulted in the elimination of *Salmonella* spp. as temperature equilibration may be achieved throughout the product.

P1-58 Development and Validation of a Finite Element Heat Transfer Model for Pasteurization of Shell Eggs with Radio Frequency Heating

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Undergraduate Student Award Competitor

Introduction: *Salmonella* Enteritidis outbreaks have been associated with shell egg contamination. Recipes that call for the use of raw shell eggs and the consumption of undercooked shell eggs are potential risk factors for salmonellosis. Current methods for pasteurizing shell eggs either take too long or induce albumen coagulation.

Purpose: Evaluate the feasibility of using radio frequency (RF) heating to pasteurize shell eggs through a computer simulation and subsequently a validation study.

Methods: Computer simulations of RF heating of shell eggs at different positions were performed using the finite element method in COMSOL Multiphysics. The process time and temperature distributions from the simulations were analyzed with curves plotted using D-values obtained

from literature to acquire theoretical log reductions of *Salmonella* Enteritidis. The simulations were then validated with a Strayfield 6kW radio frequency heater and microbiological studies.

Results: Computer simulations have shown theoretical reductions of 6 and 8 log of *Salmonella* Enteritidis in egg yolk and albumen, respectively, with a process time of 7 min. Validation studies are being performed, but preliminary results showed negligible coagulation of albumen. Observed temperature agreed well with predictions of the heat transfer model.

Significance: When compared to hot water treatment, RF processing significantly reduced the come-up time. With a faster process time and significant reductions of *Salmonella* Enteritidis, RF heating would allow production of pasteurized shell eggs and reduce the risk of salmonellosis.

P1-59 Development and Validation of a Heat and Mass Transfer Model for Air Cooling of Poultry Carcasses

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Developing Scientist Competitor

Introduction: Rapid cooling immediately after slaughter and during storage are critical factors to assure microbial safety of poultry carcasses. Computer models for simulating cooling of poultry carcasses are valuable tools to develop safe cooling procedures. However, current models make simplifications that limit applicability for industrial use and are not available to poultry processors.

Purpose: Develop an accurate heat and mass transfer model for predicting carcass temperature during air cooling of poultry carcasses.

Methods: The coupled heat and mass transfer model considered heat conduction, convection, radiation, and moisture evaporation. Three-dimensional geometries of poultry carcasses were generated from computer tomography images obtained from multiple carcasses. The effect of non-uniform carcass composition and non-uniform thermal properties corresponding to the meat and bone sections of the carcasses was considered. The model was developed using a combination of computer aided engineering software (e.g., COMSOL Multiphysics® and Materialise Mimics) and custom-made computer algorithms. Model validation was conducted under laboratory and industrial settings, following normal processing conditions.

Results: The developed model was in agreement with experimental data. Comparisons between the predicted and observed temperatures resulted in an RMSE of 2.3±1.5 °C, and a 0.08±0.05 log CFU/g deviation in the predicted net growth of *Salmonella* spp. The model was successfully adapted to provide accurate predictions using input parameters such as air relative humidity, air velocity, cooler set-point temperature, and carcass weight.

Significance: The developed model can be easily accessed and integrated with predictive microbial models through the food safety website: *numodels4safety.unl.edu*. It can be used to support hazard analysis, development of critical limits, estimation of potential impact of cooling deviations, and simulation of multiple processing scenarios for quantitative microbial risk assessment.

P1-60 Proficiency Testing of Laboratories Analyzing *Shigella flexneri* and *Shigella dysenteriae* from Spiked Sausage

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Introduction: Foods are known to be more challenging matrices than clinical samples for *Shigella* detection.

Purpose: A proficiency testing (PT) study was conducted to evaluate performance of laboratories in detecting *Shigella* in spiked sausage utilizing qPCR and/or culture methods.

Methods: Prior to shipping the samples were tested for in-house validation of sample preparation techniques, homogeneity and stability testing. At least 3 replicates of samples containing *S. flexneri* or *S. dysenteriae* at 66 - 100, 665- 1000 and 6650 – 10000 CFU/25g with or without confounding enterics were tested by qPCR and culture methods. A set composed of 4 *Shigella* spiked (10000 CFU/25g) samples, two non-*Shigella* spiked samples and two un-spiked samples were distributed to 49 laboratories for *Shigella* analyses using qPCR for screening and/or culture method for confirmation.

Results: During in-house validation, a total of 22 and 30 samples at each inoculation level were tested for *Shigella flexneri* and *Shigella dysenteriae*, respectively. *S. flexneri* (17% - 67%) and *S. dysenteriae* (<34%) were accurately identified with Biolog *Shigella* and R&F *Shigella* chromogenic, MacConkey, Xylose Lysine Deoxycholate and/or Hektoen media; however 100% of the samples were accurately detected with qPCR. In the proficiency study, 22% and 78% of participants analyzed samples by culture and qPCR procedures, respectively. Culture methods detected 36 – 82% and qPCR method detected 79 – 100% of the spiked samples. Of the positive qPCR results, only 29 - 87% of were culturally confirmed.

Significance: The real-time PCR method is a highly sensitive and selective assay which can be used as a screening tool. The low recovery rate of *Shigella* isolates indicated that the current culture method needs to improve in two areas: (1) Develop more selective enrichment to cut down background flora. (2) Adopt or develop differential and/or selective agars for better isolation.

P1-61 Thermal Inactivation of *Escherichia coli* O157:H7 (ECOH) and Non-O157 Shiga Toxin-producing *Escherichia coli* (STEC) in Mechanically Tenderized Veal

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Introduction: Among the veal samples tested by FSIS from June to September 2012, 3 of 11 (27.3%) were confirmed positive for STEC, compared to 5 of 729 (0.69%) beef trim samples. This difference in detection of confirmed positives from veal samples compared to that from beef is striking and raises the question of whether consumption of veal, particularly if tenderized, poses a greater risk to public health than beef.

Purpose: Quantify thermal destruction of ECOH and STEC in mechanically tenderized veal cutlets following cooking on an electric skillet.

Methods: For each of five trials, flattened veal cutlets (ca. 71.6 g, ca. 1/8 inch thick) were surface inoculated with ca. 6.8 log CFU/g of multi-strain cocktails of ECOH or STEC and mechanically tenderized by passing once through a "Sir Steak" tenderizer. For each cooking time, in each trial, three inoculated and tenderized cutlets were individually cooked for 0.75, 1.0, 1.25, 1.5, or 2.25 min per side on a skillet set at 191.5°C. Canola oil (ca. 15 ml) was added onto the skillet prior to cooking, and the temperatures of the meat and of the skillet were monitored and recorded using a Type J thermocouple.

Results: With longer the cooking time, higher internal temperature of the meat was achieved, along with greater reduction of ECOH and STEC. The mean final internal temperature of the meat ranged from 60.5 to 89.2°C. Microbial reductions of ca. 1.9 to 6.3 log CFU/g and ca. 2.7 to 6.1 log CFU/g were achieved for ECOH and STEC, respectively.

Significance: To deliver a 5.0-log reduction of ECOH or STEC, and to achieve the recommended internal temperature of 71.1°C, it would be necessary to cook mechanically tenderized veal cutlets on a pre-heated skillet set at ca. 191.5°C and containing 15 ml of cooking oil for at least 2.25 min per side.

P1-62 Ultraviolet Light as a Post-lethality Treatment against *Listeria monocytogenes* on Bologna and Its Impact on Quality Attributes

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Developing Scientist Competitor

Introduction: *Listeria monocytogenes* is an important foodborne pathogen and a serious public health issue due to its severity of infection and high fatality rate. These high incidences are due to consumption of *Listeria*-contaminated foods, especially Ready-To-Eat (RTE) foods that do not require any additional heating/cooking post-processing, mandating post-lethality treatment to mitigate this pathogen.

Purpose: To evaluate the efficacy of ultraviolet light against log and stationary phases of *Listeria monocytogenes* on bologna and its impact on quality and shelf life.

Methods: *Listeria monocytogenes* serotype 4a was cultured in Brain Heart Infusion (BHI) broth at 37°C. Cells were harvested at log and stationary phase, subjected to low (3–4 mW/sq. cm) and high (7-8 mW/sq. cm) intensity of UV. *Listeria monocytogenes* was inoculated on bologna followed by 30 min of attachment time; and then subjected to UV radiation from 0 to 300 s. Cells were recovered on Modified Oxford agar (MOX) after 24 h of incubation at 37°C. Additionally, shelf life along with quality attributes such as color and lipid oxidation (TBARS) were assessed over a period of 8 weeks on bologna stored at 0 and 4°C under vacuum.

Results: Populations of *L. monocytogenes* were significantly reduced ($P < 0.05$) after 150 s of UV exposure and subsequent reductions ($P < 0.05$) were observed up to 300 s irrespective of UV intensities. Higher ($P < 0.05$) reductions were observed in the log phase as compared to the stationary phase cells. Significant differences ($P < 0.05$) were observed in the L, a, b values at different storage temperature and exposure time over 8 weeks of storage. However, UV did not affect the lipid oxidation of bologna irrespective of the storage temperatures and time.

Significance: Significant reduction in *L. monocytogenes* population without affecting the quality attributes of bologna suggest the potential use of UV light as a post process intervention for RTE meats.

P1-63 Resistance of Parent and Nalidixic Acid Adapted (NA) *Escherichia coli* O157:H7 and Other Shiga Toxin-producing Non-O157 *Escherichia coli* to Ultraviolet Treatment

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Developing Scientist Competitor

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) known to cause severe life threatening health conditions like HUS is linked to a wide variety of foods in general, but beef is a predominantly identified source of serious *E. coli* O157:H7 and non-O157 STEC outbreaks.

Purpose: Current research aims at determining the D and Z of different STECs to UV at three different intensities. The research also aims at validating the usage of Nalidixic acid adapted (NA) strains to replace the parent strains for UV radiation treatments of beef.

Methods: A total of 20 parent strains and 20 NA strains of *E. coli* O157:H7, O26, O45, O103, O104, O111, O121 and O145 along with 4 nonpathogenic surrogates were used in this study (individual/cocktail). Overnight grown cultures were centrifuged and suspended in phosphate buffered saline and treated with UV at three different intensities (1.04, 1.92 and 3.02 mW/cm^2/sec) and appropriately enumerated.

Results: The D-values were determined from the linear portion of the survival curves. The results show that among the five *E. coli* O157:H7 strains tested, strain 5 was the most resistant strain with a D-value of 86.8 sec at 1.04 mW/cm^2 and *E. coli* O104 was the most resistant non O157 serotype with D-value of 65.8 sec. All the NA strains were significantly less resistant to UV than the parent strains except for *E. coli* O157:H7 strain E932 which had a D-value of 64.6 sec.

Significance: Results revealed that the D-values of parent strains were significantly more resistant than the NA strains which suggest that NA strains might not be a good choice to substitute the parent strains for UV experiments and also high intensity short time UV treatments were more effective than low intensity long time treatments for the same dosage.

P1-64 Nonthermal Plasma Treatment of Packaged Inoculated Poultry Breast Fillets for the Reduction of Spoilage Bacteria and Zoonotic Pathogens

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Developing Scientist Competitor

Introduction: Raw poultry is susceptible to certain pathogens as well as spoilage after approximately 7 days at 4°C. This research involved the development of a novel nonthermal plasma generation system with modified atmosphere (MA) packaging to improve the safety and quality of poultry fillets from a local processing facility.

Purpose: This research evaluates the effectiveness of reducing natural microflora and *Campylobacter jejuni* on the surface of packaged poultry fillets treated in a dielectric barrier discharge (DBD) plasma system.

Methods: Uninoculated poultry breasts were used to determine the effect of plasma on the natural flora. Then triplicate fillets were inoculated by 30 min. soaking in *Campylobacter* inoculum and individually packaged with air or MA (65% O_2, 30% CO_2, 5% N_2). The fillet in the polyolefin bag was positioned with the empty half of the bag was within the plasma field and the fillet outside the field. Samples were treated for 3 min. at 75 kV then stored at 4°C before microbial recovery. Samples were rinsed/plated on nutrient agar (natural flora) and campy-cefex agar (*Campylobacter*) and compared to untreated controls.

Results: Treatment of uninoculated fillets in MA resulted in a mean log of 5.53 CFU/ml at 14 d (2 log lower than untreated controls). This level falls below the generally accepted spoilage limit of 10^7 CFU/ml. Treated fillets inoculated with *Campylobacter* resulted in a 2.01 CFU/ml log reduction (MA) and a 1.35 CFU/ml log reduction (air) compared to controls.

Significance: This research suggests that nonthermal DBD plasma has the ability to reduce the natural microflora on the surface of poultry fillets resulting in shelf-life extension and the ability to reduce *Campylobacter* by 2.01 CFU/ml log which can have significant implications in the field of food safety. Furthermore, treating sealed packages with a plasma that reverts to air within a day provides an effective method to treat poultry without further exposure to contaminants.

P1-65 Development and Validation of a Dynamic Predictive Model for Growth of *Salmonella* spp. in Scrambled Egg Mix

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Developing Scientist Competitor

Introduction: Liquid egg is widely used as an ingredient in various food products. *Salmonella* spp. contamination of liquid egg products can be a major human health concern. Tools to evaluate potential risk of *Salmonella* spp. growth and the resulting safety of the liquid egg product are needed.

Purpose: Develop and validate a dynamic predictive model for the growth of *Salmonella* spp. in Scrambled Egg Mix under continuously varying temperature conditions.

Methods: Scrambled Egg Mix was inoculated with ca. 2.0 log CFU/ml of a five serovar cocktail of *Salmonella* spp. The inoculated product (10 ml) was distributed in sterile vacuum bags and immersed in water baths set at specific temperatures. *Salmonella* spp. growth data at isothermal temperatures (10, 15, 20, 25, 30, 35, 37, 39, 41, 43, 45, and 47°C) was collected. The Baranyi model was used as a primary model to fit growth data; modified Ratkowsky model was fitted to the secondary model, and a dynamic model was developed using 4^{th}-order Runge-Kutta method. The dynamic model was validated using two sinusoidal temperature profiles, 5-15°C for 480 h and 10-40°C for 48 h, respectively.

Results: The mean Root Square Mean Error (RMSE) and pseudo-R^2 value for primary model were 0.33 log CFU/ml and 0.98, and for the secondary model were 0.06 and 0.99, respectively. The RMSE values for the sinusoidal low and high temperature profiles were 0.31 and 0.46 log CFU/ml, respectively.

Significance: The developed model can be used to evaluate the risk of *Salmonella* spp. growth in Scrambled Egg Mix during egg processing, storage and distribution.

P1-66 Direct Observational Study of the Risks of Cross Contamination during Raw Poultry Handling: Practices in Private Homes

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Introduction: Substantial proportion of foodborne outbreaks have been associated with foods prepared or consumed in homes. Salmonellosis in the United States has not declined over the last ten years. Poultry products have been often implicated as the major source of salmonellosis infections. Improper food handling practices in the kitchens can create conducive environments for the regrowth of *Salmonella* leading to the increase chance of cross contaminations.

Purpose: To conduct direct observational study of individuals handling raw poultry in their homes to determine the various risk factors that can contribute to the spread of *Salmonella* in the kitchen environments. In addition, administer survey questionnaires to ascertain individual's knowledge of safe poultry handling practices.

Methods: A convenient samples of 51 individual households were included in the study. Participating subjects were asked to prepare any meal of their choice starting with fresh raw poultry. Followed the observational study, survey questionnaires were administered. Notational analysis was used to transcribe the observed food handling behavior into quantifiable risk factors.

Results: Participating individuals were knowledgeable of poultry handling practices, but their observed poultry handling practices were significantly inferior to their knowledge of food safety. Less than 25% of individuals were observed properly washing their hands before and after handling raw poultry. All of the individuals on the survey reported that they wash their hands before and after handling raw poultry. Food handling practices leading to direct and/or indirect cross contaminations were observed in 100% of the observations. While, only less than 5% of individuals measured final cook temperatures before consumption, all of the individuals, checked for internal consistency of cooking and duration of time.

Significance: Cross contamination of hands, kitchen utensils, the environment, and devices (e.g., cell phones, I-pods, laptops), continue to occur during poultry handling. People's knowledge of food handling does not appear to translate fully into practice.

P1-67 Development of Kinetic Models to Compare *Staphylococcus aureus* Growth among Fresh Cheese

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Introduction: *Staphylococcus aureus* growth might be different among different cheeses because of their different physicochemical characteristics.

Purpose: This study developed kinetic models to compare *S. aureus* growth on Brie and Camembert cheeses from two different companies.

Methods: Two fresh cheeses (Brie and Camembert) were purchased from two different companies (Company A and B), and 15 g of each fresh cheese was inoculated with 0.1 ml of a five-strain mixture of *S. aureus* to obtain 4 log CFU/g. The samples were then stored at 4, 10, 15, 25 and 30°C for 2-60 days. Total bacterial and *S. aureus* cell counts were enumerated on tryptic soy agar and mannitol salt agar, respectively. The growth data of *S. aureus* from Brie (company A and B) and Camembert (company A and B) cheeses were fitted to the Baranyi model to calculate maximum specific growth rate (μ_{max}; log CFU/g/h) and lag phase duration (LPD; h). The kinetic parameters were fitted to the square root model as a function of temperature. To evaluate model performances, root mean square error (RMSE) was calculated.

Results: *S. aureus* growth was observed at 10, 15, 25 and 30°C for the cheese. μ_{max} values were increased, but LPDs were decreased as storage temperature increased. No significant differences of μ_{max} and LPD were observed among Brie and Camembert cheeses from two companies. The

Poster

developed secondary model was acceptable (R^2 = 0.918) to describe the effect of storage temperature on the kinetic parameters. In addition, the prediction of the developed model also showed acceptable performance (RMSE = 0.351).

Significance: The results indicate that the developed models in this study should be useful in describing kinetic behavior of *S. aureus* on Brie and Camembert cheeses.

P1-68 Validation of Radio Frequency Dielectric Heating (RFDH) System for Destruction of *Cronobacter sakazakii* and *Salmonella* spp. in Nonfat Dry Milk (NDM)

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Developing Scientist Competitor

Introduction: *Cronobacter sakazakii* and *Salmonella* spp. have been associated with human illnesses from consumption of contaminated nonfat dry milk (NDM), a key ingredient in powdered infant formula and many other foods. *C. sakazakii* and *Salmonella* spp. have been reported to survive spray drying if milk is contaminated after pasteurization.

Purpose: Determine the thermal processing parameters (D- and z-values) for *C. sakazakii* and *Salmonella* spp. in NDM and validate radiofrequency dielectric heating (RFDH) system for decontamination of NDM.

Methods: High-heat (HH) and low-heat (LH) NDM were separately inoculated with a 5-strain cocktail of either *C. sakazakii* or *Salmonella* spp., transferred to thermal death time (TDT) disks and held at 75, 80, 85 or 90°C for 0 to 80 min. Surviving populations of the organisms were determined by plating on tryptic soy agar. Three replications were conducted and linear regression was used to determine the D- and the z-values. The inoculated NDM was heated using an RFDH system and held at specific temperatures in a convection oven to validate the RFDH system.

Results: The D-values of *C. sakazakii* or *Salmonella* spp. were similar within the HH- or LH-NDM at respective temperatures using TDT disks and RFDH combined with convection oven used for the holding period; except for *Salmonella* spp. in HH-NDM at 85°C. D-values of *C. sakazakii* and *Salmonella* spp. varied from 23.00 to 26.25 min at 75°C, 7.52 to 13.75 min at 80°C, 6.03 to 8.68 min at 85°C, and 3.05 to 5.82 min at 90°C. The z-values of *C. sakazakii* or *Salmonella* spp. in HH- or LH-NDM were similar using TDT disks and RFDH.

Significance: Radio frequency heating can be used as a post-process lethality treatment for NDM prior to the packaging; however, the effect of RFDH on the functional properties of NDM should be evaluated to enhance adoption by the dry milk industry.

P1-69 Heat Resistance of *Escherichia coli* Strains in Raw Milk at Different Subpasteurization Conditions Tested in a Pilot Plant Pasteurizer

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Introduction: Many cheeses in Europe are typically made from unpasteurized milk with the natural enzymes and microflora responsible for enhancing desirable flavor characteristics. On the other hand, raw milk might be contaminated with various spoilage or pathogenic bacteria. To reduce bacterial loads in raw milk, short-time application of heat at subpasteurization levels under continuous flow is often used (thermisation).

Purpose: Due to the finding of an increased thermotolerance in some *Escherichia coli* strains from raw milk cheese, the aim of this study was to investigate the efficacy of different subpasteurization conditions for the reduction of *E. coli* in raw milk using a pilot plant pasteurizer.

Methods: Nine *E. coli* strains, including four Shiga toxin-producing *E. coli* (STEC) isolates, were examined. Eight strains originated from raw milk cheese and one from vat raw milk. To reflect conditions applied by cheese manufacturers, a pilot plant pasteurizer was used (milk heated in continuous flow by passing two plate heat exchangers). After inoculation of whole raw milk, bacterial reductions at heating temperatures between 60 and 70°C (steps of 2.5°C) and holding times of 15, 20, and 25 s were determined.

Results: Six of the nine *E. coli* strains, including the four STEC strains, were similarly reduced at 60, 62.5, and 65°C, while an increased thermotolerance was observed for the remaining three strains. At 60 and 62.5°C, the reduction of all strains was below 2 log after 25 s. Reductions of at least 5 log after 25 s were observed at 65°C for six strains and at 67.5°C for eight strains. But one strain was reduced after 25 s at 67.5°C by less than 1.0 log.

Significance: For certain *E. coli* strains, time-temperature combinations above 65°C were required to obtain substantial reductions within the applied thermisation treatment. On the other hand, the examined STEC strains did not show increased thermotolerance.

P1-70 Safety Assessment of Hard and Semi-hard Cheeses Stored for Up to 15 Days at 25°C

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Introduction: Many cheeses are defined as time/temperature control (TTC) foods by the FDA, and extended room-temperature storage requires a product assessment. Extended room-temperature storage of cheeses would improve marketing and reduce energy inputs, but safety must be assured.

Purpose: Pathogens that have been implicated in illness outbreaks due to post-processing contamination of cheeses include *Listeria monocytogenes* (LM), *Salmonella spp.* (Salm), *Escherichia coli* O157:H7 (ECO157) and *Staphylococcus aureus* (SA). This study identified hard and semi-hard cheeses that inhibit pathogen growth on storage at 25°C, while identifying intrinsic factors affecting pathogen survival.

Methods: Strains of Salm (6), ECO157 (5), LM (10) and SA (5) were grown separately in broth. Strains were combined into single-pathogen cocktails, and serially diluted to ~10^7 CFU/ml. 24 types of sliced-cheeses were inoculated (~10^4 CFU/g), vacuum-packaged, and stored at 25°C up to 15 days. At day 0, 3, 6, 9, 12 and 15, sample pH was measured, followed by enumeration of pathogens on specific agar: modified Eosin Methylene Blue (Salm and ECO157), *Listeria* Selective (LM), and Baird Parker (SA). Δlog CFU/g over 15 days was determined. Cheese % moisture and % salt were determined on day 0; titratable acidity (TA) and an enumeration of flora able to be grown on deMan-Rogosa-Sharpe (MRS) agar were determined on day 0, 6 and 15.

Results: pH ranged from 4.34 (Feta) to 5.98 (Gruyere); water-phase salt from 0.76% (Lacy Swiss) to 6.96% (Parmesan); and TA from 0.94% (Havarti) to 2.83% (Feta). For LM, Δlog CFU/g ranged from -4.04 (Feta) to +0.23 (Gruyere); Salm, -4.12 (Feta) to +1.57 (Gruyere); ECO157 -3.99 (Feta) to -0.23 (Colby); and SA, -3.49 (Feta) to +2.25 (Gruyere). Cheeses supporting growth were Gruyere, Provolone, and low-sodium Provolone.

Significance: These results provide scientific validation in support of extended room temperature storage of most hard and semi-hard cheeses tested. Further assessments are needed to determine the safety of room temperature storage of Gruyere and Provolone.

P1-71 Impact of Sodium Reduction on Survival of *Listeria monocytogenes*

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Introduction: Health risks associated with high sodium intact is prompting cheese manufacturers to develop low-sodium alternatives. However, these changes require a thorough evaluation of the potential food safety implications as salt is involved in controlling microbial growth. *Listeria monocytogenes*, frequently related to dairy foods, is the most likely candidate to assess the impact of salt reduction on its survival and growth.

Purpose: The purpose of this project was to assess the ability of *Listeria monocytogenes* to survive in low-sodium commercial sliced process cheeses.

Methods: The experiments were conducted with three different brands of commercially available sliced cheeses (brands A, B, C) packaged in bulk (slice on slice) and as individually wrapped slices. Brands A, B and C had sodium contents 44, 34 and 29% less, respectively, than their corresponding standard formulations. The effects of salt content and temperature were measured using surface inoculation of slices with mixtures of 5 strains of *L. monocytogenes* and determining bacterial counts during long term storage. The changes in *Listeria* counts were correlated with those factors as well as with pH, water activity and moisture content.

Results: At 4°C, the count of *L. monocytogenes* remained at approximately 4 log CFU/*g* for the entire 60 days of storage in any of the three brands of regular and reduced salt processed cheese in both SOS or individually wrapped slices. As the storage temperature increased, the *Listeria* counts declined reaching undetectable levels at any condition after 25 and 15 days at 23 and 30°C, respectively. At those temperatures, the survival rate in brand C appeared to be shorter than the other two brands.

Significance: The main finding of this research was that sodium reduction in processed cheese did not enhance survival nor promoted growth of *L. monocytogenes* at any temperature or brand tested.

P1-72 The Contamination of Antibiotics Residues and Microorganisms in Raw Cattle Milk Collected from Cha-Am District, Phetchaburi Province, Thailand

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Introduction: Antibiotics were used widely for treatment of bovine mastitis in Thailand. The improper use of antibiotics in dairy cattle farms may lead to antibiotic residue contamination in milk. Antibiotic residues can have adverse effects on some allergic individuals. Therefore, milk quality can be determined by the occurrence of antibiotic residues. Moreover, one of the requirements in the production of high quality raw milk is maintaining acceptable bacterial counts which meet the official milk quality standards.

Purpose: This study aimed to monitor antibiotic residues and microbiological quality of individual raw milk samples from dairy cattle farms in Cha-Am district, Phetchaburi province, Thailand.

Methods: Ten individual raw milk samples from six dairy cattle farms were randomly collected every month from September 2011 to January 2012. In total, 300 raw milk samples were tested. Antibiotics residues were tested using 3M Antibiotics Detection Kit for detection of Beta-lactams, Quinolones, Sulfonamides and Tetracyclines. The contamination of aerobic bacteria, Coliforms and *E. coli* were determined by 3M Petrifilm Aerobic Count Plate and 3M Petrifilm *Escherichia coli*/Coliform Count Plate, respectively.

Results: Percentages of antibiotic residues that were found in each farm over the five-month test period ranged from 0% to 14% for Beta-lactams, 2% to 6% for Quinolones, 0% to 14% for Sulfonamides and 0% to 12% for Tetracyclines. More than 80% of samples from each farm collected over the five-month period had aerobic plate counts < 200,000 CFU/ml. In 90% of raw milk samples, contamination of *E. coli* and Coliforms were < 10,000 CFU/ml.

Significance: Antibiotic residues were detected in raw cattle milk in Thailand. Therefore, farmers should be educated about how to use antibiotics properly for preventing disease in their farms.

P1-73 Identification of Biogenic Amines Production by Bacteriocinogenic Lactic Acid Bacteria Isolated from Raw Goat's Milk

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Introduction: Biogenic amines are non-volatile organic compounds produced from the amino acids decarboxylation that occur in different foods, including dairy products. Biogenic amines production is often attributed to lactic acid bacteria, mainly *Enterococcus*, and they constitute an important cause of food poisoning.

Purpose: This study aimed the characterization of the potential biogenic amines production by bacteriocinogenic lactic acid bacteria isolated from raw goat's milk.

Methods: From a lactic acid bacteria culture collection obtained from raw goat's milk, 57 isolates were characterized as bacteriocinogenic strains and identified by molecular methods (32 *Enterococcus* spp. and 25 *Lactococcus* spp.). These 57 isolates were subjected to PCR reactions to detect genes associated to the production of the following biogenic amines: tyramine, histamine and putrescine. In addition, the same isolates were subjected to phenotypic tests (using decarboxylase culture media) to verify the production of the same biogenic amines.

Results: Among the isolates, all *Enterococcus* and 7 *Lactococcus* presented PCR amplification products expected for tyramine gene while all 57 isolates showed negative reaction for histamine and putrescine genes. Considering the phenotypic results, 30 *Enterococcus* and 12 *Lactococcus* were capable to produce tyramine, when histamine and putrescine were not produced by any isolate.

Significance: The identification of lactic acid bacteria that are carriers biogenic amines related genes is a relevant finding, once these isolates can transfer these genes to other microorganisms in foods. In addition, the presented data highlighted the relevance of studying biogenic amines production and related genes in lactic acid bacteria isolates obtained from foods, in order to identify their potential risk and leading their proper application as biopreservatives or starter cultures in dairy industries. Acknowledgments: CAPES, CNPq, FAPEMIG

P1-74 Virulence Characteristics and Antibiotic Resistance of Bacteriocinogenic *Enterococcus* Isolated from Raw Goat Milk

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Introduction: *Enterococcus* is a prevalent genus in the autochthonous microbiota of raw goat milk. Some strains are particularly interesting due to the capability to produce bacteriocins and potential use as biopreservatives in foods. However, some strains present virulence potential, hampering their application in foods.

Purpose: The present study aimed the characterization of the virulence potential and antibiotic resistance of bacteriocinogenic *Enterococcus* strains isolated from raw goat milk.

Methods: Thirty-two *Enterococcus* isolates obtained from raw goat milk previously identified by PCR and capable to produce bacteriocins were selected and subjected to rep-PCR for fingerprinting. Fourteen strains were selected and tested for the presence of virulence genes (gelatinase, hyaluronidase, aggregation substance, surface protein, cytolisin, endocarditic antigen, adhesion and vancomycin resistance) using PCR and for antibiotic resistance using Etest™ (vancomycin, chloramphenicol, ampicillin, rifampicin and gentamicin). Finally, the 32 isolates were subjected to phenotypic tests to identify the gelatinase and lipase production, and desoxyribonuclease and haemolytic activity.

Results: All tested strains presented at least one of the investigated virulence genes. None of them contained gene for hyaluronidase and vancomycin resistance. In counterpart, all strains were positive for enterococcal surface protein gene. All strains were sensitive to the antibiotics vancomycin, chloramphenicol, ampicillin and rifampicin, and three strains were resistant to gentamicin. Nine strains presented α-haemolytic activity, and one was capable to produce lipase. Three strains that presented positive results for the gelatinase gene were also capable to produce it.

Significance: The study demonstrated that *Enterococcus* isolated from raw goat milk may present virulence genes and antibiotic resistance. Despite no reported foodborne infections by *Enterococcus*, these strains can transfer virulence genes to other pathogenic microorganisms. Therefore, is important to test these characteristics before using the strains for biopreservation of foods.

P1-75 Probiotic Fermented Cow's and Goat's Milks: Determination of Biogenic Amines and Sensory Acceptance

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Introduction: Fermented milks are a traditional food created as a means of preserving fresh milk, and the addition of probiotic bacteria into fermented milks adds value with respect to their potential functional benefits. However, some genera of microorganisms with potential probiotic characteristics possess the ability to form bioactive amines.

Purpose: The behavior of biogenic amines (tyramine, putrescine, cadaverine, spermidine and histamine) in fermented milks – usually cow's and goat's raw milk - added with probiotic bacteria during ten days of chilled storage was evaluated. Additionally, a quantitative consumer test was conducted with 40 consumers to assess the products' acceptability.

Methods: For the fermentation process, using $4x10^8$ CFU/ml of lyophilized *Lactobacillus acidophilus* LA-5®, *Bifidobacterium lactis* BB-12®and *Streptococcus thermophilus*(Chr.Hansen), the samples remained in the oven at 40 ± 2°C for 8 hours, and the fermentation process was interrupted when the pH reached 4.5. Finally, the product was packaged in 200ml plastic pots and stored at 4 ± 1°C for 10 days. Biogenic amines were quantified during 10 days of chilled storage by HPLC-PDA. The sensory evaluation was assessed by a hedonic test. The results were subjected to one-way ANOVA followed by Tukey test using the software GraphPad Prisma 5.

Results: Initial elevated tyramine levels were observed in both products and its content increased throughout the storage period. The highest yield of biogenic amines occurred between the first and fifth day of storage, and a decrease in the content of such compounds occurred thereafter. A higher overall sensory acceptance of the cow's fermented milk was observed.

Significance: The results suggest that, even in a preliminary assessment, the content of biogenic amines may be a criterion for selecting (probiotic) lactic acid bacteria to develop fermented milks.

P1-76 More Than Just a Processing Nuisance: Looking at Psychrotolerant Coliform Bacteria in Pasteurized Fluid Milk

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Developing Scientist Competitor

Introduction : Coliform bacteria are significant post-pasteurization contaminants (PPC) in fluid milk. The abundance of these aerobic/facultatively anaerobic gram negative, non-sporeforming rods, their ability to ferment lactose, and to form biofilms in the processing environment result in the spoilage of pasteurized fluid milk. Product spoilage typically results from survival and growth of psychrotolerant coliforms at refrigeration temperatures.

Purpose : Assess the overall ecology of psychrotolerant coliforms in pasteurized fluid milk and identify common psychrotolerant coliform genera associated with refrigerated, pasteurized fluid milk.

Methods : Coliform bacteria were isolated from milk samples submitted over the course of one year to the Voluntary Shelf-life (VSL) Program at the Milk Quality Improvement Laboratory at Cornell University. Packaged pasteurized products collected were representative of products processed at each facility, including whole fat, reduced fat, lowfat, and nonfat milk in quart, half gallon, or gallon containers. Samples were plated in duplicate on Petrifilm Coliform Count plates according to manufacturer's directions on initial day of sample arrival and days 7, 14, and 21 of refrigerated shelf-life (6°C). Coliform-positive isolates were selected from each duplicate Petrifilm plate and subsequently characterized using 16s rDNA PCR and Sanger sequencing.

Results : A total of 467 coliform isolates were collected. Preliminary molecular characterization of 96 isolates identified 9 different coliform genera in the samples: *Raoultella* spp., *Serratia* spp., *Citrobacter* spp., *Klebsiella* spp., *Kluyvera* spp., *Pantoea* spp., *Enterobacter* spp., *Hafnia* spp., and *Buttiauxella* spp. Of these genera, the most frequently isolated were *Raoultella* spp., which accounted for 26% (25/96) of the isolates; *Hafnia* spp.

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which accounted for 16% (15/96); *Serratia* spp. which accounted for 15% (14/96); *Enterobacter* spp. which accounted for 13% (12/96); and *Citrobacter* spp. which accounted for 9% (9/96) of the isolates.

Significance : The coliform genera isolated from the milk samples are associated with sensory defects in fluid milk and in other dairy products including cheese, cottage cheese, butter, and ice cream. Identification of specific organisms responsible for product spoilage is an essential first step toward development of targeted strategies for control and elimination of key spoilage organisms.

P1-77 An Independent Laboratory Verification of an Antibiotic Assay for the Rapid Detection of β-lactam and Tetracycline Residues in Raw Milk

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Introduction: The BetaStar® Combo 3.0 is a rapid detection assay for both β-lactam and tetracycline antibiotic residues in raw milk. The test utilizes binding reagents linked to gold particles to bind to antibiotic residues in milk during a three minute incubation period. After incubation the particles are transferred onto an immunochromatographic medium where the results are interpreted either visually or using an automated reader. The test employs a three line detection system, to verify the presence of tetracyclines, β-lactams and an internal control to ensure the validity of the test.

Purpose: The purpose of this independent evaluation was to verify the detection of select antibiotic residues in low concentrations in raw, co-mingled cow's milk by the antibiotic assay.

Methods: During the evaluation, 16 antibiotic residues, including 7 Penicillins (β-lactams), 6 Cefalosporins (β-lactams),) and 3 Tetracyclines, were analyzed using 3 different lots of the assay. Two operators analyzed each antibiotic twice over a 10 day test period to result in a total of 10 analyses per antibiotic. Findings were interpreted visually by each operator and using the Reveal® AccuScan III palm reader.

Results: For each of the 16 antibiotic residues analyzed using the new method, all 10 test portions were correctly identified using the palm reader or visually by one of the two operators. No visual false negative or false positive results were reported using any of the three test lots. The palm reader reported two false positives for tetracycline. A false positive was reported for lot 2 using Cloxacillin and lot 3 using Cefazolin.

Significance: This new method demonstrated reliability as a rapid, easy to use method to detect closer to safe levels for penicillin, cloxacillin, ceftiofur and cephapirin than other rapid method tests in raw, comingled cow's milk.

P1-78 An Integrated Cell Culture-PCR (ICC-PCR) Assay for Comparing Thermal Inactivation of *Coxiella burnetii* in Skim and Whole Milk

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Introduction: An Integrated Cell Culture-PCR (ICC-PCR) assay has been developed as a potential alternative to animal bioassays for evaluating *Coxiella burnetii* (*Cb*) inactivation in milk. This assay may allow accurate and sensitive evaluation of *Cb* inactivation by novel non-thermal processes.

Purpose: To demonstrate the usefulness of ICC-PCR assay for comparing *Cb* inactivation in skim and whole milk.

Methods: *Cb* resuspended in skim or whole milk at ~7.2 log genome equivalents/milliliter (ge/ml) was treated in sealed vials submerged in a circulating water bath at 62°C or 64°C for various times. After serial dilution of milk to 10^{-6}, triplicate Vero cell monolayers were infected at each level for 48 h followed by 9 day incubation after inoculum removal and addition of fresh RPMI + 1% FBS media. Infected cells were freeze-thawed followed by DNA extraction and qPCR for the *Cb* IS1111a gene. *Cb* viability was considered positive if the Day 9 post-infection (PI) level increased by ≥ 0.5 log *Cb* ge/ml from the most concentrated Day 0 PI sample. The numbers of positive wells from each dilution were used to calculate the remaining viable *Cb*/ml by MPN method.

Results: The ICC-PCR assay demonstrated that the thermal inactivation of *Cb* in skim milk was faster than in whole milk regardless of treatment temperature. For the 62°C treatment, the infectious *Cb* in skim milk was reduced by 1 log at 10 min. and was no longer infectious after 20 min., whereas *Cb* in whole milk by decreased 0.3 log after 10 min., 3.1 log after 20 min., and was no longer infectious after 26 min. After 8 min. treatment at 64°C, infectious *Cb* was reduced by 5.4 log for skim milk vs. 3.1 log for whole milk with complete inactivation after 10 min. for both milk types.

Significance: This ICC-PCR assay is a specific and sensitive method to detect differences in the inactivation of *Cb* in skim and whole milk, and may be useful for the evaluation of thermal and novel non-thermal processes for *Cb* inactivation in milk.

P1-79 Capture and Detection of *Bacillus anthracis* Spores Using Aptamer Based Surface Enhanced Raman Spectroscopy

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Undergraduate Student Award Competitor

Introduction: *Bacillus anthracis* is a highly pathogenic spore forming bacterium and a class A potential bioterrorism agent. Since spores of *B. anthracis* can survive pasteurization, it is considered a major threat for food defense, as well as in US mail threats, and its detection in is critical for preventing a potential terrorist attack.

Purpose: The goal of this project was to evaluate a method for the capture and detection of spores of *B. anthracis* in foods/dry powders quickly with high accuracy, (#spores/ml). In this study, we demonstrate a system which combines aptamer based capture and Surface Enhanced Raman Spectroscopy (SERS) using a silver dendrite nano-surface as a method of signal enhancement. This methodology has been previously shown to detect ricin in foods such as milk and orange juice.

Methods: Two published aptamer sequences (BAS-6F and BAS-6R) were constructed. These are single strands of DNA sequences, forming unique structures that bind to surface proteins of *B. anthracis*. The aptamers were initially bound to silver dendrites and then used to capture the spores from solution. The coverage of the aptamers on silver dendrites was optimized. SERS Raman spectra before and after capturing spores were acquired and by principle component statistical analysis (PCA).

Results: Plate count determination (#spores/ml) and spectral analysis indicated that both aptamers effectively captured *B. anthracis* spores from water. Plate counts found a capture efficiency of 70-80%. Using the superior BAS-6F aptamer, the limit of detection was 10^3-10^4 spores in water and 10^4 spores in orange juice. We could discriminate between *B. anthracis* and *B. mycoides* spores.

Significance: This aptamer-capture-SERS process can be completed in less than 40 minutes, which makes it a rapid method that can be used in food security and potential mail bioterror threats.

P1-80 Determination of the Heat Resistance Characteristics of *Salmonella* Typhimurium in a Range of Low A_w Commodities

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Introduction: It is known that the dry heat resistance of *Salmonella enterica* is greatly increased at low water activity, potentially allowing resistant populations to survive thermal decontamination processes. This effect is known to vary among different low moisture foods, but little published information is available offering specific heat resistance data for *Salmonella* in these matrices.

Purpose: To determine the heat resistance characteristics of *Salmonella* Typhimurium ATCC 14028 in a range of low A_w foods across a 40°C temperature range, providing data demonstrating the effect of dry heat on *Salmonella* in these commodities and facilitating the design of thermal decontamination treatments for such products.

Methods: *Salmonella* Typhimurium ATCC 14028 was subjected to dry heat treatments at 105, 115, 125, 135 and 145°C. Surviving cells were recovered on both Tryptone Soya Agar (TSA) and Xyline Lysine Desoxycholate Agar (XLD) to compare the recovery of stressed cells between non-selective and selective media. Survivor curves were plotted and D- and z-values calculated.

Results: Low moisture foods investigated were whole wheat, sesame seeds, walnuts, pecan nuts, pumpkin seeds and brazil nuts. The average z-value calculated for *S.* Typhimurium across the range of low A_w foods was 19.7C° (ranges: TSA 17.6-20.9 C°, XLD: 18.1-22.2C°). TSA cells grown on Cells recovered on TSA medium gave higher D-values across the range of temperatures and matrices than those on XLD medium. Average D-values ranged from 176.3 minutes at 105°C to 1.9 minutes at 145°C.

Significance: These data suggest that for *Salmonella* Typhimurium ATCCC 14028 z-values remain relatively constant across the low moisture foods investigated. A greater recovery of surviving organisms is possible on non-selective media, possibly due to the inability of heat-injured cells to grow under selective pressure. D-values calculated for each matrix provide valuable heat resistance data for *Salmonella* Typhimurium in these commodities.

P1-81 Determination of Thermal Processing Conditions for Acidified Foods with a pH of 4.6 or Below

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Introduction: Acidified foods with a pH at or below 4.6 must be processed to achieve a 5-log reduction for vegetative bacterial pathogens. Published research does not exist to support FDA process filings for products with pH 4.1-4.6. This lack of documentation is challenging to all areas of the industry but especially for small processors.

Purpose: To develop models and data for the thermal destruction of acid resistant vegetative microbial pathogens in acidified foods with a pH above 4.1 (pH 4.1-4.6), and generate Z and F value for the safe processing of acidified foods.

Methods: Five strain cocktails (3 independent replications each) of *Escherichia coli* O157:H7, *Salmonella enterica,* and *Listeria monocytogenes* were incubated at 56°C to 66°C in a cucumber juice medium (CJ) at pH 4.6 containing acetic acid (100 mM), a non-inhibitory buffer. After heating, surviving viable cells were diluted at room temperature prior to plating on non-selective agar. Z and F values were then determined.

Results: Log CFU/ml vs time data exhibited non-linear kinetics. A Weibull model was used to generate 5-log reduction times. Unlike previous results for thermal processing at pH 4.1 or lower, we found that *L. monocytogenes* strains were significantly more heat resistant than *E. coli* O157:H7 ($P < 0.05$). Overall, *S. enterica* was the least heat resistant organism. The predicted Z value was 9.98°C with a standard error of 1.80. The corresponding F value at 71.1°C ($F_{71.1,}$ or F_{160}) was 5.68 min.

Significance: With the advent of the Food Safety Modernization Act, producers of acidified foods will be required to determine if production processes assure a 5-log reduction in vegetative pathogens. These data may be used by process authorities to aid in determining safe production practices, although an additional safety margin, based on the standard error for the Z value, may be included.

P1-82 Student Perceptions of International Agriculture: The Effects of Vicarious International Experience Intergration in High School Agriculture Education Curriculum

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Introduction: Awareness of globalization is increasing, and students are being encouraged to prepare to enter a more internationalized job market. The agriculture industry is no exception. If agriculture students are going to be competitive in the internationalized job market, they must understand the role that globalization plays in the agriculture industry. Studies have indicated deficient knowledge of high school and college undergraduate students regarding international issues, agricultural policies, people and cultures.

Purpose: This study sought to identify high school students' perceptions of international agriculture. Additionally, the purpose of this study was to determine the effect of curriculum integrated with vicarious international experience on students' perceptions of international agriculture.

Methods: A high school agricultural educator used his international professional development experience teaching meat processing in Tbilisi, Georgia, to develop internationally integrated curriculum for his secondary agriculture education course in meat science. A census of the students present in the class was used for pre-test/post-test data collection. A ten question survey was used to assess students' perceptions of international agriculture and its relevance to them.

Results: A paired samples t-test indicated a significant difference in the pre-test and post-test summated scores (M = 37.64, M = 44.14, respectively). As indicated by the mean scores, the post-test summated scores were significantly higher than the pre-test summated scores, $t(21) = -10.04$, $P < 0.01$. A Pearson coefficient indicated a large effect size, $r = 0.91$. This suggests that students' perceptions of international agriculture were positively impacted by the internationally integrated curriculum.

Significance: The results of this study indicate that high school curriculum integrated with vicarious international experience can positively impact students' perceptions of international agriculture. Internationally integrated curricula related to food protection and public health should be developed to positively influence secondary agriculture education students' perceptions.

P1-83 Results of a Baseline Knowledge Survey of Students at a Predominantly Minority Chicago High School

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Introduction: High school students are at an age where food handling may occur for themselves and as entry level workers in food service. An estimated 21% of all food and beverage service workers are aged 16-19 years.

Purpose: The objective of this study was to determine baseline food safety knowledge and associated factors among high school students.

Methods: A convenience sample of 231 Chicago high school students was approached to participate in a 34-question knowledge survey to obtain information about their food safety knowledge, behaviors, and personal hygiene. Frequencies of correct answers to each knowledge question were examined to determine the knowledge gaps. Bivariate analyses were performed to identify student variables associated with the knowledge score and regression models were used to examine the associations between eligible factors and knowledge score.

Results: Among the 195 participating students, 70% described themselves as Hispanic/Latino and 15% as Non-Hispanic Black. Twelve percent of the students had prior restaurant employment experience. The overall student mean knowledge score was 37%. Students demonstrated substantial knowledge gaps regarding the optimal temperatures for cooking, proper mechanisms for thawing food, cross contamination, and vulnerable populations for foodborne disease. Overall, only 5% of students could explain cross-contamination in a kitchen and only 12% accurately identified that the only way to be certain that a frozen chicken breast is safe to eat is by checking its internal temperature with a metal stem thermometer. In the final linear regression model, Hispanic ethnicity and experience cooking seafood were significantly associated with lower knowledge score and experience cooking meat and cooking alone were significantly associated with higher knowledge score ($P < 0.05$).

Significance: These data demonstrate substantial knowledge gaps in a predominantly minority high school student population. Given that high school students are a substantial proportion of the food service workforce, they are especially important to target for food safety education.

P1-84 Identification of Core Competencies for an Undergraduate Food Safety Curriculum Using a Modified Delphi Approach

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Introduction: The need for an increasing, competent food safety workforce is well documented. Identification of competencies needed for professional success is critical to assure that courses and curricula are appropriate.

Purpose: The purpose of this study was to identify and prioritize core competencies relevant to post-secondary food safety education using a modified Delphi method.

Methods: Twenty-nine experts representing food safety professionals in academia, government and industry were given two rounds of questionnaires that included food safety competencies, core domains, and subdomains. Competencies are a set of skills, knowledge and abilities that correlate to success of a trainee. The framework for which competencies were classified consisted of (1) core domains, defined as broad food safety subjects; and (2) subdomains, or more specific topics. The expert panel used a 5-point Likert scale with a consensus criterion of 75%, with a rating of "4" or greater. After each round, revisions were made to the original document after considering the responses. The objective of the first-round survey was to identify and define core domains and subdomains. The objective of the second round was to refine the specific competencies and identify neglected competencies.

Results: Twenty-one (72%) surveys were completed in the first round. Five core domains were accepted by all panel members: (1) Food Production, Manufacturing and Retail; (2) Foodborne Hazards; (3) Legislation and Policy; (4) Epidemiology; and (5) Communication and Education. Most original subdomains were also accepted by the panel. Twenty (69%) surveys were completed in the second round, after which 100% consensus was reached regarding specific competencies. Feedback from the experts highlighted areas in which further curriculum revision would be beneficial.

Significance: This study provides a framework for the development of a standardized undergraduate food safety curriculum. The Delphi method, with its inclusion of professionals representing all sectors of food safety, provided relevant perspectives for curriculum design and provided participants an opportunity to participate in the education of future food safety professionals.

P1-85 Gaps in Food Safety Professionals' Knowledge about Noroviruses

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Introduction: Norovirus is the most common cause of acute gastroenteritis and foodborne-disease outbreaks in the United States. Each year, it causes about 21 million illnesses and contributes to about 70,000 hospitalizations and 800 deaths. Food safety professionals must be knowledgeable about proper control strategies if we are to begin reducing the burden of illness from this highly contagious microorganism.

Purpose: The purpose of this study was to assess food safety professionals' understanding about noroviruses and identify gaps in their knowledge.

Methods: We conducted a web-based survey of food safety professionals (n=300). The survey was distributed through various professional associations representing food safety professionals. The 18-item questionnaire used true-false and open-ended questions to assess food safety professionals' knowledge about noroviruses, including attribution, transmission, prevention, control, and food handling.

Results: Respondents represented a variety of occupations, with environmental health specialists (37%) being the largest responding segment. Seventy percent (70%) of respondents correctly identified noroviruses as one of the three most common causes of foodborne disease in the United States. About 64% of respondents had the misperception that cruise ships are one of the three most common settings for norovirus infections; only 5% knew the three most common settings. Seventy-two percent (72%) of respondents answered at least 15 of the 21 true-false questions correctly and only 3% answered all 21 questions correctly. The subject area with the most incorrect answers was food handling. Only

14% of respondents correctly answered the 6 items on food handling (e.g., answered "false" to "noroviruses can be eliminated by cooking foods to 140°F or higher").

Significance: The survey findings identify gaps in food safety professionals' knowledge about noroviruses, particularly in the area of food handling control measures. The study findings will be used to inform the development of web-based educational materials targeting food safety professionals.

P1-86 Online Purveyors of Raw Meat, Poultry, and Seafood Products: Delivery Policies and Available Consumer Food Safety Information

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Introduction: Online sales of perishable meats, fish, shellfish, and other seafood products is a large and growing business. Hundreds of companies now market directly to consumers, permitting them to purchase products online and have them shipped via common carriers such as FEDEX and UPS. While usually packed in coolers with ice, gel-packs, or dry ice, these packages are transported, stored, and delivered using the same methods as non-perishable items. This presents the possibility that the contents of the packages may be subject to temperature abuse.

Purpose: The study examined online vendor websites to determine what food safety information they provide to their customers, and whether packages may be delivered without requiring a signature and therefore, potentially left outside for long periods.

Methods: Vendors were identified using *Google* searches including combinations of the terms: online, delivery, meat, game, fish, shellfish, and seafood, as well as specific products in these categories. The websites were coded as to what (if any) food safety information is provided, and whether a signature is required for delivered packages.

Results: The websites of 429 online vendors of fresh (uncooked) meat, finfish, and shellfish who ship directly to consumers using common carriers were identified. Of these, only 12 (3%) specifically require a signature for package delivery. The remainder (97%) appears to permit packages to be delivered without requiring a signature, with many adding a disclaimer that they will not be responsible for the contents of the packages once delivered. Moreover, 251 (58%) offer no information related to the safe handling, thawing, storage, or cooking of their raw products.

Significance: The policy of permitting deliveries without requiring a signature, (and therefore left outdoors at ambient temperatures) may subject perishable products to temperature abuse. Providing no food safety information to consumers may make them more vulnerable to such problems.

P1-87 Enhancing the Safety of Locally Grown Produce through Extension Education for Farmers and Market Managers

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Introduction: Surveys of practices on small to medium farms and in farmers markets in Georgia, Virginia and South Carolina conducted in 2009 identified practices that may put consumers at risk of foodborne illness and identified a need for training to enhance safety of locally grown produce.

Purpose: The objectives were to develop training materials for farmers and market managers, to train County Extension Agents to implement training, and to evaluate curriculum effectiveness in improving knowledge of factors affecting produce safety in local markets and motivating changes to enhance safety.

Methods: Two curriculum packages were designed with an instructor guide, PowerPoint® slides, instructor dialogue, video interviews with farmers or market managers as positive deviants to educate others, evaluation questionnaires, IRB information letters, county report forms and certificates of completion. Factsheets for farmers and market managers were developed. Each curriculum was designed to provide two hours of training. County Extension Agents were trained, and workshops were implemented with farmers and market managers and evaluated by 217 farmers and 55 market managers.

Results: The percentage of farmers improving knowledge ranged from 54-73% for each of seven factors that affect produce safety. Intended changes ranged from 8% for difficult, expensive changes (e.g., irrigation methods) up to 59% for easier, less expensive changes (e.g., conducting self-inspections, improving cleaning and sanitizing, providing handwashing and toilet facilities and keeping records.) The percentage of market managers improving knowledge ranged from 56-94% for each of 14 factors that affect market safety. Intended changes ranged from 31-75% for individual practices such as enforcing a "no pets" policy, asking questions about production methods, improving handwashing and toilet facilities, providing training for vendors and workers, keeping records, etc.

Significance: The successful training increased knowledge of factors that enhance the safety of locally grown produce and motivated intent to change.

P1-88 Translating Guidance into Skills for Cooking Fish and Shellfish to Safe (≥ 63°C) Internal Endpoint Temperatures

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Introduction: Effective guidance for cooking fish and shellfish to a safe endpoint temperature that retains quality parameters is limited. Cookbook descriptors provide only marginal guidelines on assessment techniques (flaking, color changes) and some provide temperature guidance that does not provide for safety from target pathogens.

Purpose: The purpose of this study was to determine if additional verbal/visual cues improved cooking guidance and assisted consumers in developing analytical skills to properly evaluate appropriate endpoint temperature of fish/shellfish for microbial safety/product eating quality.

Methods: Participants (n=6; ages 18-70; one male, five females) completed a series of pre-training cooking sessions during which they prepared fish (salmon, tilapia) and shellfish (shrimp) using different preparation methods in a laboratory setting. A group training session was conducted, followed by series of post-training cooking sessions. Training included visual pictures, a training video, and verbal descriptions of safe food preparation behaviors, use of thermometers, and raw and cooked product characteristics. Final internal endpoint temperatures of products were measured. Count data of behaviors presented by participants was measured by video observations. ANOVA determined differences in mean internal temperatures before and after training. Chi-square test was used to determine proportion of participants able to prepare products to correct internal temperature (63°C). Poisson Regression to determine behavioral change before and after training a = 0.10.

Results: Participants cooked products to significantly different temperatures ($P = 0.015$) and greater proportion of products (n = 72) met correct internal temperature (≥ 63°C) ($P < 0.0001$) after the training. Behaviors significantly changed (unwanted $P = 0.009$; wanted $P < 0.001$; checking temperature = 0.026) after training.

Significance: More details about visual/textural product changes concerning temperature are needed. Behavior modification is possible and educational materials potentially decrease risk of foodborne illness associated with cooking fish/shellfish.

P1-89 Comparative Study: Steam and Boiling Water Canners for Home Processing Low pH Foods

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Developing Scientist Competitor

Introduction: Home canning has gained popularity with consumers in recent years. The USDA recommends boiling water canners (BWC) for home processing of acid foods. Consumers are increasingly inquiring about the safe use of atmospheric steam canners (ASC) for home canning of acid foods due to their reported ability to save time and reduce energy costs. Thermal distribution studies were conducted to determine the heating rates in both canner types.

Purpose: Heat distribution studies in ASC compared to BWC allow for a determination of come-up time in both canner types. Such information is critical in establishing safe-use instructions for consumers wishing to use this new type of canner.

Methods: Recording thermocouples were inserted in three locations in the top of each canner. Tomato juice, room temperature (75°F) or preheated to 180°F, was packed into three different jar sizes: quarts (QT), pints (PT), and half-pints (HP), and the time for the heating medium to reach the processing temperature was recorded. Come-up time was the time that it took for the temperature of the heating medium (water or steam) to reach the processing temperature, 211 + 1°F or 100°C, starting with room temperature water (77 + 2°F) or pre-heated water (180°F), for atmospheric or boiling water canners, respectively.

Results: The time for the ASC to reach the process temperature ranged from 10.3-16.2 min across jar size and juice temperature. The time for the BWC to reach the process temperature, from the preheat temperature (180°F), ranged from 4.3-21 min across jar size and juice temperature. When preheat temperature was included in come-up time, BWC processing time ranged from 23-49 min.

Significance: Home food preservation can be an enjoyable but resource intensive process. The preheat time can be a lengthy component of a BWC process and may discourage consumers from following proper canning methods. Consumers who fail to completely follow a research-tested, up-to-date recipe put the health of their family at risk. ASC may offer a convenient method for preserving low pH foods and enables consumers to adopt appropriate food preservation practices.

P1-90 Identifying Consumer Attitudes and Concerns about Fish and Shellfish Culinary Preparation Techniques and Food Safety

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Introduction: Information and guidelines concerning seafood consumption benefits, risks, and proper product handling and preparation are not effectively reaching consumers. Limited research has been conducted concerning consumer perception of current culinary guidelines for preparing fish and shellfish. Research is needed to determine if a lack of preparation descriptions causes consumers to limit fish and shellfish intake or feel incompetent in their preparation.

Purpose: The purpose of this study was to determine consumer beliefs and concerns regarding fish/shellfish consumption and preparation as related to food safety and quality.

Methods: An electronic survey and focus group discussions (FGDs) were conducted. FGDs were completed in three different settings representing an academic community, an inland community, and a coastal community. Focus group questions and probes centered on concerns and beliefs about fish/shellfish consumption and preparation. FGDs were video and audio recorded, transcribed, and coded using video-coding software. Inter-coder reliability for software coding was validated.

Results: Concerns about preparing fish and shellfish, as reported by survey respondents, included foodborne illness, proper handling and cooking, freshness/quality/source, safety, smell, and cost. Only 37% of survey respondents identified the appropriate temperature range to meet safety and quality criteria. Nine main FGD theme categories emerged: experience, trust, confidence, quality of product, motivation, concerns, cooking procedures, cooking instructions, and knowledge. Experience with fish/shellfish increased confidence in preparation. Trust in product quality and accuracy of information influenced consumer actions. There are both positive and negative motivators for fish and shellfish consumption. Consumers have concerns regarding fish/shellfish products and preparation. Current guidelines need additional instructions to help consumers increase knowledge and confidence. There is confusion and a lack of knowledge regarding preparation of fish/shellfish.

Significance: More educational materials need to be developed to help increase consumer confidence in preparing fish and shellfish products and to increase their knowledge regarding these products.

P1-91 Growth of *Listeria monocytogenes* in Ready-to-Eat Foods: Re-enactment of Observed Domestic Storage Practices Implemented by Older Adults

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Introduction: Internationally, *Listeria monocytogenes* has the highest rate of reported foodborne disease hospitalizations (< 95%) and related mortality (< 40%). European incidence has doubled almost exclusively among older adults (≥ 60years) since 2001. Majority of incidence is reported to be sporadic; the domestic kitchen is associated with sporadic foodborne disease. Older adults are more susceptible to foodborne disease, and are also reported to frequently consume ready-to-eat (RTE) foods commonly associated with *L. monocytogenes*. Consequently, implementation of safe food-storage is of paramount importance to reduce the risks of listeriosis in the home.

Purpose: Determine older adults' food-storage practices of RTE-foods associated with *L. monocytogenes* in the domestic kitchen and re-enact such practices in a laboratory to assess *L. monocytogenes* growth.

Methods: Evaluation of older adults (≥ 60 years) (n = 100) food-storage practices in the home (recording of refrigeration temperatures, observation of food-storage practices and microbiological analysis of food contact surfaces) informed the laboratory re-enactment trials. Identified

Poster

common storage behaviors of older adults were re-enacted with soft-cheese and RTE meat inoculated with ~3.71 log CFU *L. monocytogenes*, stored at ≤ 5°C (n = 110), > 5°C (n = 110) and ambient-temperature (n = 55). Food samples were analyzed every 24 hours for up to 21 days.

Results: Key common practices implemented by older adults included prolonged storage of RTE foods and/or refrigeration temperatures > 5°C. Majority (72%) of older-adults' domestic refrigerators operated at temperatures > 5°C. Most refrigerators (70%) contained foods associated with *L. monocytogenes*, 54% of which were reportedly stored beyond UK recommendations (2 days after opening) some up to 21 days. Results indicate *L. monocytogenes* grew at all re-enactment temperatures. Average generation times revealed *L. monocytogenes* growth was slower at ≤ 5°C (94 h t^{-1}) than at > 5°C (21.5 h t^{-1}) and ambient-temperature (11 h t^{-1}), suggesting older adults' prolonged storage of RTE-foods at > 5°C/ambient temperature increased *L. monocytogenes*levels, making such foods unsafe for consumption.

Significance: Findings indicate that many older adults do not implement practices that concur with food-safety recommendations required to reduce the risk of *L. monocytogenes* in the home. Re-enactment findings demonstrated *L. monocytogenes* growth resulting from such practices which may result in RTE-food becoming unsafe for consumption. Findings may be used to inform risk based, targeted consumer food-safety initiatives for older adults to increase implementation of safe food-storage practices to reduce risk of listeriosis in this consumer group.

P1-92 Educating Older Adults about Food Safety Using an Annotated "Tasty and Safe" Cookbook

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Introduction: The number of adults over age 60 is increasing rapidly in the United States. Since they are more likely to become ill from something they ate and are at a greater risk of experiencing serious health complications from foodborne diseases, educating seniors about safe food handling is a critical step in the prevention of a possible foodborne illness.

Purpose: This study was designed to see if including food safety instructions in recipes and supplemental materials within a cookbook designed especially for older adults would be an effective method of presenting information.

Methods: A cookbook was developed that emphasized: reasons seniors are susceptible to foodborne illness, steps to take if a foodborne illness is suspected, foods to avoid, hand washing, kitchen cleanliness, cross-contamination, thermometer usage, cooking of eggs and seafood, handling of leftovers, thawing of meat and poultry, and washing of fresh fruits and vegetables. Seniors (n = 309) representing 30 states completed a pre-test, after which they received the cookbook binder, the divider pages and a set of recipes. Each month for three months another set of recipes was sent to participants. One month after mailing the last set of recipes a follow-up survey, which included questions about the cookbook, was sent to each respondent.

Results: 258 seniors completed the follow-up survey. A majority reported that the cookbook was easy to read, attractive, and interesting. Almost all found the cookbook informative and learned something new. Most would share the information with someone else. Over half reported making a change in their practices after reading the recipes.

Significance: With a growing aging population, and more aging Americans wishing to remain independently living, it is important that they have resources that will assist them with this process. Following food safety recommendations in this cookbook could help prevent foodborne illness.

P1-93 Food Safety Knowledge and Self-reported Practices of UK University Students

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Introduction: Changes in food consumption patterns of university students, particularly those living away from home has been reported; however, emphasis is given to the nutritional intake of such changes as opposed to food safety implications. Previous observational food safety research has indicated young adults implement more food safety malpractices during domestic food preparation than other consumer groups; furthermore, widespread microbial contamination has been determined in student kitchens. There is a need to determine food safety cognitive behavioral influences of university students to inform targeted strategy development to improve food safety during food preparation.

Purpose: To determine university students' food safety knowledge of four critical areas to ensure food safety and associated self-reported domestic kitchen practices.

Methods: A self-complete questionnaire, administered to university students, ages 18–25 years (n = 100) to determine food safety knowledge and self-reported practices in the domestic kitchens, was structured using key food safety principles outlined in United Kingdom (FSA) and United States (FDA) strategies to promote safe food handling in the home.

Results: Overall, university students' food safety knowledge was lacking; self-reported practices indicate implementation of food safety malpractices. Although a majority of students identified cleaning (93%) and cooking (86%) as critical requirements for food safety in the home, 42% were unaware of the need to implement hand washing before handling ready-to-eat-foods and 23% failed to report usage of hot water and soap when hand washing; furthermore, 72% did not know what core temperature ensured food safety. Fewer students believed cross-contamination (62%) and chilling (55%) to be critical; 78% failed to report that poultry would not be washed prior to cooking and 33% did not know that a domestic refrigerator should operate between 1-5°C to ensure food safety. Additionally, less than a third (32%) identified all four core practices as important for food safety. No significant differences ($P > 0.05$) were determined between knowledge and self-reported practices of male and female students. Students aged 22–25 years had significantly ($P < 0.05$) more knowledge of food safety than students aged 18-21 years.

Significance: This study illustrates a lack of food safety knowledge and frequent reporting of food safety malpractices by university students. Findings can be used to inform targeted risk communication initiatives to improve university students' domestic food safety practices.

P1-94 The Impact of Food Safety Training for Volunteers at the Ronald McDonald House

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Introduction: Food safety education has become vital to all establishments that prepare food since improper food handling practices can lead to foodborne illness outbreaks. This is especially true for establishments that serve an immunocompromised population.

Purpose: The goal of this study was to determine whether food safety education increased knowledge and safe food handling practices among volunteers at the Ronald McDonald House in Houston, Texas. The Ronald McDonald House program provides bedrooms and daily meals for families with children that are being treated at the Texas Medical Center at little or no costs. Meals are prepared by a rotating core of volunteers on a daily basis.

Methods: For this study, thirty-four volunteers completed a pre-test questionnaire concerning their current knowledge and practice of safe food handling. Next, they attended two hour food safety training seminar which addressed the following topics: cross contamination, time and temperature practices, thermometer use, and proper personal hygiene. A post-test questionnaire was administered two months after attending the training seminar. Paired t-tests were conducted to determine if food safety knowledge and behaviors changed after attending the training seminar.

Results: While there was no significant change in overall knowledge scores before and after training, specific temperature-related questions showed a significant change in knowledge between the pre and post-training evaluations (M = .64, SD = .11; M = .75, SD = .12). There was a significant change in pre-test behavior compared to posttest food handling behavior at home (M = 3.12, SD = .85, M = 3.22, SD = .75) and at the Ronald McDonald House (M = 3.27, SD = .91) M = 3.43 SD = .82).

Significance: These findings indicate that even basic training can increase awareness which may in turn influence behavior. This study has provided the Ronald McDonald House with a basic framework for the development of a food safety culture among volunteers which may contribute to the reduction of risk of foodborne illness among immunocompromised individuals.

P1-95 An Evaluation on Food Safety Performance in Louisiana School Foodservice Operations

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Introduction: Food safety issues are critical in school foodservice operations as they serve an over 32 million meals daily through the National School Lunch Program. There are 163,357 cases of foodborne illnesses being reported in Louisiana (LA) each year. Of approximately 30 foodborne outbreaks investigated, 27% was originated from education institutions (i.e., schools, daycare, and colleges).

Purpose: The purpose of this study was to identify the food safety training needs of school foodservice operations in Louisiana by reviewing the frequencies and types of food code violations using multiple health inspection data within January 1 to December 31, 2011.

Methods: School foodservice operations' inspection data from ten parishes with highest number of population was derived from Department of Health and Hospitals, entered into Excel spreadsheets and converted to SPSS for analyses. The computer function was used to evaluate food safety violations. Descriptive statistics were calculated to summarize the data.

Results: A total of 281 health inspection reports from 31 schools in 10 Louisiana parishes were reviewed. At 31 school foodservice operations, 91 violations were identified. The most frequently violated food codes categories were "receptacles for garbage, rubbish and refuse" (n = 9, 9.89%), followed by "floors" (n = 7, 7.69%), "walls and ceilings" (n = 7, 7.69%), "sanitization" (n = 7, 7.69%), "food storage" (n = 5, 5.49%), "equipment" (n = 5, 5.49%), and "labeling" (n = 5, 5.49%). Of 91 violations, 18 (19.78%) were critical violations, with "sanitation", "labeling", and "storage and display" (n = 2, 2.20%) being the most frequently violated critical categories of food codes.

Significance: The findings of this study suggested the food safety training focus areas for food handlers in school foodservice operations. Through the investigation results, school foodservice managers may gain insights on what food safety practices related to food code violations they should emphasize on while managing their operations.

P1-96 Factors Affecting Food Safety Training in U.S. Chinese Restaurants: Exploration of Chinese Cultural Values

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Introduction: Foodborne illnesses remain a challenge in ethnic restaurants. The large number of establishments and cultural differences may present unique food safety challenges for Chinese restaurateurs.

Purpose: The purpose of this study was to explore factors influencing behavioral intention to provide food safety training and to identify preferred food safety training methods among Chinese restaurateurs in the U.S.

Methods: Randomly selected 500 owners/operators of independent, traditional, full-service Chinese restaurants across the U.S. received questionnaires via mail, personal visits, or faxes. The survey instrument was developed based on the literature review and results of the elicitation study (individual interviews), validated, and pilot-tested prior to data collection. Variables were Chinese cultural values (CCVs) and food safety training related variables: Chinese restaurateurs' attitudes, personal influences, perceived barriers, and past food safety training experience. Descriptive statistics for data summary and inferential statistics such as an exploratory factor analysis (EFA), hierarchical multiple regression, and ANOVA were conducted to explore core CCVs and relationships among variables.

Results: A total 261 restaurateurs completed the survey (52.2%). Seventeen CCVs related to food safety behaviors, training, and relationships with health inspectors were identified. 'Courtesy', 'respect', and 'harmony' were the three most important CCVs; and customers, family members, and business partners were the most important personal influences for Chinese restaurateurs. Employees' physical exhaustion, learning capability, and financial resources were the top three barriers to providing food safety training. EFA grouped all CCVs into five CCV factors. Of those, 'Customer Relations' and 'Interpersonal Relations' had significant effects on the intention to provide food safety training. Food safety training manuals in Chinese was the preferred training method among Chinese restaurateurs.

Significance: Results may help food safety educators and inspectors better understand Chinese restaurateurs by learning cultural differences and develop more effective strategies to encourage safe food handling and training in these restaurants.

P1-97 Effectiveness Evaluation Study of Person In-charge (PIC) Program towards Food Safety across Dubai Food Industry

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Introduction: In an objective of reducing food poisoning incidents and somehow tackle down food safety issues; Food Control Dept. (FCD) of Dubai Municipality (DM) have launched Person in-charge (PIC) program in 2011 as an appropriate food safety alternative program that so far suits the need of this city. Hence the PIC is accountable to his employer and the government for making sure that he and the employees on that shift are following effective policies and procedures for food safety.

Purpose: To evaluate the effectiveness of PIC program implementation in food establishments and measure out its contribution to food safety standard.

Methods: A standard interview took place with all the nominated PICs for the entire 48 randomly selected food establishments using a questionnaire designed to have open ended questions to serve the purpose and shall be used in two parts; before and after the program implementation.

Results: By looking at the two stages of the study; before and after PIC implementation and comparing the results for each question in the questionnaire whether it was a direct question to evaluate food safety knowledge or a question to observe food safety practices; there was a tangible knowledge increase after the implementation and practices as well have seen certain positive changes like using the smart checklist provided by the food control department and understanding the cost of food poisoning incidents; therefore we have seen less violations in cross contamination practices by 40% compared to the first stage of the study, personal hygiene practices also got enhanced by 60% compared to the results of the first stage.

Significance: The findings of the study will highlight the areas that FCD have to focus on to improve the program implementation and make the modifications accordingly so as to be as robust as possible.

P1-98 Food Safety Certification Status, Provider, and Validity: How are They Related to Food Safety Knowledge?

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Introduction: Public health agencies are increasingly encouraging or requiring restaurant kitchen manager food safety certification, in which managers receive food safety training and demonstrate knowledge learned from the training by passing a food safety certification exam.

Purpose: The purpose of this study was to develop a better understanding of the relationship between food safety certification and food safety knowledge. We also wished to develop a better understanding of the relationship between certification provider (accredited vs. non-accredited) and certification validity (certification is current vs. certification is expired) and food safety knowledge.

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 better understand the environmental causes of foodborne illness. EHS-Net environmental health specialists collected data in 399 randomly selected restaurants. The EHS-Net specialists interviewed the managers in these restaurants about their food safety certification. The managers also took a 10-item food safety quiz.

Results: Managers who had never been certified had higher odds of failing the food safety quiz (failing equals a score of < 80) than did managers who had been certified (OR = 2.41 [1.45-3.99], $P < .001$). Managers who had been certified, but not by an accredited organization, had higher odds of failing the quiz than did managers who had been certified by an accredited organization (OR = 3.38 [1.93-5.92], $P < .0001$). Managers whose certification was not valid did *not* have higher odds of failing the quiz than did managers whose certification was valid (OR = 0.71 [0.33-1.56], $P < 0.40$.

Significance: Our data suggest that food safety certification is related to food safety knowledge. They also suggest that receiving certification from an accredited organization is important, while maintaining a valid certificate may not be. Additional analysis is needed to explore the relationships among certification status, certification provider, and certification validity.

P1-99 Food Worker Characteristics Associated with Working While Ill

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Introduction: Transmission of foodborne pathogens from ill food workers to diners in restaurants is an important cause of foodborne outbreaks. The Food and Drug Administration (FDA) recommends that food workers ill with vomiting and diarrhea, symptoms of foodborne illness, be prevented from working.

Purpose: Little is known about the factors that influence whether food workers work while ill. We conducted a study designed to identify factors related to workers working while ill.

Methods: We conducted workplace interviews with food workers (N = 491) from 391 randomly selected restaurants located in the Centers for Disease Control and Prevention's Environmental Health Specialists Network sites. These interviews assessed whether the workers had worked while ill with vomiting or diarrhea in the previous year, the workers' characteristics, job responsibilities, and beliefs about the influence of specific factors on their decisions to work while ill.

Results: Twenty percent of workers had worked while ill with vomiting or diarrhea on at least one shift in the previous year. Factors significantly ($P < .05$) related to the decision whether to work while ill with vomiting or diarrhea included worker sex, years of work experience, job responsibilities, concerns about leaving coworkers short-staffed and concerns about job loss.

Significance: The findings from this study suggest that the decision to work while ill is complex and multifactorial. Factors that influence these decisions may be personal, social, or financial. Efforts to curtail ill workers' role in food preparation should take these factors into consideration.

P1-100 Application of a Knowledge Transfer Model for Implementation of Government Food Strategies for Innovation, Safety and Quality in the Food Sector

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Introduction: Achieving a sustainable food supply chain is noted as a key priority in strategic plans, policies and programs outlined by the European Union, UK and Welsh Government. A decline in numbers of food technologists in Wales (UK) and critical need to for food sector small and medium-sized enterprises (SMEs) to meet technical demands required for business sustainability resulted in development/application of a model to transfer food science/technology expertise into food sector SMEs in Wales to deliver business needs, enable sustainability and meet Government strategy priorities.

Purpose: Assess the impact of a knowledge transfer model according to Government strategy key priorities with specific reference to increased food science/technology knowledge, food safety management, 3rd party accreditation standards, innovative new-product-development (NPD) and novel processing capabilities in Welsh food-sector SMEs.

Methods: A Knowledge, Innovation, Transfer, Exchange (KITE) Model, based on a collaborative partnership between SME, knowledge partner and affiliate, was implemented in Wales, UK. During 2009-2013 the KITE model was implemented in 32 SMEs involving 45 programs (each lasting

12-24 months). Measured evidence of the impact of the model (cumulative/for individual programs) has been recorded and analyzed according to government key-strategic-performance-indicators related to the food sector.

Results: Embedding HACCP, food safety/quality skills and specialist technical knowledge among food sector SME workforces occurred using a four-phase food safety culture approach: defining training, verification, validation and review stages. Application of the KITE model resulted in SME KITE partners achieving 46 global/retail accreditations (including BRC). Improved food safety/technical capabilities in SMEs resulted in; A) significant market development including development/launch of 632 new products (including application of novel processing technologies); B)improved food safety culture; C) development of sustainable, local supply chains and ecological efficiency; and, D) improved business sustainability with increased sales > £21million (> $33million), safeguarding 649 jobs, creating 69 food technology/quality assurance and 254 manufacturing jobs.

Significance: Application of the KITE model has facilitated improved food safety/technical compliance in food sector SMEs, implementation of innovative processes and NPD. The positive impact of the model has significantly contributed to Government strategies for food sector businesses and has the potential for international application.

P1-101 Infrastructure and Hygiene in the Production of School Food: An Exploratory Study in Indigenous Paxot Communities, Porto Seguro, Bahia, Brazil

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Introduction: Despite being one of the oldest food programs in Brazil, the National School Food Programme (NSFP) still has its weaknesses regarding implementation. Studies, that reflect this perspective, have raised concerns about the safety of food offered and there is very little research on the reality of the programs application, in indigenous communities.

Purpose: Given the high density of the indigenous population in southern Bahia, this study sought to characterize the structural and hygienic conditions in the production of school food within Pataxó communities in Porto Seguro, Bahia, Brazil.

Methods: This is an exploratory study, conducted amongst three villages - Old Village, Jaqueira and Juerana. For data collection, we used a checklist based on Resolution 275/2002 and RDC 216/2004, of the National Agency of Health Surveillance, Ministry of Health. Rates of compliance were then calculated with technical requirements for evaluation dimensions.

Results: The results revealed irregularities among school kitchens in indigenous communities, with regards to the safety of food purchased and supplied by NSFP. Overall in this assessment, non-conformance in school canteens within the villages, Jaqueira, Old Village and Juerana reached 80%, 76% and 71%, respectively. Amongst the dimensions evaluated, structural and environmental conditions in relation to hygiene in food preparation were critical factors in contributing to the low performances recorded. Indicators related to the hygiene of food handlers and levels of care in cleaning procedures were also unsatisfactory.

Significance: The study revealed loopholes in the safety of the production of school meals in the indigenous Pataxó communities, indicating the need for intervention. Measures are required to improve the production of meals and to protect the health of beneficiaries.

P1-102 Monitoring of Hygiene Indicator Microorganisms in Frozen Foods

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Introduction: Frozen food in Korea Food Code is defined as a food made by filling the manufactured, processed, cooked food into container and packaging materials after freezing treatment for the purpose of long-term storage. There are three types of frozen food: (1) Frozen food not requiring heating before consumption, (2) requiring heating before consumption and heated food before freezing, and (3) requiring heating before consumption but not-heated food before freezing.

Purpose: The purpose of this study was to evaluate hygiene indicator organisms and to improve of specifications.

Methods: A total of 128 samples of frozen food were collected from different producers and retailers. We obtained 25 samples of type 1, 49 samples of type 2, and 54 samples of type 3. Aerobic viable bacteria, coliform group and *Escherichia coli* were determined by methods in Korea Food Code.

Results: Aerobic viable bacteria (62.5%) and coliform group (21.9%) in frozen foods were detected at the level of 0.2~6.9 log CFU/g and 0.3~3.3 log CFU/g, respectively. *E. coli* was not detected in all samples.

Significance: The results of this study can be utilized as basic data for improving standards and specifications in frozen foods.

P1-103 Heat and High Hydrostatic Pressure Resistance of *Escherichia coli* Isolated from a Beef Processing Facility

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Developing Scientist Competitor

Introduction: High hydrostatic pressure processing is a promising non-thermal food preservation technology and is used commercially for preservation of meat products. *Escherichia coli* AW 1.7, which was isolated from a beef carcass, is an exceptionally heat resistant organism. Little is known about the heat and pressure resistance of *E. coli* isolated from meat or meat processing facilities.

Purpose: To determine the resistance of *E. coli* AW1.7 and other isolates from beef to heat or pressure. The impact of NaCl on resistance was also determined.

Methods: *E. coli* were grown and heated to 60°C in LB broth with addition of 0 to 4% NaCl. Cultures were treated in LB broth at 400 MPa at 40°C.

Results: Of the 11 strains of *E. coli*, 4 showed a resistance to heat that was comparable to *E. coli* AW1.7, corresponding to a reduction of cell counts of less than 5 log (CFU/ml) after 20 min at 60°C. All 11 strains were as pressure resistant as *E. coli* AW1.7, corresponding to a reduction of cell counts of less than 6 log (CFU/ml) after 15 min at 400 MPa and 40°C. When 2 or 4% NaCl was added to the growth- and treatment medium, after treatment at 60°C for 40 min cell counts of *E. coli* AW1.7 were higher by 3.5 log (CFU/ml) compared to strains grown and treated with no

NaCl added. The heat resistance of all other strains responded similarly with the addition of NaCl. Remarkably, the resistance to pressure was not affected by addition of NaCl.

Significance: Heat resistance of *E. coli* is not correlated to pressure resistance. Strains of *E. coli* that resist 60°C for 5 min or 400 MPa at 40°C for 15 min are part of the natural population that can be isolated from beef processing facilities.

P1-104 Influence of Packaging on Spore Inactivation during Pressure-assisted Thermal Processing

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Developing Scientist Competitor

Introduction: Pressure-assisted thermal processing (PATP) is an emerging technology for sterilizing low-acid foods. Different laboratory groups utilize different packaging types for evaluating microbial safety of food.

Purpose: The objective of this study was to assess the role of different packaging in the inactivation of *Bacillus amyloliquefaciens* TMW 2.479 Fad 82 spores in HEPES buffer (pH 7) during PATP treatment.

Methods: Experiments were conducted using a laboratory scale high pressure unit. Propylene glycol was used as the pressure transmitting fluid. Aliquots (2.25ml) of *B. amyloliquefaciens* spore samples (~2.09 x 10^6 spores/ml) used as a surrogate for *Clostridium botulinum* were packaged into polyethylene pouch, polyethylene tube , and semi-rigid polypropylene cryogenic vial. The spore samples with minimal headspace were pre-heated at 45°C before loading into pressure vessel. The samples were treated at 600 MPa and 105°C from 0 to 20 minutes holding time in triplicate. Processed samples were cooled immediately in ice water before counting survivors.

Results: The computed D-values from linear portion of the survivor curve for *B. amyloliquefaciens* spores treated in pouch, vial, and tube packages were 2.92, 2.09, and 2.83 minutes, respectively. Within the experimental conditions of the study, spores processed in semi-rigid vials had 0.48 to 0.9 log more inactivation ($P < 0.05$) than those processed in pouch and tube in 5, 10 and 20-minute treatments. The difference in spore inactivation could be attributed to uncontrolled experimental variability in different polymer properties. In summary, in comparison to other processing variables (such as pressure, temperature and holding time), the packaging type did not have major contribution to spore reduction during PATP treatment.

Significance: The findings of the study demonstrate that the packaging type may introduce some variability in PATP spore inactivation studies. A more systematic study is needed to determine the relationship between process efficacy and package material, thickness and geometry.

P1-105 Method to Determine Differences in Thermal Tolerance of *Salmonella* Serotypes at Low Water Activity

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Introduction: FSMA requires that facilities identify and evaluate foreseeable biological hazards as well as identify and implement preventive controls to provide assurances that those hazards will be minimized or prevented. To validate processes that may involve low moisture conditions to achieve safety targets it is necessary to identify the most resistant target pathogen.

Purpose: To develop a simple method to study the thermal resistance of *Salmonella* serotypes inoculated in oat flour at low water activity.

Methods: Outbreak and non-outbreak-associated salmonellae (n = 16) were used for the inoculation of oat flour. Dry matrices in Whirlpak bags were inoculated with lawn-grown cells at a 1:1 ratio (~18 g total) and massaged by hand until thoroughly mixed. Inoculum was packed in aluminum crucibles and stored open at 11% relative humidity and ambient temperature (23 ± 2°C). Crucibles were sealed after equilibration was achieved (3 to 6 days). A differential scanning calorimeter (DSC) was used to heat treat crucibles at 90°C for 5, 7 and 10 min and followed by cooling to 20°C for 2 min. Lids were aseptically removed from crucibles and samples diluted in buffered peptone water (BPW), then plated on tryptic soy agar with yeast extract (TSAYE) and incubated aerobically at 37°C for 24 h. Counts were expressed as CFU/g.

Results: Triplicate samples showed little variation (mean SD = 0.2 log). All serotypes exhibited enhanced thermal resistance at lower a_w (~0.2) and linear inactivation behavior from which D-values were calculated that were comparable to published data. *Salmonella* serotypes Tennessee and Agona appeared to be among the most heat resistant with $D_{90°C}$-values of 16.0 and 15.5 min, respectively.

Significance: Results indicate that a DSC can be used to generate reliable thermal kinetic data for *Salmonella* serotypes under low-moisture conditions. This simplified method may allow for rapid identification of the most thermally tolerant pathogens for validation of preventive controls.

P1-106 Development of Thermal Surrogate Cultures for In-plant Validation Studies of Pet Food Products

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Introduction: Outbreaks of *Salmonella* have been associated with dry pet food products. Low-moisture pet food products do not support the growth of *Salmonella*. However, the pathogen can persist for prolonged periods of time in dry products. In-plant HACCP programs commonly identify preconditioning, extrusion, baking and drying processes as kill steps for pet food products. An appropriate surrogate can be used in the plant setting to validate the safety of multi-variable thermal processes.

Purpose: The objective of this study was to determine if a biosafety level 1 *Pediococcus acidilactici* could be utilized as a surrogate microorganism for in-plant thermal processing validation studies for pet food products.

Methods: A generic preservative-free dry pet food product (kibbles) was used to compare the thermal death time kinetics of *P. acidilactici* (ATCC 8042) to that of a cocktail of seven *Salmonella* strains associated with low moisture products. The test product was inoculated with lyophilized cultures and heat treated at 170°F to190°F.

Results: The D-values were determined by least squares linear regression. Test results showed that *P. acidilactici* was slightly more heat resistant than the *Salmonella* cocktail.

Significance: These data suggest that inactivation characteristics (D- and z-values) of *P. acidilactici* could be utilized to predict the response of *Salmonella.*

Poster

P1-107 Application of Network Theory to Microbial Biofilms

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Introduction: 'Sociomicrobiology' has been applied to a number of microbial systems to describe how microorganisms interact and communicate. Biofilms are complex communities that may be characterized in this way. The use of network theory to describe biofilm interactions such as co-aggregation may prove useful in describing and quantifying the structure of such communities and inform control strategies for industry.

Purpose: The aim of this research was to assess the application of network theory to the investigation of microbial biofilm (bacterial biofilm co-aggregation networks).

Methods: Network modeling was performed on data from 2 previously published studies on freshwater biofilm systems. Adjacency and connectivity matrices were initially constructed and the following parameters were calculated: clustering co-efficient; characteristic path length; average degree of graph; links per species; connectedness and diameter of graph. The effect of node loss was also determined.

Results: Both biofilm systems showed comparable path lengths and clustering co-efficients above those of associated random graphs. Biofilm networks contain hub organisms and properties resembling those of other ecological networks. Preferential removal of the most highly connected nodes results in a greater number of secondary node loss than random node removal.

Significance: Network modeling may have applicability to biofilm studies. Co-aggregation information may be useful for the development of control strategies as organisms known to co-aggregate with bacteria within a biofilm, but which are detrimental to the biofilm could be introduced to the community. It also identifies 'hub' organisms essential to biofilm formation which can be preferentially targeted. This work suggests that in biofilm communities all the members are close neighbors and that a disinfection protocol that focused on one particular organism may be unnecessary as perturbation effects have the potential to propagate very quickly through an aquatic biofilm system. A focus on the whole biofilm system may still be a preferable option.

P1-108 The Effect of Sporulation Temperature on the Heat Resistance of *Clostridium botulinum* Type A Spores

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Introduction: Spores produced by proteolytic strains of *Clostridium botulinum* are extremely resistant to high temperatures and thus are the primary food safety risk for thermally processed food products. The temperature at which bacterial endospores are formed is believed to significantly impact the thermal resistance.

Purpose: To determine the effect of sporulation temperature on the heat resistance of *C. botulinum* type A spores.

Methods: Spore crops of *C. botulinum* type A strains 62A and GiorgioA were produced at four different growth temperatures (20, 27, 37 and 41°C) using TPGY broth as the medium. Spore heat resistance was determined by thermal death time (TDT) studies of each spore crop using 105°C (for spores of 62A) and 100°C (for spores of GiorgioA). The D-values were calculated from the negative slope of the survivor curves.

Results: The heat resistance of *C. botulinum* strain 62A spores was greatest when produced at 27°C and decreased for spores produced above or below 27°C ($D_{105°C}$ values for spores produced at 20°C, 1.64 min; 27°C, 4.00 min; 37°C, 3.68 min, and 41°C, 3.49 min; $P < 0.05$). Unlike 62A the heat resistance behavior of GiorgioA spores increased with sporulation temperature with spores formed at optimum growth temperatures of 37°C being the most resistant to temperature ($D_{100°C}$values for spores produced at 20°C, 3.47 min; 27°C, 4.03 min; and 37°C, 5.68 min; $P < 0.05$). Time to sporulation for GiorgioA was shorter and resulted in higher spore concentrations than for 62A at 20°C, 27°C and 37°C, and no spores of GiorgioA were produced at 41°C.

Significance: Sporulation temperature greatly affects the heat resistance of *C. botulinum* spores, and varies by strain. Knowledge of the effect of sporulation temperature on the heat resistance of *C. botulinum* spores is vital for the production of spores utilized in thermal inactivation studies.

P1-109 Reduction in the Enrichment Population of *Listeria monocytogenes* by Competitive Food Spoilage Microflora When Using Buffered *Listeria* Enrichment Broth

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Introduction: The use of molecular-based detection technologies for initially screening food samples for the presence of *Listeria monocytogenes* is becoming increasingly popular. Growth of and competition from non-target background microflora, during selective enrichment, may exert a negative effect on subsequent detection of *L. monocytogenes* when using molecular-based platforms.

Purpose: The purpose of this study was to isolate, identify and quantitate the effects of background microflora capable of growth under conditions used to select for *L. monocytogenes*.

Methods: Dairy products, produce, seafood and grain products were subjected to the U.S. FDA BAM procedure for *L. monocytogenes* enrichment. Standard isolation techniques using non-selective media were then used to isolate microorganisms capable of growth under these conditions. These potential competitive microorganisms were identified using both traditional and molecular-based approaches including 16S rRNA sequencing. Competitor microorganisms were then used with *L. monocytogenes* to perform food product challenge studies.

Results: Four species of the family *Enterobacteriaceae,* including *Citrobacter braakii*, *Enterobacter aerogenes*, *E. cloacae*, *Klebsiella pneumoniae* and *E. coli* were capable of growth under selective conditions typically used for isolating *L. monocytogenes*. In food product challenge studies, the presence of competitors reduced the enrichment populations of *L. monocytogenes* by 3.1 ± 0.3, 2.4 ± 0.7, 3.1 ± 0.7, 2.4 ± 0.9 and 2.6 ± 0.4 log CFU/ml, respectively, compared to enrichments without competitors. This effect did not appear to be dependent on initial competitor levels. One isolate of *L. monocytogenes* was extremely sensitive to the presence of competitor microorganisms. In challenge studies, the presence of *C. braakii* reduced its enrichment population by 6.6 ± 0.6 log CFU/ml.

Significance: The presence of acriflavin- and nalidixic acid-resistant background microflora can reduce the enrichment populations of *L. monocytogenes*. In certain strains the sensitivity to competition could prevent *L. monocytogenes* from reaching minimum threshold levels for some detection platforms resulting in false negative reporting.

P1-110 Citrus Extracts Inhibit Quorum Sensing and Expression of *fla*A-B and *cad*F in *Campylobacter jejuni*

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Developing Scientist Competitor

Introduction: *Campylobacter jejuni* is recognized as one of the most common cause of foodborne bacterial gastroenteritis in humans, which can range from asymptomatic to dysentery type illnesses, with severe complications such as Guillain Barre Syndrome. *fla*A-B and *cad* F genes are involved in motility and invasion which are crucial steps in *C. jejuni* pathogenesis. Quorum sensing (QS) regulates several virulence factors including changes in gene expression. Thus, compounds capable to inhibit QS present a novel target in the development of new antimicrobials.

Purpose: To investigate the effect of citrus extracts in the activity of AI-2 molecules which mediate QS in *C. jejuni* and their effect on the expression of genes involved in motility and invasion.

Methods: Peel, seeds and bagasse of *Citrus limon, C. medica,* and *C. aurantium* were extracted with ethanol. Minimum bactericidal concentrations (MBCs) of citrus extracts against *C. jejuni* were determined by the microplate-dilution method. The effect of extracts (at subinhibitory concentrations, 25%, 50%, and 75% MBC) on AI-2 activity of *C. jejuni* was determined by a bioluminescent method using *V. harveyi* BB170. To measure the relative expression of *fla*A-B and *cad*F, RNA from cultures treated with sub-inhibitory concentration of extracts was obtained, and gene expression was determined by real-time quantitative RT-PCR.

Results: The MBC of the citrus extracts ranged from 1.3 to 3.25 mg/ml. Concentrations lower than the MBC did not affect growth of bacteria, however, AI-2 activity decreased in most cases at levels of 90% (Relative light units). The relative expression of *fla*A-B and *cad*F was significantly ($P \leq 0.5$) reduced or totally inhibited by citrus extracts.

Significance : Extracts from citrus waste are an alternative to control *Campylobacter* by inhibiting growth and virulence factors mediated by QS.

P1-111 Canada Geese as Possible Vectors of Antimicrobial-resistant Bacteria

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Developing Scientist Competitor

Introduction: The development of antimicrobial resistance (AMR) in bacteria constitutes a serious threat to human health, and the use of antibiotics in agricultural production systems is a contributing factor. Wild birds, such as Canada geese (*Branta canadensis*), are highly mobile and frequently found in close proximity to food production animals, suggesting they may serve as a pervasive environmental reservoir of AMR. Currently, the role of wild birds in the spread of AMR to and from food production systems is poorly understood.

Purpose: This study aimed to investigate the occurrence of clinically and agriculturally relevant AMR phenotypes in a synanthropic wild bird population.

Methods: Fecal samples (n = 75) from Canada geese were collected from public parks in Vancouver, Canada (Oct.-Dec. 2012). Samples were screened for a panel of AMR phenotypes including extended-spectrum β-lacatamase (ESBL)-producing *Escherichia coli*, flouroquinolone-resistant *E. coli*, erythromycin-resistant *Enterococcus* spp., and methicillin-resistant *Staphylococcus aureus*(MRSA). Minimum inhibitory concentrations (MICs) were determined by broth dilution assay, resistance determinants were identified by molecular characterization, and isolate relationships were assessed by BOX-PCR.

Results: ESBL-producing *E. coli* were recovered from 22.7% of fecal samples, while 12.0% carried ciprofloxacin-resistant *E. coli*, 6.7% carried erythromycin-resistant *En. faecalis*, 5.3% carried erythromycin-resistant *En. faecium*, and no MRSA was recovered. *E. coli* isolates from 16 samples harbored a CMY-2 β-lactamase gene, three samples possessed TEM, two contained OXA-1 and CTX-M, and four samples carried *E. coli* harboring multiple β-lactamase groups. Highly erythromycin-resistant *Enterococcus* spp. (MIC >128 µg/ml) were identified in nine samples and *E. coli* with ciprofloxacin MIC >16 µg/ml were observed in three samples.

Significance: The presence of enteric bacteria with resistance to clinically important antibiotics in the feces of wild geese poses a public health risk and justifies further study of the transmission of AMR between wild birds, food producing animals, and food production facilities.

P1-112 Distribution and Genetic Diversity of *Salmonella enterica* Isolated from Irrigation Water in the Suwannee River Watershed

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Developing Scientist Competitor

Introduction: *Salmonella enterica* is responsible for approximately 42,000 cases of foodborne infection annually in the U.S. (CDC, 2012). Prior research indicates sustained prevalence of *Salmonella* and unusually high disease incidence associated with the upper Suwannee River Watershed (SRW).

Purpose: This project examined factors that may contribute to the regional contamination of irrigation water by *S. enterica* and characterized strains isolated from this area.

Methods: *S. enterica* levels were determined monthly in irrigation water and sediments from a diverse set of farms (n = 10) within the SRW. Various (n = 21) physical/biological parameters were assessed, including fecal indicators. Molecular typing (DiversiLab rep-PCR) was used to determine the relationship of pond isolates (n = 96) to clinical vs. environmental strains (n > 300). Multiplex PCR was performed to predict serotypes (n = 104), and antibiotic resistance of strains (n = 193) was also evaluated.

Results: To date, all 10 ponds were positive for *Salmonella* from both water and sediment samples. Levels ranged from non-detectable to > 110.0 MPN/100g and frequency from 11.1 to 50%, with some ponds significantly higher than others ($P < 0.05$). Significant correlations between *Salmonella* levels and fecal coliforms ($r = 0.28$, $P < 0.01$) and *E. coli* ($r = 0.27$, $P < 0.01$) were observed, although correlations for any single parameter were all $r < 0.3$. Multiplex PCR patterns corresponded to 10 known serotypes, including Newport, but most (81%) showed no identifiable patterns. Pond isolates clustered into 12 genotypes (based on > 85% similarity by rep-PCR), and more strains (50%) were associated with isolates

from clinical infections than with strains from the Suwannee River (35%), while some strains (15%) were unique to this study. Antibiotic resistance was observed mostly to streptomycin (100%) and kanamycin (10.4%), with 19.7% resistant to two or more antibiotics.

Significance: These data suggest that these ponds harbor a diverse population of *Salmonella,* which may pose potential health risks due to their genetic similarity to strains from clinical origin.

P1-113 Bacteriophages Specific to Human Pathogens from Environmental Water Samples

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Developing Scientist Competitor

Introduction: Understanding bacteriophage diversity in the environment helps in the potential application of bacteriophages as bacteria indicators and in bacterial detection.

Purpose: The purpose of this present study was to isolate bacteriophages specific to *Salmonella* and *E. coli* from water samples in the Metro Detroit area.

Methods: Water samples collected from rivers and waste water treatment plant (WWTP) were centrifuged, filtered, and mixed with indicator strains (*S.* Typhimurium ATCC 13311, *S.* Typhimurium ATCC BAA-712, *S.* Typhimurium MZ 1260, *S.* Typhimurium MZ 1261, *S.* Typhimurium MZ 1262, *S.* Enteritidis MZ 1263, *E. coli* O157:H7 ATCC 700927, *E. coli* ATCC 13706 and ATCC 23631) in double strength Tryptic Soy Broth (TSB). Overnight cultures were centrifuged, filtered, and treated with chloroform. Ten microliters of resultant lysate were spotted to Tryptic Soy Agar (TSA) plates which has soft agar layer containing indicator stains and examined for phages after 18-hr incubation. Lysis- positive supernatants were purified using soft agar overlay technique with indicator strains.

Results: Phages were isolated from 3 out of 35 water samples, including 1 sample from rivers and 2 from waste water treatment plant (WWTP). Two bacteriophage samples were able to infect all 6 *Salmonella* indicator strains and 3 *E. coli* indicators, suggesting a broad host range of bacteriophages.

Significance: Bacteriophages with broad host ranges are prevalent in water and able to infect pathogens of human public health significance. These bacteriophages have potential to be applied to bacterial detection in water.

P1-114 Fate of Indicator Microorganisms on Oranges Following Application of Low Microbial Quality Water in Foliar Sprays

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Introduction: The use of water of poor microbial quality to prepare agrochemicals is a possible route of fruits and vegetables contamination.

Purpose: The aim of this study was to evaluate the fate of foliar applied indicator microorganisms on the surface of grove oranges.

Methods: Three orange trees were sprayed with water of low microbial quality (~10^6 CFU *E. coli*/ml), and three trees served as the controls. Three replicates of 10 pooled oranges each were harvested from each tree; coliforms and *E. coli* were enumerated by plate count and MPN. Populations were determined at 0, 2 and 6 hours after spraying and was continued until *Escherichia coli* could not be detected by enrichment. Field trials were completed with Valencia variety in April, May and June, and with Hamlin variety in October, November and December 2012. Solar radiation, temperature, relative humidity (RH) and rainfall data were obtained.

Results: In April, *E. coli* populations were detected at day 12, but not beyond day 17. In May, *E. coli* populations were undetectable on day 8, detectable on days 10 and 13 and undetectable at day 15. In June, there were significant increases in *E. coli* populations on days 2 and 3, populations were detected on day 16 but became undetectable beyond day 23. In October, *E. coli* populations increased significantly on day 2 and remained detectable up to day 20. In November and December, populations of *E. coli* became undetectable on days 12 and 8 respectively. Fluctuations in RH were correlated with declines in *E. coli* populations. Rainfall and high RH were correlated with the increase in *E. coli* populations at the initial stages of sampling as well as recovery of *E. coli* during the late stages of sampling.

Significance: Low microbial quality water used in foliar sprays may introduce microorganisms to the surface of citrus. Wet weather conditions can extend the survival of indicators on surfaces of oranges.

P1-115 Use of Edible Films Containing Plant Antimicrobials to Inhibit *Pseudomonas fluorescens* in Bagged Organic Lettuce

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Introduction: The storage of fresh produce under refrigerated conditions does not inhibit the growth of psychrotrophic *Pseudomonas fluorescens,* one of the most prevalent types of spoilage microorganisms found on fresh produce. Previously, we found that edible films incorporated with natural antimicrobials can control pathogenic microorganism contamination in bagged organic salads.

Purpose: The objective of this study was to further investigate the antimicrobial effects of carvacrol (the main ingredient in oregano oil widely used as a salad dressing) and cinnamaldehyde (a major food flavoring agent) incorporated apple, carrot and hibiscus-based edible films against *P. fluorescens* in bagged organic lettuce.

Methods: Romaine or iceberg lettuce samples were washed, dip inoculated with *P. fluorescens* (10^7 CFU/ml) and dried. Each sample (10 g) was put into a Ziploc® bag. Edible films pieces (0.3 g) were then added into the bag and mixed well. The bags were sealed and stored at 4°C. Samples were taken at day 0, 3, and 7 for enumeration of survivors.

Results: On romaine and iceberg lettuce, 3% carvacrol films showed the best inhibiting effects against *Pseudomonas*. At day 0, the populations were reduced 2.2-2.3, 2.6-4.3, and 2.3-2.6 log CFU/g by 3% apple, carrot and hibiscus films, respectively. Films with 1.5% carvacrol decreased the population by 1-1.5 log CFU/g at day 7. The 3% cinnamaldehyde films showed 0.7-1.3, and 1.7-3.0 log reductions on romaine and iceberg lettuce, respectively. Films containing 1.5% cinnamaldehyde reduced *Pseudomonas* population by 0.4-1.3 log CFU/g. In general, carvacrol films were more effective than cinnamaldehyde films. Edible films showed better inhibiting effects on iceberg than on romaine lettuce.

Significance: This study demonstrates the potential of edible films incorporated with carvacrol and cinnamaldehyde to inhibit *P. fluorescens* on organic lettuce. These antimicrobial edible films could provide the organic fresh produce industry with additional options to control the spoilage microorganisms and assure quality.

P1-116 Functionality Assessment of *Bacillus* Species Isolated from Iru, Fermented African Locust Bean Seeds

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Developing Scientist Competitor

Introduction: Iru is a popular alkaline fermented soup condiment in West Africa, produced by spontaneous fermentation of African locust bean. The dominance of *Bacillus* species during the fermentation process of locust beans to produce iru has been emphasized. Culturing of this bacterium as potential starter culture can contribute functional traits, including bacteriocin production to prevent growth of pathogenic and spoilage microorganisms for the production of safe food condiments in West Africa.

Purpose: The aim of this study was to evaluate the functional and physiological properties of *Bacillus* species isolated from iru, which can be developed as autochthonous starter cultures for the production of safe, consistent and optimized condiments in West Africa.

Methods: Bacterial strains isolated from 20 iru samples were characterized genotypically, including sequencing of almost complete 16S rRNA genes of representative strains. These strains were tested for functional and physiological parameters, which include antimicrobial production potential, sugar fermentation profile using API kit, anaerobic growth, starch, protein and lipid hydrolysis and substrate adaptability.

Results: The bacterial strains were identified as close relatives of *B. subtilis*, *B. amyloliquefaciens*, *B. cereus*, *B. licheniformis, B. pumilus* and *Brevibacillus formosus* based on 16S rRNA gene sequences. Combined gel fingerprints of genotypic techniques used revealed *B. subtilis* and *B. amyloliquefaciens* as dominant species. The dominant *Bacillus* strains were able to produce antimicrobial compounds, presumably bacteriocin that inhibits pathogenic *Escherichia coli* ATCC 11229 (8.00 ± 2.83 mm), *B. cereus* MTCC 430 (3.67 ± 1.86 mm) and *Staphylococcus aureus* subsp. *aureus* ATCC 11632 (4.00 ± 0.01 mm), grew anaerobically, produced amylase, protease and lipase enzymes and adapted to locust bean substrate.

Significance: The data generated from this study will support the development of *Bacillus* strains as starter cultures that can be adapted in the fermentation process to produce optimized condiments with desirable functional attributes (bio preservative) that will solve the problem of food safety in W. Africa.

P1-117 Rapid Automated Identification of New *Bacillus*, *Geobacillus* and *Paenibacillus* Species with the Updated VITEK® 2 BCL Card Knowledge Base

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Introduction: The VITEK 2 Advanced Colorimetry™ BCL card is intended for use with VITEK 2 Systems for the automated identification of aerobic endospore-forming organisms of the family *Bacillaceae* and *Paenibacillaceae*.

Purpose: The BCL card knowledge base was expanded for the rapid automated identification of an additional 13 *Bacillus, Geobacillus* and *Paenibacillus* species.

Methods: The BCL card knowledge base contains 2517 profiles comprised of 59 species of *Bacillus* and related genera. Strains included isolates from stock cultures and culture collections. Cards were filled with organism suspensions prepared in 0.45% aqueous NaCl to a turbidity equivalent to a McFarland #2 standard. Inoculated cards were incubated for approximately 14 h and a computer-assisted algorithm was optimized and used to generate test and identification results.

Results: Of the 2517 tests in the database, 95.6% gave a correct identification with 14.4% low discrimination results requiring supplemental testing to discriminate between multiple (up to three) choices. An incorrect identification was observed with 3.6% of the isolates and 0.8% were unidentified. Results for the 13 new claims of *Bacillus, Geobacillus* and *Paenibacillus* species showed 97.2% gave a correct identification (including 24.2% low discrimination), 2.6% incorrect identification and 0.2% unidentified.

Significance: The updated VITEK 2 Advanced Colorimetry™ BCL card identified 59 species of *Bacillus* and related genera, including the 13 new species added to the claims.

P1-118 Co-production of Surfactin and a Novel Bacteriocin by *Bacillus subtilis* subsp. subtilis H4 Isolated from Bikalga, an African Alkaline Hibiscus sabdariffa Seeds Fermented Condiment

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Developing Scientist Competitor

Introduction: There is an increased interest in searching for bacteria with new antimicrobial properties. Many studies have reported that the predominant *Bacillus* spp. isolated from African fermented food products such as *Soumbala*, *Netetu*, *Kinema*, and *Maari* exhibit potent antimicrobial effects toward harmful bacteria and fungi.

Purpose: The purpose of this study was to screen strains of *B. subtilis* subsp. *subtilis* (six isolates) and *B. licheniformis* (four isolates), representing the predominant *Bacillus* spp. during *Bikalga* (*Hibiscus sabdariffa* fermented seeds) fermentation for the production of antimicrobial substances and to characterize the antimicrobial compound(s) produced by a selected *B. subtilis* subsp. *subtilis* isolate.

Methods: Antimicrobial activity of the strains was determined by agar spot and well assay. Antimicrobial substance produced by the selected isolate was tested for sensitivity to heat, pH, enzymes and chemicals. Direct detection of antimicrobial activity was carried out by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and the inhibitory compounds were identified by PCR and Ultra High Performance Liquid Chromatography-Time of Flight Mass Spectrometry (UHPLC-TOFMS).

Results: Cell-free supernatants of three *B. subtilis* subsp. *subtilis* (G2, H4 and F1) showed the best activity being inhibitory to *Listeria monocytogenes*, *Micrococcus luteus*, *Staphylococcus aureus* and *Bacillus cereus*. The antimicrobial substance produced by *B. subtilis* subsp. *subtilis* H4 was further characterized showing sensitivity to protease, trypsin, α-amylase, lipase II and mercapto-ethanol. PCR detection of *yiwB*, *sboA*, *spoX*, *albA* and *spaS*, *etnS*genes and genes coding for surfactins and plipastatins indicated a potential for subtilosin, subtilin and lipopeptide production, respectively. UHPLC-TOFMS analysis of the single band associated with antimicrobial activity in SDS-PAGE allowed identification of surfactin and a protein with a monoisotopic mass of 3346.59 Da, which is dissimilar in size to subtilosin and subtilin.

Poster

Significance: These results suggest that H4 in addition to surfactin, may produce a novel bacteriocin which may be relevant in food applications.

P1-119 Analysis of the Microbial Population of *Makgeolli* (Traditional Korean Rice Wine) during the Wine's Fermentation Period

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Developing Scientist Competitor

Introduction: *Makgeolli* has a unique taste and flavor due to fermentation, with many types of microorganisms being involved in the fermentation of the wine. Filamentous fungi hydrolyze the starch to glucose and yeasts convert the glucose to alcohol. Carbohydrate-metabolizing lactic acid bacteria (LAB) are also present in the beverage. It is important to understand the fermentation-specific microbial consortia present in *Makgeolli* for improving the wine's quality and extending its shelf life.

Purpose: Changes in the microbial communities of bacteria and fungi were monitored during the entire fermentation period of *Makgeolli* (12 days).

Methods: We brewed *Makgeolli* in our laboratory. Samples were collected every 2 days during the fermentation period, and chemical indexes such as pH, alcohol content, and reducing-sugar levels were measured. The microbial community was analyzed using barcoded pyrosequencing with bacteria- and fungi-specific primers targeting the genes encoding 16S rRNA (bacteria) and 28S rRNA (fungi). In all, 46,986 sequence reads for bacteria and 17,945 sequence reads for fungi were used for the microbial analysis.

Results: During the fermentation process, LAB belonging to the genera *Pediococcus*, *Weissella*, and *Lactobacillus* dominated the microbial population from day 4 until the end of fermentation, and *Saccharomyces* dominated significantly during the entire fermentation period. The pH was 5.12 at the beginning of fermentation and gradually decreased until the end stage. Alcohol content increased rapidly from day 2 and remained constant from day 6, while the reducing-sugar content increased on day 1, but decreased dramatically on day 2.

Significance: The changes in chemical indexes correlate with the changes in the microbial population during *Makgeolli* fermentation. These results suggest that the microbial population influences the quality of *Makgeolli*.

P1-120 Assessment of Cellular Immune Crosstalk of Lysogenic *Salmonella* Typhimurium with Chicken Macrophage

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Introduction: The increased prevalence of antibiotic-resistant pathogenic bacteria has led to renewed attention to the use of bacteriophage as a potential antibacterial bioagent. Bacteriophage is a promising approach for the prevention and treatment of infectious diseases due to its effectiveness against antibiotic-resistant pathogens, specificity to the target pathogen, cost effectiveness to develop therapeutic system, and no serious side effects on eukaryotic host cells. However, there have been relatively few studies on the potential role of lysogens in reducing colonization of invading pathogens underlying the cellular and molecular immune responses.

Purpose: The objective of this study was to understand the role of lysogenic and lytic phages in the pathogenesis of intracellular *Salmonella enterica* serovar Typhimurium in chicken macrophages as measured by bacterial invasiveness and cellular immune response.

Methods: The intracellular survival and invasive ability of nonlysogenic, lysogenic, and dual *S.* Typhimurium strains were evaluated at 37°C for 24 h post-infection (h p.i.) in bacteriophage P22-post-treated and pre-treated HD-11 cells. The production of inflammatory factors (NO, TNF-α, IL-1β, IL-8, IL-6, and IL-10) was determined in the *S.* Typhimurium-infected HD-11 grown in the absence and presence of P22 at 1 and 24 h p. i. by using ELISA kits.

Results: The numbers of viable intracellular nonlysogenic, lysogenic, and dual *Salmonella* Typhimurium strains were significantly decreased to 8.1%, 31.8%, and 7.7%, respectively, in the infected HD11 cells grown with P22 for 24 h. The invading ability of *S.* Typhimurium strains into HD11 cells was effectively reduced by the pretreated-P22 at 1 h p.i. (> 2 log). The production of NO, TNF-α, IL-8, IL-6, and IL-10 was increased in HD11 cells in the presence of P22. The reduction of intracellular *S.* Typhimurium was highly correlated with the enhanced production of nitric oxide (NO) ($r = 0.816$, $P < 0.001$).

Significance: These results suggest that the application of lysogenic bacteria and lytic phages could potentially be an effective way to control the survival of intracellular pathogen.

P1-121 Temperature Adaptation Alters *Salmonella* Enteritidis Heat/Acid Resistance and Stress/Virulence Related Gene Expression

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Introduction: *Salmonella* Enteritidis is one of the leading causes of foodborne disease in the world. As a typical mesophile, *S.* Enteritidis can grow in a wide range of temperatures. Although it has been suggested that environmental stresses can activate the self-defense system of pathogens and thus help them adapt and become more resistant and virulent, little is known about the responses of *S.* Enteritidis under different temperatures.

Purpose: This study aimed to investigate the acid/heat resistance and stress/virulence related gene expression of *S.* Enteritidis after adaptation to cold or heat conditions.

Methods: *S.* Enteritidis was cultivated at 10, 25, 37, and 42°C. Adapted cells were subject to simulated gastric fluid (pH 2.0) and thermal treatment (54, 56, 58, and 60°C) to determine their acid/heat resistance. *D*-values were calculated based on survivor curves. The transcription levels of stress-related genes (*rpoS* and *rpoH*) and virulence-related genes (*spvR*, *hilA*, *sefA*, and *avrA*) of adapted cells were evaluated by real-time PCR. Mean values were compared using ANOVA.

Results: Adaptation of cells at lower or higher temperatures than 37°C did not increase their acid resistance; while adaptation to higher temperatures significantly ($P < 0.05$) increased their heat resistance. For instance, $D_{60°C}$-values of cells adapted at 10, 25, 37, and 42°C were 0.3, 0.5, 1.0 and 1.4 min, respectively. The *rpoS* gene expression was 16.5- and 14.4-fold higher in cells cultivated at 10 and 25°C, respectively; while the *rpoH* gene expression was 2.9-fold higher in those adapted at 42°C. Virulence related genes-*spvR*, *hilA*, *avrA*-were significantly induced by 6.4-, 5.0-, 2.6-fold in cells cultivated at 42°C, except *sefA* gene which was induced in cells adapted at 37°C.

Significance: These data suggest that environmental temperature may have a significant impact on the heat resistance and virulence potential of *S.* Enteritidis, thus temperature should be well controlled during food storage.

P1-122 Contact Dependent Growth Inhibition of *Escherichia coli* O157:H7 by EC869 CDI System

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Introduction: Contact Dependent Growth Inhibition (CDI) is a recently discovered mechanism that microorganisms use to compete in various microecosystems. CDI systems express large cell surface exposed CdiA proteins with potent antimicrobial peptide tips. Many CDI systems also contain additional downstream orphan toxins targeting susceptible bacterial cells through direct cellular contact via CdiA binding. Genomic analyses indicated *Escherichia coli* O157:H7 strain EC869 encoded a CDI system with 11 potential orphan toxin sequences.

Purpose: In this study we examine the functionality of the EC869 CDI system in competing against other O157:H7 strains.

Methods: EC869 and target O157:H7 strain (EDL933) were co-inoculated in 10% TSB in a continuous culturing system with a volume displacement rate of 30%/h. Both EC869 and the target strain were enumerated daily by plating to determine the growth in the mixed culture. A K12 strain expressing a plasmid-encoded CdiA chimera with the EC869 orphan 11 toxic tip was examined similarly.

Results: Co-culturing with EC869 resulted in strong growth inhibition of the targeted O157:H7 strain. After 24 h of co-culturing, the presentation of the targeted O157:H7 in the mixed culture was reduced from 50% to 11%, and to less than 2% after 48 h incubation. Similar inhibition of growth was observed when a K12 strains was targeted. *E. coli* K12 strain carrying the cloned chimera CDI system strongly inhibited the growth of K12 target strains, reducing the growth of K12 target strain by more than 3 log units after 2 h exposure. However, no inhibition to the growth of O157:H7 target strain was observed, suggesting different orphan peptides may be required for the growth inhibition of O157:H7.

Significance: The CDI system of EC869 confers the host strain significant advantage in competition with other microorganisms, including other E. coli O157:H7 strains. The CDI system could be further explored for "curing" O157:H7 from the animal reservoir.

P1-123 Role of Egg-yolk Antibody (EYA) in Protection against Bacterial Association in *Salmonella*-poultry Cell Interactions

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Introduction: *Salmonella enterica* serovars Enteritidis (SE) and Typhimurium (ST) are the most common serovars isolated from egg and poultry products. To meet the growing organic poultry sector and to address antibiotic resistance in conventional poultry, alternative antimicrobial strategies are essential. Works demonstrate egg-yolk antibodies (EYA) immunogenic properties against enteric pathogens but their role in decreasing colonization of SE and ST in poultry has not been demonstrated clearly.

Purpose: The purpose of this study was to investigate the ability of the water-soluble fraction (WSF) of egg-yolks containing EYAs to decrease the association of SE and ST to chicken cells culture model.

Methods: A chicken fibroblast, DF-1, and chicken macrophage-like cell line, HD-11, were used in this study. Semiconfluent monolayers of cells were treated with four different WSFs at various concentrations. *Salmonella* suspensions were added for infection. Monolayers were washed and associated bacteria were enumerated after serial dilution.

Results: Western blot analysis revealed non-specific binding of the WSF with bacterial cell lyses of ST and SE. Association of ST in DF-1 depended on the application of WSF. For example in DF-1 cells, untreated log CFU were 5.86 ± 0.02 vs. 4.5 ± 0.33 ($P < 0.0001$). In HD-11, application of the various WSF increased and decreased association, depending on WSF applied; untreated log CFU were 3.86 ± 0.22 vs. 3.05 ± 0.03 vs. 4.52 ± 0.16 ($P < 0.0001$). Reduction was also nuanced in SE infection; in DF-1 cells, untreated log CFU were 5.60 ± 0.01 vs. 4.03 ± 0.20 ($P < 0.0001$). Similar HD-11 results were also found, where untreated log CFU were 3.86 ± 0.22 vs. 2.97 ± 0.09 vs. 4.38 ± 0.09 ($P < 0.0001$, $P = .0011$)

Significance: These findings demonstrate the need for research identifying necessary adhesins for *Salmonella* infection in chickens. While EYA application may be viable, development of encapsulation methods may enhance efficacy of the treatment.

P1-124 Extending the Shelf Life and Eliminating *Salmonella* spp. and *Escherichia coli* in Tahini-based Dressing

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Introduction: Tahini dressing is a popular Middle Eastern product prepared from sesame-paste (Tahini), water and citric acid. It has a short shelf life of 2 days at room temperature and 5 days when refrigerated, with high risk of *Salmonella* spp. contamination. Developing effective preservation means with minimal heat treatment is essential due to the high fat and low moisture content of tahini.

Purpose: Investigate a preservation method to extend the shelf-life of tahini dressing stored at 4° and 25°C and eliminate tolerant species of *Salmonella* and *Escherichia coli* while maintaining good organoleptic properties.

Methods: Samples were thermally treated at 85°C/15 minutes or 110°C/7 minutes, stored at 4° and 25°C, then analyzed in duplicates at days 0, 7, 14, 28 and 62 for total aerobic counts, *E. coli*, coliforms, yeasts and molds. Another batch was thermally treated at 85°C/15 min and inoculated with 10^3 CFU/ml of *Salmonella* spp. and *E. coli* strains isolated from tahini. Citrox (2%), sodium benzoate (5 mg/ml) and potassium sorbate (5 mg/ml) were individually added. Samples were stored at 25°C and analyzed for surviving bacteria, peroxidase value, a_w and pH at days 0, 7, 14, 30, 45, and 60.

Results: The shelf-life of tahini dressing was extended to 2 months with both thermal treatments stored at 4°C and one month with milder treatment stored at 25°C. Higher thermal treatment negatively affected the texture. Sodium benzoate and potassium sorbate completely eliminated *Salmonella* spp. and *E. coli,* whereas Citrox was ineffective. PV, a_w and pH remained stable with treatments and storage.

Significance: Mild heat treatment combined with lower storage temperature and sodium benzoate and potassium sorbate extended the shelf life of the dressing extensively, inhibited contaminating pathogens while maintaining product quality. This allows better local and international marketability and more confidence in a widely consumed dressing.

Poster

P1-125 Asian Food Safety Trends: Examining the CDC Data in the United States from 1990-2008

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Introduction: Asian food is one of the top three ethnic cuisines in the United States. However, limited food safety information is available about this cuisine.

Purpose: We analyzed CDC foodborne illness outbreak data from 1990 through 2008 in order to examine food safety trends associated with Asian foods.

Methods: We obtained foodborne illness data from the CDC, and, when possible cross-checked this data with other published reports. The data were processed and ranked based on numbers of outbreaks, cases per outbreak, etiology, outbreak locations, and outbreak vehicles.

Results: Out of 17,640 total foodborne disease outbreaks in the U.S. during this time, 1,529 (8.7 %) were associated with the three most popular ethnic food categories (Italian, Mexican, and Asian). Asian foods represented approximately 20.6% of outbreaks (315 outbreaks) and 9.6% of cases (3,529 cases) among total foodborne disease outbreaks for these three types of ethnic foods. During the 18-year period under study, fewer cases per outbreak occurred for Asian cuisine than occurred for the other two most popular ethnic cuisines (Italian and Mexican). The majority (60%) of outbreaks from Asian foods occurred in restaurants and delicatessens, and of those, 62.2% were of unknown etiology. Among the known etiologies, bacterial agents were the most prevalent (77%), followed by viruses (18%) and chemicals and toxins (5%). Bacterial etiologies in foodborne illness outbreaks associated with Asian foods included *Bacillus cereus* (45%), *Salmonella* spp. (33%), *Staphylococcus* spp. (16%), *Clostridium* spp. (3%), *Campylobacter* spp., *Vibrio parahaemolyticus*, and *Plesiomonas shigelloides* (1%). Asian foods most frequently associated with illness were Asian-style cooked, fried, steamed, or dark rice (40%), sushi (15%), noodle and lo mein dishes (8%), and eggrolls and spring rolls (7%).

Significance: This epidemiological analysis suggests contains further examination of special issues concerning ingredients, preparation, cooking, serving, and handling of Asian foods.

P1-126 Estimating the Burden of Foodborne Illness in Japan Using Clinical Laboratory Data for Whole of Japan, 2006–2010

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Introduction: In Japan, under the Food Sanitation Law, the numbers of food poisoning cases must be reported; however, these do not exactly reflect the real burden of foodborne illness due to the passive surveillance nature. We have been estimating the real burden of diarrheal diseases for *Campylobacter*, *Salmonella* and *Vibrio parahaemolyticus* in Miyagi Prefecture, Japan.

Purpose: The purpose of this study was to estimate the burden of foodborne illnesses associated with the three pathogens for whole of Japan, using data also covering other prefectures.

Methods: Data on laboratory-confirmed infections of the 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 2010. The stool submission rate and the physician consultation rate were estimated from two population telephone surveys conducted in Miyagi prefecture. Each estimate was introduced into a Monte-Carlo simulation model as a probability distribution, which was run for 10,000 iterations.

Results: The physician consultation rate and the stool submission rate of the diarrheal cases were estimated from the combined telephone survey data as 32% and 11%, respectively. The estimated mean numbers per year of foodborne illnesses for *Campylobacter*, *Salmonella* and *V. parahaemolyticus* in whole of Japan were approximately 3.4-4.7 million, 690-800 thousand, and 50-150 thousand during 2006-2010, respectively. The numbers of reported foodborne illnesses per year in Japan during 2006-2010, for *Campylobacter*, *Salmonella* and *V. parahaemolyticus*, were approximately 2,100-3,100, 1,500-3,600 and 170-1,300, respectively.

Significance: These data reveal a significant difference between our estimate of burden of foodborne illness and the reported foodborne disease cases associated with the three pathogens. Need for active surveillance system to complement the present passive surveillance is strongly suggested, in order to identify and prioritize food safety issue more precisely and to monitor the effectiveness of risk management options.

P1-127 Prevalence and Characterization of Shiga Toxin-producing *Escherichia coli* in a Pasture-based Cow-calf Production System

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Introduction: *Escherichia coli* are bacteria commonly found in the digestive tract of cattle; most are harmless, but those producing Shiga toxins (STEC) can cause human illness. Ingestion of raw or undercooked contaminated beef is a prominent source of infection.

Purpose: To assess role of bloodline, maturity, and physical proximity of cattle in presence and distribution of STEC.

Methods: Angus cows (n = 90) and their calves (n = 90) were maintained in paddocks in groups of 7 or 8 pairs. Fecal samples were collected *per rectum* at 3 time points in July, August, and September, and enriched for STEC. Screening for virulence genes *stx1*, *stx2*, *eaeA*, and *hlyA* was done using multiplex polymerase chain reaction (mPCR). *Stx*+ fecal samples were cultured on MacConkey Agar, and a maximum of 6 colonies from each sample were randomly picked. Determination of serogroups O26, O45, O91, O103, O111, O113, O121, O145, and O157 was performed for each *stx*+ colony using mPCR. Fingerprinting of *stx*+ colonies was done through repetitive sequence-based PCR. Comparison of STEC distribution across time points, maturity and paddocks was done using Pearson Chi-Square or Fisher's exact tests with Monte Carlo simulations.

Results: STEC prevalence was 93.3% (84/90) in adults, and 95.6% (86/90) in calves. Preliminary results show that calves were less likely to be *stx*+ in August ($P < 0.001$). Differences were present between paddocks for *stx*1 in July ($P = 0.027$) and September ($P = 0.011$), and *eae*A in August ($P = 0.026$). *Stx*+ *E. coli* colonies were obtained for 49.4% (68/170) of animals with *stx*+ fecal samples. Of 744 *E. coli* colonies, 335 were positive for at least one virulence gene, with 28 being positive for two virulence genes, 21 for three, and 1 for all four. Fingerprinting of *stx*+ colonies identified limited clustering of isolates by paddocks, but not by bloodlines.

Significance: Results show high prevalence of *stx*-positive animals in the herd. The role of current physical proximity (paddock) seems to be more important than bloodline in the establishment of dominant STEC populations. Animals within bloodline do not share STEC populations.

Disclaimer: Virginia Tech Institutional Animal Care and Use Committee has reviewed and granted approval to this project #09-147-FST.

Poster

P1-128 Multiple-Locus Variable-Number Tandem Repeat Analysis for Strain Discrimination of Non-O157 Shiga Toxin-Producing *Escherichia coli*

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Introduction: Non-O157 Shiga toxin-producing *Escherichia coli* (STEC) are emerging pathogens of growing concern worldwide. Rapid and sensitive strain discrimination methods are critical for quick identification of outbreaks and fast traceback to the contamination source.

Purpose: The objective of this study was to develop a multiple-locus variable-number tandem repeat (VNTR) analysis (MLVA) assay for intra- and inter-serogroup discrimination of 6 major non-O157 STEC serogroups: O26, O111, O103, O121, O45, and O145.

Methods: Twelve VNTR loci were selected, amplified in 3 multiplex PCR reactions, and the amplicons were sized using multicolor capillary electrophoresis. Validation of the developed MLVA method was conducted using 84 clinical isolates of non-O157 STEC.

Results: Sixty-five unique MLVA types were obtained among the 84 clinical non-O157 STEC isolates comprised of geographically diverse sporadic and outbreak related strains. Four of the 6 serogroups clustered separately in a minimum spanning tree. The developed MLVA method allowed a higher level of discrimination among serogroups O26, O111, O103, and O121, with discriminatory powers of 1.0, 0.96. 0.97, and 0.97, respectively, than it did for serogroups O45 and O145, with discriminatory powers of 0.90 and 0.82, respectively. The overall discriminatory power of the MLVA method for 84 non-O157 STEC isolates was 0.99. Compared to pulsed-field gel electrophoresis (PFGE), a higher level of discrimination was observed for serogroup O26.

Significance: The developed non-O157 STEC MLVA method allows inter-and intra-serogroup strain discrimination among 6 major serogroups of non-O157 STEC and provides a discriminatory power similar to slightly better than PFGE.

P1-129 Characterization of Shiga Toxin-producing *Escherichia coli* O5 Strains Received at CDC from 2011–2012

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Introduction: Laboratory surveillance for STEC is essential for identifying clusters to detect outbreaks and for monitoring trends to assess the impact of interventions and policy. Unusual phenotypic traits have the potential to negatively impact surveillance systems. Shiga toxin-producing *Escherichia coli* (STEC) are typically urease negative; however, urease-producing STEC O5 strains were recently reported in Austria.

Purpose: The purpose of this study was to characterize STEC O5 strains from the United States for their virulence genes, phenotypic markers, and genetic similarity.

Methods: STEC O5 isolates submitted to CDC by state and county public health laboratories were tested by PCR for Shiga toxins 1 and 2 (stx_1 and stx_2), intimin (*eae*) and enterohemolysin (*ehxA*) and serotyped. All isolates were inoculated to urea agar slants (Christensen's) using an 18-24 hour culture. The slants were incubated at 35-37°C and examined at 24 hours, 48 hours, and 7 days for the development of an intense pink-red color on the slant, indicating urease activity. Genetic variation among isolates was assessed by PFGE analysis.

Results: During the past two years, CDC received 75 STEC O5 strains that were all non-motile from 29 states. The majority of these, 70/75 (93%), hydrolyzed urea within 24 hours, and an additional strain within 48 hours, while the remaining strains showed no activity within 7 days. 73 strains (97.3%) were positive by PCR for stx_1, *eae*, and *ehxA* genes, while two strains (2.7%) possessed the genes for stx_1, stx_2, and *ehxA* and one strain (1.3%) was positive for stx_1, stx_2, *eae*, and *ehxA*. By PFGE analysis, 57 strains were separable into 48 unique *Xba*I patterns.

Significance: STEC strains typically lack urease activity. These findings demonstrate the urelytic nature of a subset of genetically diverse STEC strains. Continued surveillance is important to detect phenotypic variants that may impact identification schemes.

P1-130 Shiga Toxin-producing *Escherichia coli* in the United States Reported through PulseNet USA and the National *Escherichia coli* Reference Laboratory from 2006–2012

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Introduction: Laboratory surveillance for Shiga toxin-producing *Escherichia coli* (STEC) is essential for identifying clusters to detect outbreaks and for monitoring trends to assess the impact of interventions and policy.

Purpose: Laboratory findings from PulseNet and the National *Escherichia coli* Reference Laboratory (NRL) were combined to develop a comprehensive profile of STEC strains isolated from humans between 2006 and 2012 in the US.

Methods: Data in PulseNet were used to determine the number of isolates and virulence profiles for STEC O157, O26, O103, O111, O45, O121 and O145, and data from NRL were used to determine these values for all other STEC serogroups. Shiga toxin profiles were determined by EIA or PCR assays and *eae* and *ehxA* genes were determined by PCR assays.

Results: A total of 29,869 STEC strains were isolated and characterized during the study period. Ninety-one percent (27,201) expressed or were indistinguishable by PFGE to known patterns for one of the following O antigens: O157 (18,391), O26 (2,767), O103 (2,390), O111 (1,842), O45 (359), O121 (620) and O145 (501), while 9% (2668) expressed one of 125 other O antigens. The percentages of strains in the common O groups that were positive for stx_1/stx_2/stx_1+stx_2, respectively, were as follows: O157: 2/56/42; O26: 94/2/4; O103: 97/<1/2; O111: 70/1/29; O45: 95/<1/4; O121: 5/94/1; O145: 30/54/16. Over 99% and 94% of these strains were positive for *eae* and *ehxA*, respectively. Among strains from the less common serogroups, stx_2 alone or in combination with stx_1 was present in 38% of strains, and 53% and 77% of strains were positive for *eae* and *ehxA*, respectively.

Significance: Greater than 90% of STEC causing human illness likely belong to 7 serogroups. A small percentage (~3%) of diverse strains with the potential to cause severe disease falls outside these groups. Continued surveillance is important to monitor changes in STEC prevalence.

P1-131 Evidence of Non-O157 Shiga Toxin-producing *Escherichia coli* in the Feces of Meat Goats at a U.S. Slaughter Plant

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are important human pathogens and attention to non-O157 serogroups has increased in recent years. Although cattle are normally considered the primary reservoir for STEC, recent illnesses associated with goat contact has demonstrated the importance of these animals as reservoirs for the organisms. However, the prevalence of STEC, including non-O157 serotypes, in U.S. goats has not been well described.

Purpose: Our objectives were to determine the prevalence of six major non-O157 STEC serogroups in the feces of meat goats at slaughter in the southeastern U.S.

Methods: Rectal contents from 296 goats were collected post-evisceration over nine days between August and October. Samples were enriched in *Escherichia coli* broth and incubated for six hours at 40°C. After enrichment, DNA was extracted and used as template in an 11-gene multiplex PCR that detects major non-O157 serogroups (O26, O45, O103, O121, O111, and O145) and virulence genes. Samples were considered positive when at least one non-O157 STEC serogroup was present with either *stx*1 or *stx*2, the Shiga toxin-producing genes.

Results: All six non-O157 serogroups were detected by PCR in our samples. Prevalence of O26 was highest with 6.4% of rectal contents positive. The prevalence of O45 was 3.4%, O103 was 4.4%, O111 was 4.1%, O121 was 1.4% and O145 was 3.0%. Twenty-two of 296 samples (7.4%) had more than one non-O157 serogroup present. One sample had three non-O157 STEC serogroups. Twenty-four samples (8.1%) were PCR positive for a non-O157 serogroup, but lacked *stx*1 or *stx*2.

Significance: Goats appear to be an important reservoir for non-O157 STEC and further work to understand the characteristics, epidemiology and ecology of STEC in these animals is warranted.

P1-132 Analysis of the Intimin Gene from Sporadic and Outbreak-associated Shiga Toxin-producing *Escherichia coli* (STEC) O26, O45, O103, O111, O121, O145 and O157 from the United States

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Introduction: STECs belonging to seven serogroups (O26, O45, O103, O111, O121, O145 and O157) are considered adulterants by FSIS. Strains from these serogroups have the ability to attach to and efface intestinal epithelial cells due to the presence of the intimin (eae) protein.

Purpose: The purpose of the study was to examine the *eae* gene in a collection of STEC strains from humans to identify informative regions to detect, subtype and predict the serogroup to facilitate the response to public health events caused by these STECs.

Methods: We examined full length *eae* sequences extracted from the whole genome sequences of over 100 sporadic and outbreak-associated STEC isolates from serogroups O26, O45, O103, O111, O121, O145 and O157. As controls we included 18 *eae* sequences from other *Escherichia coli* and *E. albertii* deposited at NCBI. Meta-data and *eae* sequences from these serogroups were visualized and analyzed using Geneious and CLC genomics software to detect 1) conserved regions for broad detection of *eae*-positive strains 2) fixed SNPs for prediction of serogroup and 3) variable SNPs for discrimination between outbreak and non-outbreak isolates.

Results: Among the strains tested, conserved regions within the first two-thirds of the *eae* gene were identified that could detect all *eae*-positive STEC, and fixed SNPs in the latter third of the gene were identified that could predict the serogroup. No outbreak-specific SNPs were identified among the strains tested.

Significance: This study is an initial step toward the development of highly discriminatory genotyping assays for use in future detection or subtyping strategies. Improved diagnostic and tracking methods will directly impact our ability to identify and control outbreaks of existing and emerging virulent clones of this pathogen in a timely fashion.

P1-133 Assessment of Shiga Toxin-producing *Escherichia coli* (STEC) Survival and Shiga Toxin Stability in Enrichment Broths

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) strains are important foodborne pathogens capable of causing severe, life-threatening disease. The testing of enrichment broths for Shiga toxins or the genes encoding these toxins is a sensitive method for diagnosing STEC infections. Since laboratories vary in their procedures for handling and storing broths, we compared different storage temperatures for their effects on STEC detection and isolation.

Purpose: To guide the development of recommendations for handling enrichment broths, we assessed the effect of storage temperatures on the survival of STEC in these samples.

Methods: Aliquots of a master stool sample spiked ~10^5 CFU/ml with one of 20 STEC strains (O157:H7/NM(6), O26:H11(2), O111:H8(2), O121:H19(2), O145:NM(2), O103:H2(2), O69:H11, O79:H7 and O165:NM) were used to prepare replicate stool enrichment broths. Half of the enrichment broths were stored at ambient temperature (25°C), while the other half were stored in the refrigerator (3-5°C). Broths were subcultured to either CT-SMAC (O157 STEC) or washed sheep blood agar with mitomycin C (non-O157 STEC) at days O-5, 14, 21, 28 and 56 and tested for STEC by EIA and PCR. The results for each strain on days 1 through 56 were compared to those obtained initially for the different storage conditions.

Results: Over the period of the study, there was no apparent difference in survival of the 20 strains in broths stored at 4°C and 25°C as assessed by EIA and PCR. There were differences in the number of target to non-target organisms in the broths stored under the different conditions. Target organisms in broths stored at 25°C were more likely to be overgrown by day 14 (17/20) compared to broths stored at 4°C (7/20).

Significance: The success of public health surveillance systems for identifying and controlling outbreaks is dependent upon STEC isolation. While STEC can survive in stool enrichment broths for extended periods at 4°C and 25°C, their isolation is less encumbered by competing flora when stored at 4°C.

P1-134 Prevalence of *Clostridium difficile* in Canadian Rivers and Fate during Waste Water Treatment

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Introduction: *Clostridium difficile* is a Gram-positive, anaerobic, spore-forming bacterium that is an important cause of enteric disease in humans and some animal species. While traditionally considered primarily a hospital- and antimicrobial-associated pathogen, there has been an increase in community-associated *C. difficile* infection (CA-CDI) in humans internationally, for reasons that are unclear. Source of exposure outside of the hospital has not been adequately investigated, but concern has been raised about foodborne or environmental sources.

Purpose: Determine the prevalence and compare the toxigenic and genotypic profile of *C. difficile* encountered in rivers and waste water sources within Southern Ontario; investigate the survival of *C. difficile* during anaerobic sludge digestion.

Methods: Samples from rivers and waste water treatment plants (WWTPs) were collected and enriched, *C. difficile* was isolated. Molecular characterization of toxin A, toxin B, binary toxin, and sequencing of *tcdC* were undertaken to determine the toxigenic profile of the isolates, PCR ribotyping, toxinotyping and pulsed field gel electrophoresis (PFGE) were employed to detect the genotypes of the isolates.

Results: *C. difficile* was found in 39.1% (25/64) river samples and 93.0% (119/128) sludge samples; 23 (92.0%) of river isolates and 95 (79.8%) from sludge isolates were toxigenic; 69.6% (16/23) toxigenic sediment isolates could be classed as ribotype 078 and toxinotype V, and 13 were NAP7 while 3 were NAP 8, 16.8% (16/95) toxigenic sludge isolates were ribotype 078, toxinotype V, and NAP7. The 118 toxigenic *C. difficile* isolates were grouped into 41 different PCR ribotypes, 12 toxinotypes and 57 PFGE types. Anaerobic sludge digestion did not alter *C. difficile* levels or ribotypes.

Significance: Toxigenic *C. difficile* are present in raw sewage but do not proliferate during the digestion process. The effluent released from WWTPs add to the environmental burden of *C. difficile* although how this contributes to the incidence of CA-CDI remains unclear.

P1-135 Are *Campylobacter* Cases Low Risk for Public Health Follow-up?

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Introduction: The high volume of gastroenteric cases reported to Toronto Public Health (TPH) requires disease investigations of foodborne agents be prioritized through a risk-based system. Cases of diseases linked to outbreaks are followed-up with a telephone call. A passive letter-based system with a questionnaire is used to collect information from cases deemed 'low risk', but is not returned by 40% of cases.

Purpose: This study investigated the reasons why cases of one of the most common low-risk gastroenteric diseases, *Campylobacter,* did not return their questionnaires and whether there were missed opportunities for public health interventions.

Methods: Clients reported between April 3 and November 5, 2012 with a confirmed *Campylobacter* illness and who did not respond within 30 days after a questionnaire was mailed to them were included. These non-respondents were telephoned, to ask whether they remembered receiving a letter from TPH, the reason why they did not respond, what would have encouraged a response and whether they worked in a sensitive occupation.

Results: A total of 226 *Campylobacter* cases had not responded during the study period. Of these, 172 (76.1%) were reached by phone and 162 (71.7%) agreed to participate. The age and gender distribution of participants did not differ from cases who responded to the original letter. The largest proportion (54.4%) of participants reported they had forgotten to return the questionnaire. When asked what would have encouraged a response, more information on the importance of returning the questionnaire (19.1%), followed by a simpler form (15.9%) were the most common answers. Only 3 (2.4%) cases were employed in sensitive occupations; none reported working while ill.

Significance: This study validated treating *Campylobacter* reports as "low-risk" for public health follow-up. Data collected suggest a reminder, simplifying the questionnaire, and underscoring the importance of responding could increase the response rate for letter-based investigations.

P1-136 Effective Evidence-based Food Safety Materials for Restaurant Food Handlers: Interventions Designed to Target Knowledge Gaps

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Introduction: Foodborne outbreaks are commonly associated with restaurants.

Purpose: This study determined the effectiveness of a brochure versus a story-based comic book intervention targeting knowledge gaps in restaurant food handlers.

Methods: From June 2009 through February 2011 and September 2010 through March 2012, respectively, 729 food handlers from 211 participating restaurants in Lake, Kane and Suburban Cook Counties and 88 food handlers from 16 restaurants in DuPage County were interviewed in English or Spanish to determine baseline knowledge and identify which knowledge items were least known. At randomly selected intervention restaurants, an educational brochure or comic book, developed based on the knowledge gaps identified from the baseline survey, was distributed. Restaurant managers were requested to actively encourage their staff to read the materials. Thirty-five restaurants (85 food handlers) from the comic book group, 36 restaurants (54 food handlers) from the brochure intervention and 27 restaurants (56 food handlers) from the control group read the materials and participated in the follow-up knowledge survey.

Results: The mean food safety knowledge score increased from baseline by 9.6% for the comic book group and 6.3% for the brochure group ($P < 0.0001$, respectively) but did not increase for the control group ($P = 0.84$). A mean score increase was observed among the non-certified food handlers in the intervention groups also (comic 10.5% increase, $P < 0.0001$; brochure 5.0% increase, $P = 0.0007$; control, $P = 0.78$). Knowledge regarding the proper minimum internal temperature to cook chicken increased 27% in both intervention groups ($P < 0.0001$). One hundred percent of the certified food managers in the intervention groups said that these educational materials should be provided to restaurants.

Significance: These data demonstrate significant food safety knowledge increases among restaurant food handlers from targeted educational material with a relatively higher efficacy for a comic book format. Positive feedback regarding the materials suggests acceptability of this food safety training method.

P1-137 Outbreaks Associated with Antibiotic-resistant Foodborne Pathogens

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Introduction: Antibiotic-resistant foodborne pathogens are a natural consequence of antibiotic usage in food animals. Sixty-five percent of antibiotics that are sold for use in animal agriculture are similar or identical to antibiotics sold for use in human medicine, and whenever an antibiotic is used, its efficacy diminishes as certain bacterium, under selective pressure, will go on to survive and proliferate. When people consume food items that are contaminated with antibiotic-resistant pathogens, they develop infections that are more likely to lead to hospitalization and an increased risk of death. Keeping track of antibiotic-resistant foodborne illness outbreaks is a critical first step in understanding overall disease burden.

Purpose: Limited information exists on outbreaks of foodborne illness due to antibiotic-resistant bacteria. CSPI has developed a database to document the link between food products and associated human cases.

Methods: Outbreaks were compiled based on comprehensive literature review. The outbreak database catalogues illnesses, hospitalization, and death, based on etiology, state, food source (when available), food commodity category, antibiotic-resistance profile, WHO and FDA classifications of antimicrobial pharmaceuticals.

Results: A total of 55 outbreaks, in which bacterial infections were resistant to at least one antimicrobial agent, have been identified between 1973 and 2012, causing 20,571 documented illnesses, 3155 hospitalizations, and 27 deaths. Outbreaks from associated food commodity categories include: beef (causing 10 outbreaks), dairy (13), eggs (1), multi-ingredient foods (3), poultry (6), pork (1), produce (3), seafood (2), and unknown items (13). *Salmonella* spp. were identified as causative agents in 48 of 55 outbreaks, with *S.* Typhimurium implicated in 19 and *S.* Newport 15 outbreaks. *E. coli* caused 5 outbreaks, and *Campylobacter jejuni* and *Staphylococcus aureus* were each responsible for 1 outbreak. Responsible bacteria displayed resistance to a total of 15 different antibiotics and to at least one sulfonamide.

Significance: Prevention efforts are needed to combat the problem of antibiotic-resistant foodborne illnesses. These illnesses are more likely to cause longer hospital stays, and an increase in risk of death. Database development and analysis provides public health officials with the evidence to construct more effective mitigation efforts.

P1-138 The Epidemiology of Yersiniosis in Illinois and Missouri, 2005–2011

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Introduction: Few population-based epidemiological studies of yersiniosis have been performed. FoodNet recently reported a 70% reduction in incidence of yersiniosis during 1996-2009 in its 10 active surveillance states.

Purpose: To describe the recent epidemiology of yersiniosis in two non-FoodNet Midwestern states.

Methods: Yersiniosis is reportable in Illinois and Missouri. Culture-confirmed cases of *Yersinia enterocolitica* infection reported to the state health departments during 2005-2011 were analyzed to examine demographic characteristics, seasonality, and annual incidence (calculated per 100,000 population using US census data).

Results: Two hundred and twenty confirmed yersiniosis cases were reported, (160 from Illinois, 60 from Missouri). The average annual incidence was 0.17 cases (Illinois 0.18, Missouri 0.14) compared to 0.3 for FoodNet states. An overall decline in incidence was not observed ($P = 0.63$). Among the 179 cases with race information, 92 (51%) were White, 72 (40%) Black, 9 (5%) Asian and 7 (4%) other races. The average annual incidence was highest among Blacks (0.40) compared to Asians (0.19) and Whites (0.09). Seventy-one percent of the cases among Blacks occurred during November to January. No seasonality was observed in other races although 18% of cases occurred in March among Whites. Incidence was higher among children < 5 years of age (1.27) compared to persons > 64 years (0.25) and 5-64 years (0.07). Black children <5 years of age had the highest annual incidence rates (4.42). In Illinois, children < 5 years were more likely to have a history of chitterlings being served at home within 7 days before illness onset compared to persons older than 64 years (39% vs 0%, OR=∞, 95% CI=1.46-∞).

Significance: A higher incidence and a winter seasonal trend among Blacks were consistent findings with recent FoodNet published data. However, no decrease in incidence was observed in Illinois and Missouri. Additional prevention efforts are needed to reduce the incidence of this foodborne disease.

P1-139 Development of Epidemiological Investigation System Using Mobile Lab in Korea

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Introduction: Effective control of foodborne outbreaks is very important to public health, and it is necessary for early detection and identification of foodborne pathogens and immediate removal of the suspected food from the market, accompanied by preventing secondary spread.

Purpose: To ensure preventing foodborne disease and operating early warning system, KFDA established the mobile laboratory which is designed for on-site testing of 16 foodborne pathogens.

Methods: The mobile laboratory is equipped with the instruments including a DNA extraction system, Real-time PCR and reagents for DNA assay. In order to analyze foodborne pathogens in foods and feces, the PowerCheKTM 19 Pathogen Multiplex Real-time PCR Kit (developed by KogeneBiotech Co., Ltd.) has been used. The assay was carried out to detect and identify 19 specific genes of 16 pathogens, simultaneously, by using multiplex primer and probe system labeled with different fluorephores.

Results: The KFDA Mobile Lab has successfully performed on-site testing during the "2011 World Nuclear Summit" and the "Yeosu EXPO 2012" etc. A total 838 food samples were tested and food pathogens such as *Vibrio parahaemolyticus* and *Listeria monocytogenes* were detected from 11 samples. Also, it has confirmed the cause of outbreak occurring in 8 school caterings immediately and prevented the possible spread of additional foodborne outbreak in August, 2012.

Significance: The KFDA Mobile Lab can simplify on-site food testing and be applied to efficient monitoring of the outbreak of foodborne pathogen. These applications will provide early warning against the outbreak and assist food safety and regulatory system for the compliance of the relevant rules and regulations.

P1-140 A Prevalence Study Investigating the Bacteriological Hazard Associated with Live Oysters on Retail Sale in Toronto, Canada

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Introduction: Raw, live oysters are available from various retail locations in the city of Toronto, Canada, and are often consumed raw. Because of the geographical location of the city, oysters are transported over long distances from a variety of different locations. A microbiological survey of raw, live oysters sold from retail establishments in Toronto, Canada, was undertaken.

Purpose: The purpose of the study was to determine whether or not raw, live oysters sold in Toronto posed a bacteriological hazard to consumers.

Methods: Seven hundred and fifty oysters were collected from randomly selected retail locations across the city of Toronto. The oysters sampled came from various locations. Samples were examined for aerobic colony count, *Vibrio* species and *Escherichia coli*. The method for *Vibrio* species was based upon a standard method published by the UK Health Protection Agency. The *E. coli* method was based upon the International Organization for Standardization method. Aerobic colony count was carried out using a method that used plate count agar and incubation at 37°C. Biochemical tests were used to confirm identification.

Results: The results showed that none of the oysters were contaminated with *E. coli*, and the aerobic colony counts were all within acceptable limits. *V. alginolyticus* was isolated in 20% of the samples. Furthermore, *Pasteurella pneumotropica* and *Pasteurella multocida* were each isolated in 8% of samples.

Significance: It was concluded that the live oysters sold at retail in Toronto, Canada, were of relatively good microbiological quality and that none of the isolated bacterial species posed a major gastrointestinal threat to consumers, indicating that raw, live oyster consumption in Toronto is relatively safe from a bacteriological perspective.

P1-141 Prevalence of Norovirus, Hepatitis A Virus, Hepatitis E Virus, and Rotavirus in Shellfish in South Korea

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Introduction: The importance of foodborne viral infection is increasing worldwide because of the enormous economic and social loss of viral food poisoning. Foodborne viruses such as norovirus (NoV), hepatitis A virus (HAV), hepatitis E virus (HEV), Rotavirus (RoV) are transmitted through water and food contaminated with stool.

Purpose: The purpose of this study was to examine the prevalence of foodborne viruses in shellfish collected in South Korea using real-time RT-PCR.

Methods: The 152 shellfish samples (Oyster, Manila clam, and mussel) were obtained from the food suppliers for school meals and purchased at the local market in Kyounggi area from January, 2011 to November, 2011. Virus was eluted from the stomach and the digestive diverticula of shellfish using glycine and threonine buffer and concentrated with polyethylene glycol.

Results: The detection rate of NoV genogroup I, NoV genogroup II, HAV, HEV, and RoV were 22%, 6%, 1%, 0%, and 0% of shellfish, respectively. Although geographic distribution of NoV was statistically significant, the seasonal variation of NoV was not prominent in this study.

Significance: Although the viability of foodborne viruses was not confirmed with the limitation of real-time RT-PCR and lacking of cultivation technique, monitoring data against foodborne viruses in shellfish may contribute to prevent the viral food poisoning in public health.

P1-142 Evaluation of a New *Salmonella* Detection Method for Seafood

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Introduction: Thailand's exports of fresh, chilled and frozen seafood products are approximately 570,000 metric tons, 50% more than a decade ago. The Fish Inspection and Quality Control Division (FIQD) of the Thailand Department of Fisheries (DOF) provides certification and inspection of pre-export seafood products and, to respond to the growing market needs, has prioritized the qualification of simpler and shorter methods for rapid *Salmonella* detection.

Purpose: To evaluate the performance of a new *Salmonella* detection method through comparison with a cultural method for local seafood matrices, including [1] Spiked Raw Shrimp; [2] Spiked Raw Cephalopod; [3] Spiked Processed Fish; [4] Raw Shrimp; [5] Raw Cephalopod; & [6] Raw Fish.

Methods: The evaluation included pure-culture inclusivity and exclusivity testing. Food testing was performed using 141 samples comparing the performance of a molecular method and a reference cultural method. Sixty samples were spiked with a low level (1-10 CFU) of *Salmonella*: Raw shrimp (20 samples); Raw Cephalopod (20 samples); and Processed Fish (20 samples). Eighty-one samples were tested with natural contamination: Raw Shrimp (41 samples); Raw Cephalopod (20 samples); and Raw Fish (20 samples). After a shared enrichment, all samples were analyzed by two ***Methods:*** (a) 3M Molecular Detection Assay *Salmonella*; and (b) ISO 6579. Presumptive positive molecular results were culturally and biochemically confirmed following ISO 6579 procedures.

Results: The molecular method yielded inclusivity and exclusivity rates of 100%. For the 141 samples tested, the molecular method showed 100% sensitivity, 100% specificity, 100% accuracy and no significant difference from the reference method using the chi-square statistic.

Significance: For testing artificially and naturally contaminated seafood matrices from Thailand, the 3M Molecular Detection Assay *Salmonella* was found to be reliable and accurate, and to offer advantages to laboratories, including faster time to results.

P1-143 Detection of Norovirus in American Oyster (*Crassostrea virginica*) along Louisiana Gulf Coast Using Two Real-time RT-PCR Assays

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Developing Scientist Competitor

Introduction: Noroviruses (*Caliciviridae*) are the leading cause of viral gastroenteritis in the U.S. Norovirus gastroenteritis are generally linked with genogroups GI and GII, predominated by GII. About 13% of foodborne outbreaks are associated with the consumption of contaminated molluscan shellfish, in which low levels of contamination are occasionally found.

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Purpose: The purpose of this study was to detect norovirus in the American Oyster (*Crassostrea virginica*) using two real-time rt-qPCR methods, and compare the virus recovery rates.

Methods: Live oysters from commercial harvesting areas along Louisiana Gulf Coast were obtained on a biweekly basis. About 5 g of digestive diverticula homogenate from 10-12 oysters was digested in PBS (100 µg/ml proteinase-K). The extracted RNA was analyzed using two real-time RT-PCR assays (A and B), established by others. In response to a norovirus outbreak at Cameron Parish, LA (January, 2013) samples from the suspected contaminated area and a stool sample from an infected individual were analyzed. The virus recovery was determined by spiking oyster homogenate with a 20% aliquot of norovirus stock prepared from a GII positive stool specimen (7.5 log (genomic copies)/ml).

Results: No norovirus was detected in any of the oyster samples (n = 8). The stool sample of the infected individual was positive for GII. Analyzing the serial dilution of the stock showed both methods were linear from the range of 2.2 to 3.9 log (genomic copies)/µl of PCR reaction (R^2 = 0.989 and 0.997, $P > 0.05$) with PCR efficiencies of 80% and 92% for methods A and B, respectively. Low and variable recoveries were found for methods A and B (4.4 ± 1.7 and 11.3 ± 2.8 percent, respectively; $P < 0.05$).

Significance: Low recoveries were obtained that was probably due to matrix effect of the samples, and indicates the need for improving concentration methods for detecting low copy numbers of norovirus in the oysters.

P1-144 Predictive Modeling for the Thermo-ultrasound Inactivation of *Vibrio parahaemolyticus* in Shrimp during Post-harvest Washing Process

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Introduction: *Vibrio parahaemolyticus* has been identified as an important causative agent of human gastroenteritis in seafood such as shrimp. Ultrasound and mild heat treatment are effective intervention strategies to decontaminate *V. parahaemolyticus* in shrimps. However, there is limited information on their combined application to control *V. parahaemolyticus* in shrimps.

Purpose: The study aimed to develop a predictive model to evaluate the combined effect of ultrasound and mild heat on reducing *V. parahaeomolyticus*in shrimps during the postharvest washing process.

Methods: Raw shrimps inoculated with *V. parahaeomolyticus* were treated with ultrasound (96, 150, and 205 W) combined with mild heat (47, 50, and 53°C) based on a 3 × 3 full factorial design and the bacterial survival curves were fitted with a Weibull model. The time for a 4-log CFU/g of bacterial reduction (t_{4d}) was calculated based on parameter estimates of the Weibull model to evaluate the inactivation rates and a response surface model was developed to describe the changes of t_{4d} as a function of temperature and ultrasonic power. The model was validated with independent experiments within the model range.

Results: The results indicated that the models were significant ($P < 0.05$) with high adjusted coefficients of determination (R^2-*adj* = 0.89-0.98). The response surface model showed that t_{4d} was reduced when temperature and ultrasonic power increased and there was a significant positive interaction effect ($P < 0.01$) between the two factors, indicating thermo-ultrasound treatments accelerated the inactivation of *V. parahaemolyticus*. Bias factor (B_f = 1.02) and accuracy factor (A_f = 1.09) calculated based on the validation data indicated a satisfied performance of the model.

Significance: The developed predictive model could be used to design appropriate regimens of ultrasound and mild heat treatment to achieve desirable *V. parahaeomolyticus* reduction in shrimps.

P1-145 The Effects of Storage Temperature on the Prevalence of *Vibrio parahaemolyticus* and Physical and Sensory Properties of Oysters

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Introduction: During harvesting and storage, natural spoilage flora and microbial pathogens may grow affecting the composition and texture of oysters, thereby posing a two-fold problem: inferior product quality and a potential health threat to susceptible consumers. A solution to these problems would mitigate their associated negative effects on the seafood industry.

Purpose: The purpose of this study was to investigate the effects of different storage temperatures on the microbial prevalence, sensory and textural characteristics of post-harvest shellstock Eastern oysters (*Crassostrea virginica*).

Methods: Oysters harvested from the Chesapeake Bay, Maryland, during the summer months (June, July, and August, 2010) were subjected to different storage temperatures (5, 10, and 20°C) over a period of 10 days. At selected time intervals (0, 1, 3, 7, 10 days) two separate samples of 6 oysters each were homogenated and analyzed for pH, halophilic plate counts (HPC), total *Vibrio*, and *Vibrio parahaemolyticus*. Sensory evaluation was performed for acceptability, appearance and odor of oyster meats shucked after storage at the test temperatures and times. Texture analysis was performed using a texture analyzer on meats shucked from oysters held under the same conditions as for sensory.

Results: The pH of the oyster homogenate showed no consistent pattern with storage time and temperature. The HPC (4.5 to 9.4 log CFU/g) were highest on day 10 while olfactory acceptance reduced with time and increasing storage temperatures. The *V. parahaemolyticus* counts increased over time from 3.5 to7.5 MPN/g by day 10. Loss of freshness as judged by appearance and odor was significant over time ($P < 0.05$). Toughness of oysters increased with storage time at 5 and 10°C from day 1 to day 3, but was inconsistent after day 7.

Significance: The results indicate that the length of storage and temperature had a significant effect on bacterial counts and olfactory acceptance of oysters but had an inconsistent effect on texture.

P1-146 Identification of Type Three Secretion System-2 Effectors of *Vibrio parahaemolyticus*

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Introduction: Gastroenteritis produced by *V. parahaemolyticus* (Vp) is attributed to production of thermostable direct (TDH), TDH-related hemolysins (TRH), and the inflammatory response produced by effector proteins (EP) secreted by a type III secretion system (T3SS-2) contained within pathogenicity islands (PI). However, few T3SS-2 EPs have been identified and the cumulative effects of the different virulence factors are poorly understood.

Purpose: The goal of this project was to identify T3SS-2 EPs involved in the enterotoxigenicity of Vp and to determine their distribution and relationship to other virulence markers.

Methods: *In silico* analysis was performed on sequenced T3SS-2 PI regions, for which two variants have been reported, α and β. Fourteen putative EP encoding genes were identified, many with an α and β allele. PCR primers were designed to identify alleles, if present. Serotyping of

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the O and K antigens was performed using commercial antisera. A total of 186 Vp strains, which included clinical and environmental isolates, were analyzed for all targets, and for urease activity (UA).

Results: Among O3:K6 or UT:K6 clinical strains that only possessed *tdhA*, only α-EP gene targets were present. An environmental variant of this PI type was observed in O5 strains. Additionally, some strains possessed UA in the absence of*trh*. For strains which possessed *trh*, with or without *tdhA*, only the β alleles of EP genes were present (T3SS-2β PI), except for two adjacent α-allele EP genes, which was observed in some strains. A variant of T3SS-2β PI which had EP gene β-alleles and UA in the absence of *tdhA* and *trh* was observed in some O1, O3, O5, and O8 strains.

***Significance*:** These results suggest that T3SS-2 PI variants exist. Further, the synergistic contributions of T3SS-2 EPs with other virulence markers needs to be investigated, *in vivo*, to understand the enterotoxigenicity of *V. parahaemolyticus*.

P1-147 Application of Chitosan Microparticles for the Reduction of *Vibrio* Species

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Introduction: *Vibrios* are Gram-negative bacteria that commonly occur in costal estuarine environments. *V. vulnificus* (Vv), *V. parahaemolyticus* (Vp), and *V. cholerae* (Vc) are the principle pathogens and cause at least 75% bacterial seafood-borne disease in the U.S. Consumption of undercooked seafood, especially raw oysters, can result in a severe systemic vibriosis. The primary response by the seafood industry to reduce *Vibrios* relies on the implementation of postharvest processing (PHP), including thermal treatments and high hydrostatic pressure. Although effective, currently approved PHP will also kill the oysters and is not suitable for the live "half shell" market.

Purpose: This study investigated the efficacy of chitosan microparticles (CM) for reduction of Vv, Vp and Vc both *in vitro* and in live oysters.

Methods: The three *Vibrio* species were inoculated (ca. 10^4 CFU/ml) into nutrient medium with different concentrations of CM (0, 0.1, 0.3, and 0.5%) to determine effects on growth over 12 h. Artificial seawater (ASW) was also inoculated (ca. 10^7 CFU/ml) to examine vibrio survival over 48 h when exposed to the above concentrations of CM. Live oysters maintained in ASW (16ppt) at room temperature and were similarly exposed to CM for 48 h. All samples were processed, serially diluted and spread plated to culture media to enumerate viable bacteria.

Results: Growth of all species was completely inhibited within 3 hours of treatment with 0.3% and 0.5% CM. Furthermore, treatment with 0.5% CM successfully reduced all species by 7.5 log CFU/ml in ASW by 48h. Vv was the most sensitive to CM treatment, while Vc was the most resistant. Numbers of *Vibrios* also significantly declined ($P = 0.012$) in treated oyster samples (0.1, 0.3 and 0.5% CM) compared with untreated controls.

Significance: These results show that CM treatment might be an effective postharvest process to reduce spoilage or pathogenic microbial risks in seafood industry.

P1-148 An Investigation into the Temperature Variation during Hot Holding of Cooked Mussels in Domestic Coolers

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Introduction: It is relatively common during outdoor events in the province of Prince Edward Island, Canada for commercial vendors to store cooked mussels in domestic coolers to keep the product hot before sale. Provincial legislation requires that all hot products should be maintained at a minimum temperature of 60°C**.**

Purpose: A study was conducted to ascertain how long hot, cooked mussels could be stored for in domestic coolers before the temperature decreased to below 60°C.

Methods: Mussels were cooked using guidance published by Health Canada, then placed into domestic coolers and the temperature measured at five different locations every ten minutes for up to four hours. Three experiments were conducted; in the first the cooler was not opened, in the second the cooler was opened for one minute every ten minutes and in the third the cooler was opened for one minute every ten minutes and some mussels removed. Each experiment was conducted in triplicate.

Results: It was found that when the cooler was left unopened, the mean temperature dropped to < 60°C in 56 minutes. For coolers opened every 10 minutes the mean temperature decreased to < 60°C in 38 minutes. For coolers opened every ten minutes and mussels removed, the mean temperature decreased to < 60°C in 52 minutes. For all three experiments, the temperature dropped into the "danger zone" for microbial growth within the four hour experimental period.

Significance: It was clear from the results that this method of storing mussels for commercial sale is not appropriate. It was concluded that mussels should not be stored under these conditions for > 30 minutes and temperature should be regularly monitored to ensure it stays within legislated limits.

P1-149 Evaluation of the 3M™ Molecular Detection System for the Detection of *Listeria* spp. in Seafood Processing Plant Environments in Thailand

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Introduction: *Listeria* contamination in processing plant environments is a major issue for the seafood industry in Thailand; faster and more reliable results are therefore desired for early detection and monitoring of environmental *Listeria* spp. The 3M™ Molecular Detection System (MDS) uniquely utilizes isothermal DNA amplification and bioluminescence to detect *Listeria* spp. significantly faster than the traditional culture method.

Purpose: This study evaluated the MDS *Listeria* for its ability to detect *Listeria* spp. in environmental samples from seafood processing plants.

Methods: Duplicate environmental sponge samples were collected from 222 different sites within three seafood processing plants in Southern Thailand. Each duplicate set of sponges was screened for *Listeria* spp. following two ***Methods:*** (1) MDS method after 26 h and 48 h of enrichment; (2) FDA-BAM method with minor modifications. Additionally, cultures of three *Listeria* spp. (*L. monocytogenes*, *L. innocua*, and *L. ivanovii*) were separately spiked onto plastic and stainless steel surfaces at a low level (1-5 CFU). Sponge samples (n=36) from these surfaces were analyzed following the MDS method.

Results: Detection of *Listeria* spp. by the two methods did not differ significantly ($P < 0.05$). Among 222 samples, 11 and 13 samples were positive for *Listeria* spp. by the MDS and FDA-BAM method, respectively. Five samples tested positive by the MDS only and seven samples tested positive by the FDA-BAM only. The MDS was able to detect as low as 4 CFU of *Listeria* spp. in sponge samples. Overall, the sensitivity of the MDS

was 87.0% (95% CI: 77.4-96.6%), specificity was 97.6% (95% CI: 95.5-99.7%), accuracy was 95.3%, and the positive predictive value was 89.4% (95% CI: 80.5-98.2%).

Significance: The MSD *Listeria* provides rapid and reliable detection and monitoring of *Listeria* spp. that may be a source of product cross-contamination in seafood plant environments.

P1-150 Inactivation of *Listeria innocua* on Raw and Read-to-eat Shrimp by Antimicrobial Coatings and Cryogenic Freezing

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Introduction: Seafood, when adjusted for per capita consumption, causes more incidences of foodborne illness in the United States than meat or produce. Shrimp have been associated with those outbreaks linking to the contamination of *Listeria monocytogenes*. Therefore, effective intervention technologies are needed to inactivate or inhibit growth of the pathogen on shrimp post-processing and during storage.

Purpose: Antimicrobial coatings alone, or in combination with cryogenic freezing, were evaluated for their ability to inhibit the growth of *Listeria innocua,* a surrogate for *Listeria monocytogenes,* on whole raw and ready-to-eat (RTE) shrimp.

Methods: Shrimp were inoculated with *L. innocua* and treated with antimicrobial coatings and cryogenic freezing (-75°C, 2 min), used alone or in combination. The coating solutions contained chitosan (20 mg/ml), allyl isothiocyanate (AIT, 60 µl/ml), or lauric arginate ester (LAE, 50 µl/ml. The treated shrimp were stored at -18°C for 6 days before being thawed at 4, 10 or 22°C for either 24 or 48 h and the survival of inoculated *L. innocua* and natural bacteria on shrimp was investigated.

Results: Results revealed that antimicrobial coating treatments or cryogenic freezing used alone reduced the natural bacteria or *L. innocua* on raw shrimp by less than 2 log CFU/g, while antimicrobial coatings achieved *ca.* 5.5 to 1 log CFU/g reduction of *L. innocua* on RTE shrimp, depending on inoculated population levels. Chitosan+AIT coating in combination with cryogenic freezing achieved more than 5-log reduction of *L. innocua* and natural bacteria on raw shrimp. The coating-treated RTE shrimp had significantly ($P < 0.05$) less *L. innocua* than controls at each thawing temperature and time.

Significance: This study demonstrates that the hurdle technology is an effective approach to reducing microbial loads on shrimp surfaces, which may provide processors or distributors with viable options for designing non-thermal interventions to improve the microbiological safety and shelf life of shrimp.

P1-151 Pre-treatments Effects of Aluminium and Other Mineral Levels in Semi-dried Jellyfish Products

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Introduction: Jellyfish is a high valued seafood product among the Asian food markets. Consumers appreciate the unique texture of those products and treat them as a delicate dish in a banquet setting. Since the products are processed with heavy salt and alum, the residues of aluminum level in ready-to-eat jellyfish dishes may exceed the daily intake allowance. The excess aluminum residue in food products has recently caused health concerns in China and other Asian countries. How to reduce aluminum residue in the food products becomes a timely issue.

Purpose: This study determined aluminum and other mineral levels in existing processed jellyfish products commercially available in the markets in China and the United States, in order to evaluate the effectiveness of fresh water or vinegar treatment prior to serving for reduction of aluminum residue in ready-to-eat forms.

Methods: Both jellyfish products produced in both China and the United States were collected from supermarkets and manufacturers, respectively. All the jellyfish products are currently produced using different phases of brining techniques with 24% salt and 5% potassium aluminum sulfate addition. After slicing, the processed jellyfish products were treated with either fresh water or vinegar solution for 2, 4, 8, and 24 h. Aluminum levels were determined by using ICP as well as HPLC methods. Other minerals, including sodium, potassium, calcium, magnesium, iron and zinc, were also determined by using ICP technique. All data collected were analyzed using ANOVA.

Results: The commercial jellyfish products produced in both the U.S. and China contain 70% moisture, 5% protein and 24% slat. The production techniques are similar. They all used brining solutions with alum. The tested jellyfish samples treated with 3% vinegar showed a significant reduction of aluminum levels from 350 ppm to 195 ppm; while those treated with fresh water only reduced to 225 ppm. For sodium, potassium and magnesium, immersing in both vinegar and fresh water showed a significant reduction, while iron and zinc were not showing any significant changes. Since the composition of jellyfish is collagen, further study on the relationship among minerals and collagen is needed.

Significance: The vinegar treatment reduced aluminum residue more than fresh water treatment did. However, the texture of vinegar treated samples may be comprised. Optimizing the treatment with accept sensory texture will be the next study topic for ensuring a safe and healthy jellyfish product.

P1-152 The Commerce and Quality of Fish Trading: A Study in the Municipal Market of São Francisco Do Conde, Bahia, Brasil

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Introduction: In many coastal cities of Bahia, Brazil, there is a predominant artisanal fishing trade, which tends to be informally conducted and can result in concerns about product safety and conservation.

Purpose: This study aimed to characterize the trade and the quality of fish sold at the municipal market of São Francisco do Conde, Bahia

Methods: An exploratory study was carried out using quantitative methods including questionnaires from six vendors and obtaining microbiological and physical chemistry samples of 72 fresh and processed fish, comprising of: 12 sea bass (*Dicentrarchus* labrax), 12 mullet (*Mugil brasiliensis*), 12 shrimp (*Litopenaeus schmitti*), 12 oysters (*Crassotea rhizophorae*), 12 mussels (*Mytella ssp*) and 12 crabs (*Callinectes spp*).

Results: The respondents had a mean age of 38.3 years, most of whom had not completed the equivalent of a first grade high school education (83.3%), with a monthly income range of between one to three times that of the minimum wage, all of whom declared themselves as heads of households. With regards to trade, there was poor infrastructure and poor building maintenance in the market area, with hygiene inadequacies associated to the market environment, equipment and utensils. Among the samples taken, there were non-compliant rates of 41.6% (30) for *Escherichia coli* and 26.6% (19) for coagulase positive staphylococci, *Salmonella* spp was not identified. Overall, half (36) of the samples analyzed, failed to

meet microbiological standards. As for the presence of physicochemical properties, there was a non-compliance of 52.7% for pH levels and 27.7% for total volatile bases. Considering these distinct patterns, 76.3% (55) of the samples were classified as non-compliant.

Significance: The results highlight health-related concerns in the selling of fish, signifying the need for intervention in order to support the supply chain and reinforce food and nutrition security at local levels.

P1-153 Identification of Fish Samples through DNA Barcoding: A Surveillance Study of Public Health Importance

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Introduction: The DNA barcoding has been widely successful in species identification in a wide range of zoonotic species, including fish. It uses the 5' region of the mitochondrial cytochrome oxidase I (COI) gene as the genetic marker. The primary mission of FDA is to warrant a safe food supply chain and correctly label food and food products. This task is indeed very challenging as over 80% of seafood consumed in the United States is imported, and there are over 28, 000 known species of fish.

Purpose: The major objective of this study was to evaluate and validate the DNA barcoding method that can be used in our laboratory, for rapid species identification of fish samples from routine surveillance, foodborne outbreak, and in species substitution and economic fraud investigations.

Methods: In this study, we standardized the protocols for DNA extraction, and COI gene based PCR amplification and bi-directional nucleotide sequencing of the standard barcode region (655 bp). Initially, we carried out a validation study using 32 samples belonging to 4 known fish species. Later, we successfully performed the DNA barcoding for 64 surveillance import samples of 12 fish species collected from Argentina, Brazil, China, Norway, United Kingdom, and Vietnam. Data was analyzed using the GENEIOUS program. The analytical tools on BOLD and GenBank were also utilized to assess the performance of barcoding to identify species.

Results: High quality bases (>98%HQ-100%HQ) were obtained for all the samples sequenced. Using neighbor-joining distance comparison, the genetic distance was measured and the phylogenetic tree was constructed. The generated COI nucleotide sequences provided a complete species-identification to the fish samples analyzed.

Significance: The results suggest that DNA barcoding is a very effective method for species identification of fishes. This will further help in accomplishing the mission of our agency.

P1-154 The Effect of Heat and Various Salts on the Activity of the Inosinate Monophosphate-degrading Enzyme in Horse Mackerel

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Introduction: Horse mackerel is a popular fish consumed worldwide and is often salted or dried for long-term storage. Maintenance of the quality of stored fish necessitates the suppression of inosinate monophosphate (IMP) degradation. Since IMP is degraded by inosinate phosphatase (IMPase), the preservation technology of mackerel can be improved by elucidating the property of IMPase.

Purpose: This study aimed to clarify the changes in IMPase activity in horse mackerel under varying heat, salt, and pH conditions.

Methods: Fish flesh homogenate was dialyzed against water for 2.5 days, and the dialysate was filtered and diluted twice at 10°C (enzyme solution). The standard reaction mixture included buffer (0.2M succinic acid/NaOH pH 4-6 and 0.2M maleic acid/0.2M Tris/NaOH pH 6-8), 25 mM IMP, 10% of various salts (NaCl, $MgCl_2$, $CaCl_2$, or $MgSO_4$) or water, and enzyme solution. The reaction mixture was incubated overnight after heating for 2 h at 45°C or without the heat treatment. The reaction was stopped using 10% perchloric acid, and the amount of inorganic phosphate liberated in the supernatant was determined.

Results: IMPase activity decreased by heat treatment and in the presence of NaCl, $MgCl_2$, and $CaCl_2$ at pH 7-8. The enzyme that retained high activity at high pH could not withstand heat or salt treatments. IMPase activity was unaffected by heat and salt treatments at pH 5. However, the enzyme that showed high activity at low pH could withstand heat or salt treatments. The IMPase activity decreased at low pH and increased at high pH in the presence of $MgSO_4$ regardless of whether heat treatment was applied or not.

Significance: These data suggest that the quality of horse mackerel could be maintained during the heating process by the addition of NaCl, $MgCl_2$, and $CaCl_2$ at high pH or by the addition of $MgSO_4$ at low pH.

P1-155 Rapid Detection of Microorganisms in Food and Beverage by Fluorescence

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Introduction: Spoilage contaminations are implicated in many food and beverages and are a major concern for industries. Rapid microbiological methods address contamination events sooner and provide a better control on the manufacturing process for products.

Purpose: A rapid detection method was developed to significantly shorten time to detection of contaminants. The compatibility of different matrix and performances of the rapid system were challenged to detect critical spoilage contaminants in specific food and beverage samples.

Methods: The EZ-Fluo Rapid Detection System method, based on universal fluorescent staining of microorganisms, is composed of sample filtration on membrane and incubation on traditional culture medium. After a short and validated incubation time, membranes are transferred onto a cellulose pad soaked with staining solution allowing enumeration of fluorescent micro-colonies. After staining, membranes can be re-incubated onto medium to continue growth allowing species identification.

Results: Filterability, fluorescent background and potential antimicrobial activity of two products were previously checked showing full method compatibility. *Aspergillus brasiliensis* and *Candida albicans* are detected in coconut powder after 24 hours of incubation on Sabouraud Dextrose Agar at 25°C instead of 5 days. In the same way, the detection time of *Alicyclobacilli* in energy drink is 20 hours on Potato Dextrose Agar instead of 3 days. The slope of the linear regression of three levels of *Alicyclobacilli* contamination is close to 1. Using a significance level of 0.1, the Anderson-Darling normality test indicates that the microbial counts follow a normal distribution. Regarding fluorescent enumeration, the *P*-values from Student test ($P \geq 0.05$) versus reference method are 0.674, 0.188, and 0.698, respectively, for the low, medium and high level of contamination demonstrating the equivalence between both methods.

Significance: The alternative method divides times-to-result by three to five compared to control method and gives earlier detection of contaminants and would significantly improve process and product quality.

P1-156 Changes of Bacterial Growth and Water Activity Values of Sliced Cabbage, Sandwich, and Tofu Stored under Various Temperatures and Humidity Conditions

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Introduction: Temperature may have both direct and indirect impact on food safety hazards in food chain at any stages, from production through consumption. Although temperature and humidity are supposed to have direct impacts on microbes growing in foods, researches related to these factors are limited.

Purpose: The objective of this study was to investigate changes of bacterial growth and water activity of sliced cabbage, sandwich, and tofu stored under different temperature and relative humidity with or without sealing air-tightly for 7 days storage.

Methods: Water activity, total viable counts, total coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, and *Salmonella* spp. in sliced cabbage, sandwich, and tofu were analyzed under different temperature (20, 30, and 40°C) and humidity (30%, 50%, and 75%) during storage. The significance was verified through ANOVA.

Results: Initial A_w of sliced cabbages, sandwiches, and tofu were 0.999, 0.979, and 0.996, respectively. Storage temperatures, relative humidity, and air tight sealing did not significantly ($P > 0.05$) change A_w of sliced cabbage during storage. A_w of sandwiches increased significantly ($P < 0.05$) with increasing relative humidity and temperature during storage. In tofu, A_w ranged from 0.972 to 1.000 and were not significantly ($P > 0.05$) influenced by temperature and relative humidity during storage.

Storage time and temperatures affected significantly ($P < 0.05$) aerobic plate counts and coliforms in sliced cabbage and tofu, while relative humidity and sealing did not change significantly ($P > 0.05$) aerobic plate counts of sliced cabbage during storage. In case of sandwich, storage temperatures, relative humidity, and sealing did not affect coliforms forming. *Staphylococcus aureus* and *Bacillus cereus* were not detected in sandwich and tofu during study.

Significance: Thorough supervision of temperature and time is required regardless of whether sealing or not for the safety of ready-to-eat foods due to climate conditions; however, relative humidity did not affect significantly the A_w of the foods which contained high A_w originally.

P1-157 Predicting Mold Spoilage on Pastries

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Introduction: The growth of molds in food products causes food spoilage and can be responsible for health issues via the production of mycotoxins. Due to the high occurrence of molds in stored agricultural products such as wheat and corn, fungal spoilage is of particular concern for the bakery industry.

Purpose: Development of a predictive tool using challenge tests and mathematical models to determine the time of appearance of the mold on pastries, according to measurable environmental factors ($T°$, a_w).

Methods: Rosso model has been used to describe and evaluate the cardinal values of the water activity and temperature for *Aspergillus candidus*. Instead of the optimal growth rate (μopt), it is proposed to use the minimum time of appearance of mold which was determined on food product. Indeed mold growth rate is fast and industrial issues mainly refer to appearance time, *i.e.,* when is my product spoiled? The minimum time of appearance on pastries (1/Ta) was determined on cakes after storage at 25°C. Developed mathematical tool was further validated on cakes produced at a pilot scale or transferred by industrials.

Results: In the range of water activity from 0.7 to 0.9 and a temperature range from 15 to 25°C, a total of 55 cakes were inoculated with *Aspergillus candidus*. The results show that the a_w measured on cakes vary over time. The water activity value to be taken into account in the simulation was the a_w of equilibrium (after 2 weeks storage). Results of simulation were then very close to the observed data ($R^2 = 0.85$; BF = 0.98; AF = 1.10). At this stage, for formulation without preservative, developed model provide satisfactory prediction of the time of appearance of molds as a function of water activity and temperature.

Significance: This study shows that predictive models developed for simulation of bacteria growth can be used to describe the apparition of molds on bakery products. The model will be useful to food microbiologists and manufacturers whose aim is to predict the likelihood of fungal spoilage as well as the development of new formulations minimizing fungal growth.

P1-158 Comparison of the Thermal Inactivation Pattern of *Zygosaccharomyces Fermentati* Using Bigelow and Weibull Model

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Introduction: The conventional approach for determination of D and z values, developed by Bigelow, has been used for decades to predict the effectiveness of thermal processing. However, the effective prediction of thermal inactivation cannot be evaluated from classical Bigelow model any longer because many microbial thermal inactivation patterns show not only linear survival curves but also various non-linear ones. Therefore, a number of mathematical models such as Weibull have been suggested to describe various microbial survival curves.

Purpose: The objective of this study was to compare Bigelow and Weibull models for prediction of the thermal resistance of *Zygosaccharomyces fermentati* which was isolated as a putrefactive microorganism from orange juice.

Methods: The BPS buffer (pH 7.4) and orange juice (pH 3.9, Brix 12%) were used as heating menstrum and inoculated by spoilage yeast which was grown at 35°C for 3 days to achieve roughly $>10^7$ CFU/ml. The 0.07 ml of each inoculated menstrum was put into capillary tube followed by flame sealed. Then 3 tubes for each temperature were submerged in a water bath at 52, 55, 57 and 60°C, respectively, removed at various time intervals, cooled down immediately in cold water and enumerated those survivors from PDA plates at 35°C for 3 days.

Results: R^2 (0.95-0.99 in BPS, 0.93-1.00 in Juice) and RMSE (0.07-0.34 in PBS, 0.004-0.62 in juice) values of Weibull model were shown more reliable data than those of Bigelow model. Hazard plot for judging the applicability of Weibull model presented straight lines in both PBS (R^2 = 0.82-0.99) and orange juice (R^2 = 0.93-1.00). Through the 2nd fitting step, p of shape parameter and δ of scale parameter were recorded as 0.81, Log (δ) =0.24(T)-13.52 (R^2 = 0.99) in PBS respectively, on the other hand, 0.62 and Log (δ) = 0.26(T)-14.11 (R^2 = 0.98) were obtained from orange juice.

Significance: These comparative data suggest that the Weibull model is reliable to predict thermal inactivation and evaluate sterilization process against *Zygosaccharomyces fermentati*, spoilage yeast in orange juice, more effectively than the conventional Bigelow model.

P1-159 Challenge Test on Heat-resistant Fungus, *Byssochlamys striata* Inoculated in Blueberry Juice and Subjected to Heat Treatments

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Introduction: Some strains of heat-resistant fungi have been reported to tolerate food processing steps such as heating. Spoilage caused by these organisms has frequently been reported in fruit and fruit juices. *Byssochlamys striata*, initially isolated from blueberry, produces eight-spored asci and has been shown to be resistant to heat treatments.

Purpose: The objectives of this study were to investigate the response of heat-resistant fungal spores of *B. striata* ATCC 10501 at different growth age to various heat treatments and establish heat treatment time and temperature conditions to inactivate this fungus.

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 various heat treatments at 80, 85, 90, 93, 95, 96 and 99°C for different times. Mold counts were conducted and expressed as CFU/ml. Survivor curves were plotted, D-values and Z-values were determined and compared using ANOVA.

Results: The 30 days old spores of *B. striata* 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 *B. striata* at 80, 85, 90, 93, 95, 96 and 99°C were found, and the Z-values calculated from the thermal death time curves were also determined.

Significance: The results established by this study may be used by blueberry processors to prevent losses due to spoilage caused by the heat-resistant microorganisms and to establish appropriate thermal process schedules for blueberry products.

P1-160 Identifying Bacteria that Cause Spoilage of Fermented Red Hot Pepper Mash

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Introduction: Production of hot pepper sauce requires the fermentation of red hot pepper mash in barrels for 2 to 3 years. Some barrels will develop undesired changes in color, texture, and odor. We have isolated the following microbes from spoiled pepper mash *Bacillus firmus, Bacillus pumilus, Brevibacillus laterosporus, Enterococcus avium*, and *Aerococcus viridans*.

Purpose: The main goal of this project was to determine which of these strains causes mash spoilage.

Methods: The fresh mash was separated into 7 different subgroups. Subgroups 1 through 5 were inoculated with 10^7/g of the isolated bacteria individually, the 6th was inoculated with 10^7/g of a mixed culture of each isolated bacteria, and the 7thwas used as negative control. All containers were incubated at 35°C ± 2°C. All groups were observed daily for changes in odor, color and gas production. Total plate counts were carried out on day 1 and every 15 days for each subgroup.

Results: The counts in the inoculated mash increased during the first two weeks from 1.96×10^6 CFU/g to 1.96×10^9 CFU/g, and then they declined to 2.35×10^7CFU/g on day 60. The non-inoculated mash counts declined from 6.16×10^6 CFU/g to 9.61×10^3 CFU/g in the first two weeks then increased to 1.51×10^5CFU/g at 60 days. By 30 days the *Bacillus* species produced a slimy white layer on top of the mash. On day 60 the odor and a color of the inoculated mash samples were different from the control, especially for *Enterococcus avium* and *Brevibacillus laterosporus*.

Significance: *E. avium* and *B. laterosporus* were found to be the main cause for the spoilage of the red hot pepper mash. Our results can be used in the development of Good Management Practices to control spoilage of the red hot pepper mash.

P1-161 Effect of High Hydrostatic Pressure on Psychrotrophic *Clostridium* spp. Isolated from Spoiled Vacuum Packaged Fresh Beef

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Developing Scientist Competitor

Introduction: Contamination of beef with endospores of psychrotrophic *Clostridium* spp. has led to incidences of blown-pack spoiled meat in Canada. Interventions used during carcass dressing processes are not sufficient to destroy endospores. High hydrostatic pressure may be a viable option for reducing the population of endospores on fresh meat; however, little is known about the pressure resistance of psychrotrophic *Clostridium* spp. isolated from fresh meat.

Purpose: The objective was to determine the resistance endospores of psychrotrophic *Clostridium* spp. to high hydrostatic pressure in minced raw beef.

Methods: Endospores of three psychrotrophic *Clostridium* spp. isolated from blown-pack spoiled beef (BP-1, BP09-01 and BP09-13) and *Clostridium estertheticum* ATCC 51377 were used. Beef was minced using a food processor, inoculated with endospores, and transferred to pressure resistant tubes. Samples were exposed to 400 MPa at 4, 40 or 70°C for 0, ramp, 4, 8, 15, 30 and 60 min.

Results: After pressure treatment at 400 MPa at either 40 or 70°C, viable cells of BP-1, BP09-01, BP09-13 and *C. estertheticum* ATCC 51377 were not detected after 8 min of treatment. Endospores of BP09-01 were destroyed by pressure treatment for 4 min at all temperatures. Counts of BP-1 and BP09-13 were reduced by 1 log (CFU/ml) after 4 min at all temperatures. Endospores of BP09-13 were detected after 30 min of treatment at 400 MPa and 4°C but *C. estertheticum* ATCC 51377 was not detected after 15 min at 400 MPa and 4°C.

Significance: Spoilage of fresh meat due to the growth of psychrotrophic *Clostridium* spp. can result in significant economic losses for the meat industry. High hydrostatic processing of vacuum packaged beef at 40°C and 400 MPa for 8 min could mitigate the spoilage caused by psychrotrophic *Clostridium* spp.

P1-162 Effect of pH on the Germination of Spores of *Clostridium estertheticum* in Meat Juice Medium

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Introduction: Early onset blown pack spoilage (EOBPS) of vacuum packaged chilled raw beef and other meats is characterized by gross swelling of packs at early times during storage. *Clostridium estertheticum*, a strictly anaerobic spore former, has been identified as the primary cause of EOBPS. The meat must have been contaminated with spores rather than oxygen sensitive vegetative cells of *C. estertheticum* before packaging.

Therefore, to understand how EOBPS might be controlled, it is necessary to establish the conditions required for germination and outgrowth of *C. estertheticum* spores.

Purpose : The objective of this study was to determine the effect of pH on the germination of spores of *C. estertheticum*in meat juice medium (MJM).

Methods: Anaerobic MJM of pH 5.00, 5.50, 6.00, 6.50, 7.00 and 8.00 was inoculated with *C. estertheticum* spores (1×10^5 CFU/ml). Spore germination was monitored using a modified differential spore stain (Wirtz-Conklin). The spores remaining in the MJM suspensions were counted at 0 h, 4 h, 8 h, 24 h, 31 h, 48 h and 72 h. The effect of pH on outgrowth was monitored over 20 days by observation of gas accumulation in Durham tubes in suspensions of spores in MJM of different pH values.

Results: The numbers of remaining spores decreased quickly within 4 h incubation. The spores remaining after 72 h at pH 5.00 and pH 7.00 were 37% and 19%, respectively, of the initial numbers, showing that the germination of spores of *C. estertheticum* was enhanced as the pH approached neutrality. As the pH increased, the time before gas production was decreased, with gas production being apparent after 8 and 20 d incubation of MJM of pH 7.3 and 5.7, respectively.

Significance: The normal pH of beef muscle tissue (5.5-5.8) is less favorable for germination and outgrowth of *C. estertheticum* spores than are higher pH values. Modification of meat surface pH might be a possible approach to prevention of EOBPS in vacuum packaged beef.

P1-163 Isolation of DNase- and Protease-Producing Bacteria on Catfish Spoilage

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Undergraduate Student Award Competitor

Introduction: Fish spoilage is a global concern to the consumer and producer alike. It is believed that microbial enzyme activity is a major contributor to fish spoilage. Therefore, the freshness of fishery products is an important concern for aquaculture farmers, private sectors, and consumers.

Purpose: The purpose of the study is to isolate DNase- and protease-producing bacteria as well as to show their effects on catfish spoilage.

Methods: Six catfish samples were obtained from an aquaculture facility at Delaware State University and from a local retail source for comparison of microbial populations. The catfish were stored for up to 3 weeks at 4°C among various intervals and samples were taken for microbiological analysis. The duplicate samples (20g) were stomached with a saline solution (180ml) and then plated on selective and rich media for analysis of DNase- and protease-producing bacteria and total aerobic plate counts, respectively. The triplicate plates were incubated for 3 days at 28 °C, and then colonies were enumerated for comparison of the population types. DNase- and protease-producing colonies were selected for genus confirmation by Polymerase Chain Reaction (PCR) using universal primers for *Pseudomonas*.

Results: The total aerobic microbial populations of the catfish obtained from the aquaculture facility took approximately 3 weeks to reach the stationary phase at log 10 CFU/g with limited DNase producing bacteria counts around log 0-1 CFU/g, but high amounts of protease producing bacteria at log 9.8 CFU/g. The catfish purchased from a local retail source took 15 days to reach the stationary phase at log 10 CFU/g with increasing numbers of protease at log 9.6 CFU/g and DNase producing bacteria at log 9.1 CFU/g. PCR confirmations showed that 40/62 DNase- and protease-producing colonies were positive for *Pseudomonas* species.

Significance: Results obtained in this study suggest that extracellular DNase- and protease -producing bacteria play a large role in catfish spoilage and support the need for further research on bacterial identification isolated from the catfish spoilage.

P1-164 Increased Water Activity Reduced the Thermal Resistance of *Salmonella enterica* in Peanut Butter

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Developing Scientist Competitor

Introduction: *Salmonella enterica* is the leading cause of bacterial foodborne illnesses in the U.S. Our previous study suggested that desiccation-stressed *S. enterica* cells were significantly more heat resistant than freshly-grown cells in peanut butter (PB) of low water activity (a_w).

Purpose: To evaluate the effects of rehydration on survival rates and heat resistance of *S.enterica* serotypes in PB.

Methods: Relative heat resistances of serotypes *S.* Typhimurium, *S.* Enteritidis and *S.* Tennessee were compared individually and as a 3-strain cocktail at 90°C and 126°C in two PB formulations adjusted to a_w 0.2, 0.4, 0.6 and 0.8. Scanning electron microscopy was used to monitor morphological changes of cells in peanut oil. D-values were calculated using Bigelow model and compared using ANOVA.

Results: Increased a_w in PB significantly reduced the heat resistance of desiccation-stressed cells treated at 90°C ($P < 0.05$). Differences in heat resistance were observed among the three serotypes and between the two PB formulations. When treated at 126°C, differences in heat resistance among different serotypes and adjusted water activities were less notable ($P > 0.05$). Based on the Weibull model, an average of 52-132 min was required to achieve a 5-log reduction of the 3-strain cocktail at 90°C in PB with a_w of 0.2. When a_w was increased to 0.6, only 23-27 min was required to achieve the same 5-log reduction. Under scanning electron microscope, minor morphological changes were apparent in cells during desiccation and rehydration processes in peanut oil having a similar a_w to that of PB.

Significance: Results from this study collectively suggest that water activity plays a key role in determining *S. enterica* heat resistance in PB. The variability of heat resistance among different serotypes, PB formulations and water activities should be taken into consideration for developing and validating effective intervention and mitigation strategies.

P1-165 Lethality of Moist Heat and Silver Dihydrogen Citrate Sanitizer Combinations on *Listeria* Strains Adhered to Components of a Deli Meat Slicer

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Introduction: Deli meat slicers have been implicated in cross-contamination of ready-to-eat (RTE) foods with *Listeria monocytogenes* that has resulted in several listeriosis outbreaks. We investigated the lethality of moist heat and silver dihydrogen citrate (SDC) sanitizer on *Listeria* strains that were inoculated on stainless steel (SS) and cast aluminum (AL) coupons cut from actual components of a deli meat slicer.

Purpose: Combined effect of sanitizers and thermal treatment could provide better control of *Listeria monocytogenes* on food contact surfaces.

Poster

Methods: The coupons inoculated with *Listeria* strains were subjected to treatments inside and outside of meat slicer using a commercial bread proofer oven that was operated for 7 h at 66°C. Post treatment recoveries of the inoculated *Listeria* from the treated coupons were enumerated using MOX growth medium.

Results: All treatments produced significant ($P = 0.05$) reductions compared with positive and untreated negative controls. Moist heat reduced the inoculated strains to non-detectable levels when the coupons were placed inside the motor compartment of the slicer and the sanitizer plus moist heat gave same results for the coupons placed inside and outside of the slicer. Chemical sanitizer treatment alone showed average log reductions of around 5 CFU/cm^2 on AL and SS coupons, respectively, and the moist heat alone treatment on both AL and SS coupons showed a log reduction of 4.49 and 4.87 logs when placed externally and above 6 logs when placed inside the motor compartment. Sanitizer plus moist heat treatments showed highest log reductions of *Listeria* strains to non-detectable levels on deli meat slicer components when placed inside or outside the motor compartment of the slicer.

Significance: The sanitizer and moist heat combination treatments can effectively reduce the *Listeria* cells attached to food contact surfaces of a deli meat slicer.

P1-166 Cross-contamination between Deli Foods and Slicers by *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella*, and Validation of the Antimicrobial Efficacy of Sanitizers

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Introduction: Foodborne pathogens have been associated with ready-to-eat (RTE) sliced deli meats. Sanitizers are widely used to minimize the risk of cross-contamination in the food industry, but their efficacy can be reduced when used in commercial settings such as on slicers. It has been suggested that levulinic acid plus sodium dodecyl sulfate (SDS) may provide substantial antimicrobial efficacy on food processing equipment.

Purpose: The purpose of this study was to: (1) determine the levels of cross-contamination that can occur between deli foods and slicers contaminated by *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* Typhimurium, and (2) determine the killing efficacy of levulinic acid plus SDS for inactivating these three pathogens on slicers.

Methods: *E. coli* O157:H7, *L. monocytogenes* and *S.* Typhimurium were individually surface-inoculated either on deli foods (10^{4-5} CFU/cm^2) to contaminate slicer surfaces or on slicer blades (10^{7-8} CFU/blade) to contaminate deli foods, including beef, cheese and ham. The populations of pathogens on food slices and at 6 contact locations on slicers were enumerated. After application of sanitizers, either as a liquid or as foam, surfaces of the slicers were swabbed for pathogen enumeration.

Results: The three pathogens survived on the dried surface of slicers for at least 6 days. After slicing 5 to 10 slices, up to 10^3 CFU of pathogens/cm^2 from the contaminated deli foods (10^{4-5} CFU/cm^2) were transferred to slicer surfaces, including the grip, carriage tray, blade cover, blade and gauge plate. Once the slicer blades were contaminated by *E. coli* O157:H7, *L. monocytogenes* or *S.* Typhimurium, these pathogens were transferred at a rate of 10^{1-3} CFU per slice to up to 100 slices. Contaminated slicer surfaces sprayed with a sanitizer containing 1% levulinic acid plus 0.1% SDS as a foam (45-55 psi) reduced 10^{6-8} CFU of pathogens/blade, including *E. coli* O157:H7, *L. monocytogenes* and *S.* Typhimurium within 1 min at 21°C.

Significance: Results revealed that pathogen cross-contamination can occur between slicer and deli foods and that levulinic acid plus SDS sanitizer can effectively reduce foodborne pathogen populations of 10^{6-8} CFU/blade on slicers.

P1-167 Increasing Shelf Life of Injected Meats by Biofilm Removal

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Introduction: The injection of brines and marinades in meat is inherently susceptible to the introduction of spoilage organisms to finished product, as injector needles and system components are documented breeding grounds for biofilm. It is hypothesized that adding a disinfectant with biofilm removal efficacy to the injection sanitation process can result in increased shelf life of finished product.

Purpose: This study was conducted to evaluate whether a commercially available disinfectant with biofilm removal capabilities, used as a supplement to the standard injection system and needle cleaning regimen, can increase finished product shelf life.

Methods: A two week shelf-life study was conducted comparing a traditional cleaning regimen to treat an injector and components to a regimen that adds a commercially available disinfectant formulated for biofilm removal (Sterilex Corporation, Hunt Valley, MD). Baseline raw dark poultry meat samples were collected and compared against samples of raw dark poultry meat collected after injector treatment with the disinfectant product over a 14 day period. Shelf life of meat was determined by looking at both microbiological metrics (APC and Total Coliform counts) as well as organoleptic observations.

Results: An evaluation of microbiological APC counts & coliform counts as well as organoleptic properties of the poultry samples over the 14 day evaluation period yielded finished product shelf life of eight (8) days for the control sample and thirteen (13) days for the treated sample. Microbiological thresholds were reached on treated samples 4-5 days later than the control sample, leading to an increase in shelf life (10^6 CFU/ml for APC and 10^5 CFU/ml for Coliform). Organoleptic differences between the two sets of samples were even more dramatic.

Significance: These data demonstrate that the addition of a commercial disinfecting agent with biofilm removal efficacy to an injection sanitation program can increase finished product shelf life.

P1-168 Microbiota in Fish Production Facilities and Impact on Growth and Biofilm Formation of *Listeria monocytogenes*

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Introduction: *Listeria monocytogenes* remains a problem for the food production industry, especially those producing Ready-To-Eat food. The bacterium may establish itself in certain niches in food production facilities and some clones may persist for years in the same plant. Thorough cleaning is a recommended measure, but it is sometimes speculated that removing the natural competing bacterial background flora increases the problem.

Purpose: Determine the impact of the bacteria representing the background microbiota in salmon production plants on biofilm production of *L. monocytogenes*.

Methods: Bacteria were collected from typical harborage sites for *L. monocytogenes* after regular cleaning and disinfection in four salmon production plants. The temperature in the production facilities was measured during one week. *L. monocytogenes* and a non-monocytogenes *Listeria* sp. (isolated together with *L. monocytogenes*) was attached and grown as biofilm on steel coupons at 12°C and 20°C with a thin layer of salmon broth ("listeriaculture"). The same experiment was also done adding a mixture of ten different species of bacteria commonly found in the production environment together with listeria ("multiculture"). The experiment was repeated three times. In one occasion, the composition of the microbial flora was determined by 454 pyrosequensing.

Results: The listeriaculture biofilm multiplied through the incubation period reaching about 10^9 CFU/cm^3 after nine days. The *Listeria* sp. outcompeted *L. monocytogenes* at 20°C ($P = 0.011$). A steady biofilm of about 10^9 CFU/cm^3 was obtained after two days for the multiculture. The background microbiota suppressed *L. monocytogenes* ($P = 0.001$) and it only represented 0.1 - 0.01% of the total biofilm population. The multispecies biofilms were dominated by *Pseudomonas* sp. followed by *Flavobacterium* sp., *Chryseobacterium* sp., and *Acinetobacter* sp.

Significance: *Listeria monocytogenes* may grow and form biofilm at conditions simulating salmon production facilities and the natural background microbiota may inhibit but not eliminate it in biofilms. Proper cleaning and disinfection is recommended to combat *L. monocytogenes* in the food production environment.

P1-169 Push-through Sanitation of Peanut Butter Processing Equipment

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Introduction: Recent *Salmonella* contamination of nut butter products and processing facilities has led to the first facility registration suspension by FDA under the Food Safety Modernization Act. Implementation of evidence-based preventive controls and sanitation procedures are required to ensure product safety as *Salmonella* can survive in low-moisture foods, such as peanut butter.

Purpose: The objective of this study was to evaluate the efficacy of a peanut butter push-through clean of a section of previously contaminated peanut butter processing equipment. The results obtained will provide important information to the industry on validation of sanitation methods and guidance in determining lot separation of nut butters.

Methods: Uncontaminated peanut butter (~100 l) was heated to 55°C and pumped through pilot-scale nut butter processing equipment at ~3 l/min. The flow was paused, and 30 g of inoculated peanut butter (~9 log CFU *Salmonella*/g) was inserted in piping located at the end of the processing system. Pumping was resumed, and samples of peanut butter (10 g) were taken periodically from the exit piping (t = 0-65 min), and *Salmonella* enumerated via plate count.

Results: Approximately 8 log CFU/g of *Salmonella* was detected in the peanut butter samples obtained immediately after pumping resumed, with levels reduced to 3.5 log CFU/g after pumping through ~100 l of uninoculated peanut butter. In addition, *Salmonella* was detected in the peanut butter remaining in the piping (6.0 log CFU/g) and in the exit valve (5.2 log CFU/g). Physically cleaned inner surfaces of piping, appearing 'visibly clean', had 1.4-1.7 log CFU/10 cm^2 *Salmonella*, indicating that bacteria were transferred to the stainless steel surface of the piping during the push-through procedure.

Significance: Results suggest that use of uncontaminated peanut butter as a push-through to 'clean' contaminated sections of stainless steel piping is not sufficient to remove *Salmonella* contamination, and thus is not a viable sanitation method.

P1-170 Transovarial Transmission of Foodborne Pathogens by the Housefly, *Musca domestica*

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Introduction: Bacterial foodborne pathogens can be transmitted by flies either horizontally, by contact with the environment or other flies, or vertically (transovarially) from mother to progeny. Although scientific evidence supports the horizontal transmission of foodborne pathogens by flies, transovarial transmission has received little consideration.

Purpose: Determine the frequency of transovarial transmission of *Salmonella enterica* and *Listeria monocytogenes* by the housefly, *Musca domestica*.

Methods: Groups of 20-30 lab reared adult houseflies were kept in jars in a Percival growth chamber at 25°C. Flies were fed with fly food (milk with 2% sugar) containing 0, 10^4, or 10^8 cells/ml of either *S. enterica* or *L. monocytogenes* for 24 hours and with fly food containing no bacteria for an additional 48 hours. Small pieces of meat were then added to the jars for fly oviposition. Groups of ~100 eggs were collected, surface disinfected, placed in specific enrichment media, and incubated at 37°C for 24 hours. Enriched media was used to detect specific foodborne pathogens using the BAX system Q7, according to manufacturer's instructions. Fourteen and six replicates were performed for *S. enterica* and *L. monocytogenes*, respectively. Data were analyzed using the Fisher Exact Test (SAS v9.3).

Results: The frequency of transovarial transmission of *S. enterica* by houseflies fed with 10^4 and 10^8 cells/ml was 7% and 38% ($P = 0.065$), respectively, whereas the frequency of transovarial transmission of *L. monocytogenes* was 17% and 67% ($P = 0.018$), respectively. Neither *S. enterica* nor *L. monocytogenes* were detected on eggs of flies fed with only sugar and milk (0 cells/ml).

Significance: These results show an unforeseen mode of transmission that is likely to have epidemiological ramifications for the spread of foodborne pathogens by flies, emphasizing the public health significance and the regulatory importance of the presence of flies in food.

P1-171 Biofilm Formation by Shiga Toxin-producing *Escherichia coli* and Multidrug-resistant and Susceptible *Salmonella* and Their Inactivation by Sanitizers

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Developing Scientist Competitor

Introduction: Compared to planktonic cells, bacterial biofilms are more resistant to sanitizing agents, causing crucial challenges for their inactivation in various food environments.

Purpose: This study compared biofilm formation by seven serogroups of *Escherichia coli* (i.e., O157, O26, O45, O103, O111, O121, O145) and two or three phenotypes of *Salmonella* Newport and *S.* Typhimurium (i.e., susceptible, multidrug-resistant [MDR], and/or multidrug-resistant with acquired *ampC* gene [MDR-AmpC]). In addition, their reduction by water, quaternary ammonium compound-based (QAC), and acid-based (AB) sanitizers were investigated.

Poster

Methods: Seven strain mixtures of the above pathogen groups were separately spot-inoculated onto stainless steel coupon surfaces for a target level of 2 log CFU/cm^2. Coupons were stored statically, partially submerged in 10% (w/v) non-sterilized meat homogenate at 4, 15, and 25°C. Microbial counts on days 0, 1, 4, and 7 and survivors after exposure (submersion in 30 ml for 1 min) to water, QAC and AB of one-week mature biofilms were enumerated.

Results: At 4°C, pathogen counts on inoculation day ranged from 1.6 ± 0.4 to 2.4 ± 0.6 log CFU/cm^2 and changed to 1.2 ± 0.8 to 1.9 ± 0.8 log CFU/cm^2 on day 7 with no appreciable difference among the pathogen groups. Biofilm formation at higher temperatures was more enhanced. *E. coli* O157:H7, as an example, increased ($P < 0.05$) from 1.4 ± 0.6 and 2.0 ± 0.3 log CFU/cm^2 on day 0 to 4.8 ± 0.6 and 6.5 ± 0.2 log CFU/cm^2 on day 7 at 15 and 25°C, respectively. As compared to 4°C, after sanitation, more survivors were observed for 15 and 25°C treatments with no appreciable differences among the seven pathogen groups. MDR-AmpC *S.* Newport, as an example, were reduced to <1.6 ± 1.3 and 1.9 ± 0.9 log CFU/cm^2 after exposure to QAC and AB at 15°C, while at 25°C were reduced to 5.5 ± 0.5 and 6.0 ± 0.4 log CFU/cm^2, respectively.

Significance: Overall, patterns of growth and susceptibility to QAC and AB sanitizers were similar among the seven tested pathogens, with enhanced biofilm formation capability and higher numbers of survivors at higher temperatures.

P1-172 Efficacy of Chemical Treatments for Control of *Mycobacterium tuberculosis* Biofilms on Various Surfaces

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Introduction: Tuberculosis is most commonly caused by *Mycobacterium tuberculosis*. Although the natural host of the pathogen is humans, occasional cases of *M. tuberculosis* infections have been reported in animals which have had contact with infected humans. The pathogen has potentials to form biofilms on surfaces commonly used in food processing environment.

Purpose: This study assessed biofilms formed by selected strains of *M. tuberculosis* and investigated the efficacy of three different treatments for biofilm control.

Methods: Two *M. tuberculosis* strains were inoculated separately in 150 ml Middlebrook 7H9-Tween 80 (0.1%) broth with 5% liver extract and 10% oleic albumin dextrose catalase (OADC), 5% liver extract alone, or 10% OADC alone in sterile jars containing 2 x 2 cm^2 coupons of steel, cement or ceramic for biofilm development. The jars were incubated at 37°C with agitation for 2, 3 and 4 weeks, respectively. Biofilms on coupons were subsequently exposed to 10 ml of 2% Iodasteryl®, 0.5% Virocid® and sterile water at 28 and 45°C, respectively for 5 min. Biofilm mass on treated and untreated coupons were assessed.

Results: Tested strains of *M. tuberculosis* formed biofilms on all three contact surfaces. One tested strain formed more biofilms than the other strain. More biofilms were formed in media containing both liver extract and OADC than media with only liver extract or OADC. Biofilm mass increased as incubation time increased till the 3rd week of incubation. More biofilms were formed on cement followed by ceramic and steel coupons. Chemical treatments at 45°C removed more biofilms than those at 28°C. Treatments with Iodasteryl® and Virocid® were equally effective in removing the biofilms on tested surfaces. However, neither treatment completely removed the biofilms.

Significance: Results suggest that examined *M. tuberculosis* strains could form biofilms on tested surfaces, and chemical treatments used partially removed biofilms from the surfaces.

P1-173 Benzalkonium Chloride-based Antimicrobial Paper: A New Approach for the Removal of Food Pathogenic Bacteria on Human Hands

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Introduction: Foodborne diseases remain a major concern causing about 48 million gastrointestinal illnesses, 128,000 hospitalizations, and 3,000 deaths annually in the United States. Improper washing of hands and poor employee hygiene habits are the major factors which significantly contribute to the rapid spread of pathogenic microorganism in the food sector. It is estimated that 36% of foodborne illnesses originate from improper hand hygiene.

Purpose: The purpose of the present study is to evaluate *in vivo* the antibacterial activity of a new dry antibacterial paper, with benzalkonium chloride (BC) as an active compound, on hands of human volunteers.

Methods: The inhibitory activity of the antibacterial paper was first evaluated qualitatively on various food pathogenic bacteria using the Agar Diffusion Test and quantitatively using the AATCC 100-2004 Method. The antibacterial activity was then evaluated on human hands using the ASTM E1174 - 06 Standard Method. Finally, the safety of this antibacterial paper was evaluated by determining its potential to induce irritation and/or allergic sensitization on human hands using the Repeated Insult Patch Test.

Results: The dry antibacterial paper was shown to be active against various Gram positive and Gram negative food pathogens, including *Escherichia coli* and *Listeria* with reduction rates exceeding 99.97% after a persistence time of 30 minutes. Up to 3.24 log reduction on human hands was observed in the clinical trial with *S. marcescens* and *E. coli*. The clinical trial did not show any dermal irritation or allergic sensitization. Moreover, the potential of migration of BC from hands to water was negligible.

Significance: The antibacterial paper developed here, offers an easy and effective way to reduce bacterial contamination and transmission in the food sector. Its use will compensate for people's imperfect hygiene practices by reducing residual bacteria left on hands after washing.

P1-174 Enhancement of Slightly Acidic Electrolyzed Water Sanitization Efficacy on Fresh Vegetabls by Ultrasonication and Water Wash

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Introduction: Fresh vegetables are an essential part of the diet of people around the world. Despite the extensive advances in food processing methods, vegetables have been implicated in many foodborne disease outbreaks. Therefore, developing new sanitization approaches as well as improving the present techniques is necessary.

Purpose: The aim of this study was to develop a simple, environmental friendly, easy to perform hurdle approach to maximize decontamination efficacy of slightly acidic electrolyzed water (SAEW) on Chinese cabbage, lettuce, sesame leaf and spinach using ultrasonication (US) and water wash (WW) based on the fact that each hurdle applied might impose an increase in the antimicrobial action.

Methods: The SAEW used in this study was produced by electrolysis of a diluted hydrochloric acid (6% HCl) in a chamber without membrane at a setting of 2.5 A and 22.8 V. Effect of five different treatments; dipping in distilled water, SAEW, SAEW followed by WW, SAEW with US and SAEW with US followed by WW on the reduction of total bacteria count (TBC), Yeasts and molds (YM) and artificially inoculated pathogenic bacteria was investigated.

Results: SAEW treatment alone resulted in significant ($P \leq 0.05$) reductions from 1.08 to 1.53 log CFU/g in microbial numbers compared to the control (no treatment). SAEW combined with US followed by WW resulted in 2.5, 2.54, 2.6 and 2.8 log CFU/g reductions in YM, TBC, *Escherichia coli* and *Listeria monocytogenes* on cabbage, respectively. Reductions of 2.25, 2.4, 2.5, 2.6 log CFU/g, 1.76, 1.88, 2.33, 2.4 log CFU/g and 1.97, 2.08, 2.41, 2.49 log CFU/g on lettuce, sesame leaf and spinach in YM, TBC, *E. coli* and *L. monocytogenes* were also observed.

Significance: The newly developed hurdle method is a valid approach for fresh produce sanitization. Following further investigation and optimization this approach may be a useful tool for produce decontamination in the food industry.

P1-175 Inactivation of Foodborne Bacteria on a Ball-shaped Surface Model Using Bacto Agar by UVC-Assisted Titanium Dioxide Photocatalysis

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Introduction: There is an increasing interest in the application of UVC-assisted TiO_2-photocatalytic oxidation (PCO) reaction for the disinfection of food surfaces. Disinfection effects of the UVC-assisted TiO_2-PCO on fruits and vegetables are difficult to evaluate because of their inconsistency in degree of contamination and surface properties. A unified food surface model needs to be developed to overcome this drawback.

Purpose: Objectives of this study were to develop a food surface model and to analyze disinfection effects of UVC-TiO_2-PCO on bacterial load on the food surface model and morphological changes in bacteria during the treatment.

Methods: Ball-shaped food surface model was prepared using Bacto agar which is generally used as microbiological culture media. Foodborne bacteria such as *Escherichia coli* K12, *Salmonella* Typhimurium, *Listeria monocytogenes*, and *Staphylococcus aureus* were inoculated to the surface model and treated with the UVC-TiO_2-PCO reactor. Microbial inactivation and morphological changes in bacteria after the treatment were evaluated.

Results: Bacteria treated with the UVC-TiO_2-PCO reactor showed higher reduction in bacterial count compared to UVC alone or control (dark reaction). UVC-TiO_2-PCO treatment reduced microbial counts by 4.5-6.0 log CFU/cm^2 compared to 3.0-5.3 log CFU/cm^2 reductions by UVC alone after treatments for 100 s. Dark reaction as a control showed 0.7-1.6 log CFU/cm^2 reductions after 100 s. Gram-negative bacteria decreased rapidly from the beginning; however, Gram-positive bacteria showed initial time lag under the UVC-TiO_2-PCO reaction. Structural damages of bacteria were detected more markedly as the reaction time increased.

Significance: The results validate that the food surface model using Bacto agar can be applied to investigate effects of surface disinfection and the UVC-TiO_2-PCO reaction is effective to inactivate foodborne bacteria.

P1-176 Improvement of Microbial Safety of Hard-cooked Eggs Using Pulsed Light

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Introduction: Ready-to-eat (RTE) products including hard-cooked eggs are potential vehicles of foodborne illness as they are susceptible to recontamination post-processing. Thus, the evaluation of novel intervention technologies such as pulsed light (PL) for mitigation of the impact of post-processing contamination has become an urgent goal to achieve.

Purpose: The major objective of this study was to evaluate the efficiency of pulsed light on inactivating *Escherichia coli* K12, a biological indicator of unsanitary processing conditions, on hard-cooked eggs.

Methods: Hard-cooked eggs inoculated with *E. coli* K-12 (MDD 333) were positioned 5.5 cm and 9.5 cm away from the quartz window of a PL system (LH840-LMP-HSG) and treated from 1 to 30 s. Initial and survivor counts were determined by: standard plate counting (SPC) on tryptic soy agar supplemented with Rifampicin (80 µg/ml) and most probable number (MPN) for counts falling below 25 CFU/ml for SPC. Temperature was monitored during treatment in addition to color and texture determined by the CIELAB system and texture profile analysis respectively. Each experiment was conducted in triplicate and statistical analysis was conducted using Analysis of Variance.

Results: Significant reductions ($P < 0.05$) on *E. coli* K-12 populations were achieved after PL exposure. Maximum inactivation (6.4 ± 0.0 log CFU/egg) was attained after 20 s and 30 s treatment at 5.5 cm and 9.5 cm from the quartz window, respectively. Likewise, temperature increased significantly ($P < 0.05$) up to 8.8 ± 0.9 °C and 5.6 ± 0.6 °C after 30 s treatment at 5.5 cm and 9.5 cm distance, respectively. No significant differences ($P > 0.05$) were observed in color or texture post PL treatment.

Significance: The findings of this study suggest that PL can enhance the safety of hard-cooked eggs and contribute to minimize the risk associated with RTE foods.

P1-177 Assessing Effective Quality Controls of Chlorination in Postharvest Wash Water Sanitation of Fresh Produce

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Developing Scientist Competitor

Introduction: Postharvest produce sanitation is a key food safety step to control and reduce microbial loads and its importance is addressed in newly proposed rules for produce safety under FDA's Food Safety Modernization Act. There are common farm sanitization practices which require further examination for effectiveness due to variables like sanitizer concentration, efficacy lost due to turbidity, chemical dissipation, temperature, and pH. The intention is to compare and evaluate monitoring practices such as oxidation reduction potential for effective water sanitation in postharvest practices in order to aid and ease transition of industrial safety methods onto small commercial farms.

Purpose: To evaluate methods of postharvest produce sanitation management and the factors that affect the efficacy of chlorine sanitation in control of microbial loads.

Methods: Autoclaved water was inoculated with 10^6 CFU/ml *Escherichia coli BL21*. Water samples at pH of 6.0-7.0 and were exposed to different chlorine concentrations (50-75ppm), turbidity levels (1-50 NTU through the addition of autoclaved soil), and different exposure times (1, 3, 5 minutes). ORP was measured and then samples de-chlorinated with sodium thiosulfate prior to performing standard plate counts.

Results: Data shows that chlorine concentration 50 ppm measured ORP 850 RmV in low turbidity (below 10 NTU) with a 3 log reduction in microbial load. Water with increased turbidity exposed to 50 ppm resulted with decreased ORP (650 RmV to 300 RmV) and produced a 1-2 log reduction.

Significance: Data suggests ORP values 600-800 RmV with low turbidity can be used as a monitoring range for indication of chlorine efficacy required in postharvest wash water for reduction of microbial loads.

P1-178 Variation in Detection Limits of an ATP Bioluminescence Meter between Bacterial Growth Curve Phases

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Introduction: Handheld devices for measuring adenosine triphosphate (ATP) by bioluminescence are increasingly popular for rapid monitoring of surface hygiene. ATP is present in all living cells including bacteria; however, the quantity of ATP per cell is variable between bacterial species and in different growth phases. Therefore, it is not always clear how ATP measurement correlates with the presence of bacteria.

Purpose: The purpose of this study was to determine the detection limit (sensitivity) of the Hygiena SystemSure ATP meter in lag, log, stationary and death phases of the bacterial life cycle for *Escherichia coli* and *Staphylococcus aureus*.

Methods: A bacterial growth curve was performed for each organism tested. Samples were taken at 0 hours (lag phase), 4 hours (log phase), 12 hours (stationary phase), and 24 hours (death phase). Samples were diluted and tested by the SystemSure and cultured for colony counts. For each growth phase the colony count (colony forming units/ml or CFU/ml) at which the SystemSure reading fell below the "clean" cutoff of ≤10 relative light units (RLU) was determined.

Results: The limits of detection of the SystemSure in CFU/ml were as follows. For *E. coli*: lag phase: 2,175,000; log phase: 24,000; stationary phase: 2,860,000; and death phase 1,010,000. For *S. aureus*, the detection limits were: lag phase: 1,115,000; log phase: 171,000; stationary phase: 15,300,000; and death phase: 1,110,000.

Significance: The CFU/ml at which the SystemSure read ≤10 RLU indicates the concentration of living organisms present in the sample. The detection limit for the SystemSure varied by 100 fold depending on the bacterial life phase, with the greatest sensitivity during log phase and the least sensitivity during stationary phase. Users of ATP bioluminescence devices must be aware that living bacteria may be present on a surface that is determined to be "clean" by ATP detection.

P1-179 An Investigation of Restaurant Food Safety Performance: A Comparison between Ethnic and Non-ethnic Restaurants in Louisiana

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Introduction: Of 163,357 foodborne illnesses reported in Louisiana (LA) each year, 28,000 were related to foods served in restaurants. The state government implements annual restaurant inspections to ensure safe food are served to the consumers.

Purpose: This study examined health inspection data from casual dining restaurants in LA in a one-year period (January 1 - December 31, 2011) to identify frequency and types of food code violations occurring in the restaurants and compare differences of food code violations between ethnic and non-ethnic restaurants.

Methods: Restaurant inspection data from ten parishes in LA with highest number of population was derived from Louisiana Department of Health and Hospitals' website. The information was copied and pasted into Excel spreadsheets and converted to SPSS for data analyses. T-test was applied to compare food safety performance between ethnic and non-ethnic restaurants.

Results: A total of 4,393 restaurant inspection reports were retrieved from 756 restaurants (535 non-ethnic and 221 ethnic) in ten parishes. Of 7,481 inspections, most of them was routine-driven (n = 6614, 58%), followed by complaint (n = 506, 8%) and re-inspection (n = 306, 4%). On average, each restaurant violated 3.64 critical food codes and 8.20 non-critical food codes in a year period. Top three violated codes were "Food contact surfaces and utensils are not clean to sight and touch" (0.35 violation/restaurant), "Potentially hazardous food held for more than 24 hours is not date marked"(0.18 violation/restaurant) and "Food contact surface was not washed, rinsed and sanitized" (0.18 violation/restaurant). Results indicated that ethnic restaurants have significantly higher critical food code violations (3.21 vs. 1.51; $P < 0.01$) and non-critical food code violations (9.40 vs. 7.71; $P < 0.01$) than non-ethnic restaurants.

Significance: Food safety educators could utilize the results to develop food safety training materials that targeted at ethnic and non-ethnic restaurants in LA. Restaurant managers could use the data to improve food safety performance in their establishments.

P1-180 Draft Beer Seller's Perception on Sanitation and Prevalence of Foodborne Pathogens in Draft Beer in Korea

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Introduction: Beer has been covered for the largest proportion in alcoholic beverage consumption in South Korea and is supposed to be a microbiologically safe beverage due to its native antimicrobial barriers such as low pH, alcohol concentration, antiseptic action of hop acids and carbonation, etc. However, draft beer is still to be suspected of sanitation concerns because some pathogens have been found to survive.

Purpose: This study was executed to investigate microbiological contamination conditions of draft beer and awareness of employees on the draft beer sanitation management in draft beer restaurants in South Korea.

Methods: A total of 112 draft beer samples were purchased from 10 different cities in South Korea. The draft beer samples were analyzed for coliforms, *Salmonella* spp., *Staphylococcus aureus*, *E. coli* O157:H7, and *Yersinia enterocolitica*. Questionnaire surveys were conducted by 639 beer sellers to investigate perception of draft beer sanitation management.

Results: Only *Staphylococcus aureus* was detected from 2 beer samples out of 9 samples which were purchased from Cheonan City and 2 beer samples out of 19 samples from Iksan City. Other foodborne bacteria were not detected from any beer samples. According to the data of questionnaire survey, 72.6% of beer sellers answered that draft beer can be contaminated by any bacteria. Approximately forty-eight percent of beer sellers were aware of hygienic practices, while 51.6% did not. It has been suggested that presence of *Staphylococcus aureus* in draft beers may be caused by poor personal hygiene. Generally, draft beer sellers realize that the quality of draft beer can be affected by bacteria but more than half of them did not know how to sanitize draft beer apparatus such as keg, coupler, faucet, and beer line.

Significance: Therefore a manual or a guideline on the sanitation management of draft beer should be established for the training of the beer sellers in South Korea.

P2-01 Differential Survival of Turkey-derived *Campylobacters* in Vehicles (Feces, Water) of Special Relevance for Pre-harvest Transmission

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Developing Scientist Competitor

Introduction: *Campylobacter jejuni* and *Campylobacter coli* are leading bacterial agents of human foodborne illness in the United States and other industrialized nations. There is a high prevalence of both species in conventionally grown turkeys pre-harvest, and many strains exhibit resistance to multiple antibiotics. However, little is known about relative survival of different *Campylobacter* strains outside their animal host, in vehicles such as feces and water that are of high relevance for pre-harvest transmission.

Purpose: The purpose of this study is to examine the differential survival of turkey-derived *Campylobacter* in vehicles of special relevance for pre-harvest transmission.

Methods: Feces from *Campylobacter*-positive turkey flocks and a suspension of such feces in water were stored at 4°C and total *Campylobacter* populations were enumerated on CCDA at 48-hour intervals. Isolates from each time point (total, n = 548) were purified on Mueller Hinton Agar. For each isolate, species designations were determined by multiplex PCR and antibiotic susceptibility profiles were determined for five antibiotics: tetracycline (T), streptomycin (S), erythromycin (E), kanamycin (K), and ciprofloxacin (Q).

Results: *C. coli* survived longer than *C. jejuni* both in feces and in water ($P = 0.0005$ and $P < 0.0001$, respectively). At the initial enumeration (T0), TK and TKQ *C. coli* accounted for 19 and 21%, respectively, of total *C. coli* in both feces and water. These were the longest-surviving strains, accounting for 48% (TK) and 35% (TKQ) of *C. coli* at ≥ 4 days. *C. coli* resistant to all five antibiotics (TSEKQ) appeared unable to survive longer than 4 days in feces.

Significance: This study can further our understanding on relative survival of different species and strains of *Campylobacter* outside their avian host. Results will inform efforts to reduce transmission of this pathogen in turkeys pre-harvest.

P2-02 Withdrawn

P2-03 Influence of Compost Particle Size on Pathogen Survival under Greenhouse Conditions

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Developing Scientist Competitor

Introduction: Animal waste directly applied to an agricultural field is one of the possible contamination sources of fresh produce. Although pathogens can be eliminated by proper composting process, pathogens are able to survive, recolonize and regrow on compost heap surfaces. Furthermore, bioaerosols can be generated on compost surfaces with different particle sizes which can carry pathogens, travel via air and contaminate fresh produce nearby.

Purpose: This study was to investigate the survival of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in compost with different particle sizes as affected by initial moisture content and seasonality under greenhouse condition.

Methods: The mixture of avirulent *S.* Typhimurium and *E. coli* O157:H7 were inoculated into the finished compost with initial moisture content of 20%, 30% and 40%. Then, the finished compost samples were sieved into three portions with particle size of >1000, 500-1000 and <500 µm, and stored in greenhouse for 30 days. At selected intervals, compost samples were tested for pathogen population.

Results: The moisture contents in compost samples dropped to under 10% within 5 days of storage followed by gradual decline till 30 days in all treatments. For moisture contents of 20 and 30%, the average *Salmonella* reductions in compost with particle sizes of >1000, 500-1000 and <500 µm were 2.15, 2.27 and 2.47 log CFU/g within 5 days of storage in summer, respectively, as compared with 1.60, 2.03 and 2.26 log CFU/g in late fall, and 2.61, 3.33 and 3.67 log CFU/g for winter, respectively. *E. coli* O157: H7 reduction in compost with particle sizes followed the same trend as *Salmonella*.

Significance: Our results revealed that compost with larger particle size supports pathogen survival more than the compost with small particle size, and the initial rapid moisture loss in compost contributes to fast inactivation of pathogens in the finished compost.

P2-04 Survival of *Salmonella* in Food as a Function of Water Activity and Fat Level

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Introduction: *Salmonella* is known to survive in low water activity (a_w) foods for extended time. The combination of high fat and low a_w might have a protective effect in enhancing *Salmonella* survivability. However, there is a lack of information on how fat content will influence the survival of stressed *Salmonella* cells under these conditions.

Purpose: The purpose of this study was to evaluate survival of thermally-stressed *Salmonella* in a model food system (peanut paste) with various combinations of a_w and fat levels.

Methods: Four model peanut pastes with combinations of two different levels of fat (47 and 56%) and a_w (0.3 and 0.6) were inoculated with a dry inoculum of *Salmonella* Tennessee, *Salmonella* Typhimurium DT104 or *Enterococcus faecium*. Inoculated pastes were heat-treated at 75°C for 30-50 min to obtain approximately 1.0-log reduction of each organism and stored at 20 ± 1°C for ~ 1 year. Monthly monitoring of survivors was conducted using tryptic soy agar plate counts.

Results: Greater log-reductions of *Salmonella* Tennessee, *Salmonella* Typhimurium and *E. faecium* occurred in pastes at higher a_w than lower a_w regardless of the fat levels during 10 months of storage. *Salmonella* Tennessee showed a ~1.7 log CFU/g reduction in 0.3 a_w pastes at both fat levels; a greater reduction (2.7 and 2.3) was observed in the 0.6 a_w pastes at 47 and 56% fat, respectively. *Salmonella* Typhimurium showed a similar survival trend, with enhanced survival occurring in 0.3 a_w pastes when compared to the 0.6 a_w pastes. While a similar trend occurred in *E. faecium*,

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higher survival rates were observed for the surrogate in all tested conditions; the decline of *E. faecium* in all formulations was within the range of 1.1-1.8 log.

Significance: The study provides information on the influence of fat on *Salmonella* survival in low a_w foods. Higher levels of fat in a peanut paste matrix appear to decrease the survival of *Salmonella*.

P2-05 Modeling the Physiological Response of *Salmonella* to Heat Shock during Slow Cooking Processes

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Undergraduate Student Award Competitor

Introduction: Sublethal heating, as can occur during slow cooking of meat products, is known to induce increased thermal resistance in *Salmonella*. However, very few studies have addressed the kinetics of this response. Several recent studies have reported improved thermal inactivation models that include the effect of prior sublethal history on subsequent thermal resistance; however, none of these models were based on cellular-level responses to the sublethal conditions.

Purpose: To quantify heat stress regulation gene transcript levels in *Salmonella enterica* subsp. *enterica* serovar Montevideo strain S5-403 subjected to various time-temperature profiles, in order to better model the physiological response to sublethal heat stress that can occur during slow cooking operations.

Methods: *Salmonella* culture (100 µl) was incorporated into 0.2 ml PCR tubes containing 100 µl of TSB . Samples were then subjected to various heating profiles (including a sublethal holding period at 40 or 45°C for 5, 10, 15, 30, 60, or 180 min) using a PCR thermocycler. RNA was then extracted from *Salmonella* in the heat-treated samples and its quality assessed on an Agilent 2100 Bioanalyzer. cDNA synthesized from the extracted RNA was used to assay for and quantify heat stress regulation gene transcript levels for stress genes *rpoH, dnaK, clpB, ibpA, htpG,* and *degP* using the ABI Prism 7000 Detection System (Applied Biosystems).

Results: The transcript levels were evaluated in terms of the influence of sublethal holding temperature, holding time, and their interaction. For example, in comparing the initial, maximum, and final log copy numbers for each gene, *ibpA* displayed significant changes in transcript level over time, with a significant increase during the first 15 min at 40°C ($P < 0.05$), followed by a decrease to a final level (at 180 min) that was still greater than the initial level ($P < 0.05$).

Significance: The quantitative description of the cellular-level responses to sublethal thermal injury will enable improved population-level models formulated on these underlying kinetics, rather than purely mathematical constructs.

P2-06 Heat Resistance of *Salmonella* Tennessee in Model Peanut Paste Formulations at Four Different Levels of Fat and Water Activity

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Introduction: It is well recognized that the heat resistance of *Salmonella* is markedly increased when a_w is reduced by addition of the solutes or by removal of water. It has been also suggested that *Salmonella* have increased heat resistance in foods with high fat concentration.

Purpose: The objective of this study was to evaluate how water activity (a_w) and fat level affect the thermal destruction of *Salmonella* Tennessee in model food systems made from peanut flour and oil.

Methods: Sixteen peanut paste combinations of four fat concentrations (47, 50, 53 and 56%) and four a_w (0.3, 0.4, 0.5 and 0.6), were inoculated with dry inoculum on talc (~1 x 10^6CFU/g) and tested for thermal resistance at 70 and 75°C. A modified thermal death test method using magnetic copper plates to compress the samples at ≤ 1 mm thickness was used. Cell counts were obtained by plating samples onto tryptic soy agar. Counts of survivor cells were fitted to the Weibull model using the Geeraerd and Van Impe Inactivation Model Fitting Tool.

Results: Greater inactivation of *Salmonella* Tennessee was achieved at 75 than 70°C. After 50 minutes of treatment at 70°C, the greatest heat resistance (≤ 2.1 log-reduction) of *Salmonella* Tennessee was observed in the formulations at 47% fat and 0.3 and 0.6 a_w, and in the formulation containing 53% fat and 0.6 a_w. At 75°C, significantly greater heat resistance occurred in the formulations at lower fat levels (47 and 50%) than at the higher levels (53 and 56%). The inactivation curves were upwardly concave, indicating rapid death in the first 3-6 min, followed by lower death rates and an asymptotic tail.

Significance: Understanding how the properties of food, such as fat content and a_w can affect the heat resistance of *Salmonella* in food will allow the food processors to formulate foods and food processes that will destroy the organism. The inactivation data from this study will be used in predictive modeling.

P2-07 Thermal Inactivation of Desiccation-adapted *Salmonella* spp. in Aged Chicken Litter

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Developing Scientist Competitor

Introduction: Heat-treated chicken litter is recycled as an organic fertilizer or soil amendment for agricultural production. However, chicken litter may contain loads of human pathogens, such as *Salmonella*. Some populations become acclimatized to desiccation environment during stockpiling and develop heat resistance during subsequent high temperature processing.

Purpose: The objective of this study was to investigate the thermal inactivation of desiccation-adapted *Salmonella* spp. in aged chicken litter and to examine potential cross-tolerance of desiccation-adapted *Salmonella* spp. to heat treatment.

Methods: A mixture of four *Salmonella* serotypes was inoculated into the finished compost with 20, 30, 40, and 50% moisture contents for a 24-h desiccation adaptation. Afterwards, the compost with desiccation-adapted cells was added into the aged chicken litter with the same moisture contents for heat treatments at 70, 75, 80, and 150°C. Recovery media were used to allow injured cells to resuscitate.

Results: A 5-log reduction of the desiccation-adapted *Salmonella* cells in chicken litter with 20% moisture content required >6, >6, and 4~5 h exposure at 70, 75, and 80°C, respectively, whereas the same reduction in non-adapted control with 20% moisture content was achieved within 1.5~2, 1~1.5, and 0.5~1 h at 70, 75, and 80°C, respectively. Time required to obtain a 5-log reduction in desiccation-adapted cells gradually became shorter as temperature and moisture content were increased. At 150°C, desiccation-adapted *Salmonella* survived for 50 min in chicken litter with 20% moisture content, whereas control cells were detectable by enrichment until only 10 min.

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Significance: Our results demonstrated that the thermal resistance of *Salmonella* in aged chicken litter was increased significantly when the cells were adapted to desiccation. Therefore, the chicken litter processors need to validate and modify their heating process in order to eliminate *Salmonella* that may be subjected to dry stress.

P2-08 The Effect of UV Radiation on Survival of *Salmonella enterica* in Dried Manure Dust

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Introduction: Animal manure has been shown to harbor *Salmonella enterica*, an enteric pathogen known to be resilient to environmental stresses such as desiccation and solar UV radiation. In farm settings, it has been observed that unintended aerosolization could occur when manure becomes dehydrated, resulting in the exposure of leafy crops to wind-driven manure dust. In order to appraise the risk contributed by aerosolized particulate manure to produce fields, it is important to determine whether these particles can act as a barrier to protect *Salmonella* from damaging effects of UV light.

Purpose: This study investigated the effect of UV radiation on the survival of *Salmonella* when present on dried manure particles.

Methods: *In vitro* survival under UV-A (365 nm) of *Salmonella* inoculated into manure dust and dispersed as a thin layer on a petri dish covered with filter paper was compared to exposure under similar conditions using a thin layer of cells that were directly applied to the test surface. The dust was obtained by dehydrating turkey manure to < 5% moisture content and processing until particle sizes of approximately 125 µm were achieved.

Results: Analysis showed that the presence of manure particles significantly ($P < 0.05$) protected *Salmonella* from UV exposure. *Salmonella* cells exposed to UV in a control medium showed a 5-log decline within 80 min compared to the 1.5-log decrease in the manure dust matrix. This was in spite of the higher initial inoculum level of control samples (control inoculum 7.5×10^{10} CFU/ml; dust inoculum 6.75×10^{6} CFU/g).

Significance: These data suggest that manure dust particles can provide protection from lethal UV rays to *Salmonella* cells, thereby increasing the risk of edible-crop contamination in pre-harvest settings. The ability of manure dust matrix to shield *Salmonella* from damaging UV effects could increase this pathogen's survival on leafy greens during cultivation.

P2-09 A Meta-analysis of *Salmonella* Inactivation Parameters and Data for Thermal Pasteurization of Low-moisture Foods

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Introduction: There has been a recent rapid expansion of research into thermal pasteurization of low-moisture foods. However, the utility of such research is hindered by a lack of standard methods (experimental and analytical), the special challenge of dynamic product moisture, and limited accessibility and characterization of results. As a result, the industry lacks adequate data and tools to reliably address the pressing need for process validation embodied in proposed federal rules for preventive controls.

Purpose: The purpose of this project was to conduct a meta-analysis of existing thermal inactivation data and parameters for *Salmonella* in low moisture foods.

Methods: Data were compiled from a range of available studies that included thermal inactivation of *Salmonella* in/on low-moisture food matrices. A minority of the data were acquired directly from ComBase, while other publications were identified and acquired through searches of various indexes and journals. Data from 20 representative studies were characterized in terms of product, conditions, and the extent to which data variability and model uncertainty were reported

Results: The representative studies encompassed ~600 inactivation data sets that included ~4,200 individual data points. The fraction of these data involving particulates (e.g., nuts), powders (e.g., flour), and pastes (e.g., peanut butter) were 55% (n = 2,286), 42% (n = 1.763), and 3% (n = 144), respectively. Only 10% of the studies reported any measure of replication error to characterize uncertainty; 75% reported some indication of parameter estimation error. Of the selected studies, 65% were performed under isothermal conditions, and 30% (n = 6) included dynamic water activity. However, of the 70% that reported an inactivation model, only one study reported a model accounting for dynamic water conditions.

Significance: The resulting database of *Salmonella* thermal resistance data for low-moisture foods meets an important need for an industry, which currently lacks access to and analysis of data and models sufficient to use them to validate thermal pasteurization processes.

P2-10 Sodium Chloride Habituation Increased Thermal Resistance and Caco-2 Cell Invasion of *Salmonella*

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Developing Scientist Competitor

Introduction: Many processed foods contain sodium chloride, and *Salmonella* spp. could be exposed to sodium chloride. The pathogen may resist to thermal process and then invade intestinal cells, resulting in salmonellosis.

Purpose: Therefore, the objective of this study was to evaluate the effect of sodium chloride on thermal resistance and Caco-2 cell invasion of *Salmonella*.

Methods: Ten strains of *Salmonella* were inoculated in tryptic soy broth (TSB) at 35°C for 24 h. The cells were then challenged to 60°C for 1 h, and survivals were enumerated on tryptic soy agar (TSA). *Salmonella* cells were also examined for Caco-2 cell invasion. Two strains of *Salmonella* were then selected for the highest thermal resistance/Caco-2 cell invasion efficiency (*Salmonella* Enteritidis NCCP10812) and the lowest antibiotic resistance/biofilm formation (*Salmonella* Enteritidis NCCP12243). Two strains were subjected to TSB plus 0, 2, and 4% NaCl for 24 h at 35°C, or exposed to sequentially increased NaCl concentrations up to 4% NaCl. Subsequently, the cells were challenged to 60°C for 1 h and inoculated on to Caco-2 cells. *Salmonella* cells were then recovered on TSA.

Results: *S.* Typhimurium NCCP10812 showed increased thermal resistance ($P < 0.05$) as sodium chloride concentration increased. However, the thermal resistance of the pathogen was not increased by sequential increase of NaCl concentration. Regarding Caco-2 cell invasion efficiency, the invasion efficiency of *S.* Typhimurium NCCP10812 was not increased, but the efficiency of *S.* Enteritidis NCCP12243 was increased ($P < 0.05$) as sodium chloride concentration increased. Moreover, *S.* Enteritidis NCCP12243 had increased Caco-2 cell invasion efficiency ($P < 0.05$) by sequential increase of sodium chloride concentration.

Significance: These results indicate that sodium chloride in food may increase thermal resistance and cell invasion efficiency of *Salmonella*.

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P2-11 Mitigation of *Salmonella* in Cattle Lymph Nodes in a Commercial Feedlot Setting Using NP51, a *Lactobacillus*-based Pre-harvest Intervention

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Introduction: Our laboratory previously conducted a two year surveillance of cattle lymph nodes from multiple regions which indicated that up to 70% were positive for *Salmonella* during certain seasons of the year. *Salmonella* harbored in the lymph nodes of cattle can evade carcass decontamination procedures in slaughter plants. Some of the lymph nodes including the subiliac are located in the carcass trim utilized for ground beef thereby potentially contaminating the product.

Purpose: This study was conducted to evaluate a pre-harvest direct-fed microbial intervention including *Lactobacillus acidophilus* NP51 at a rate of 10^9/head/day (NP51) on the reduction of *Salmonella* prevalence in cattle lymph nodes.

Methods: Approximately 1,800 cattle were randomized into two treatments in a commercial feedlot with 12 pens/treatment and 75 head/pen. The treatments included control cattle and cattle supplemented with 10^9/head/day NP51. Subiliac lymph nodes were obtained from approximately 25 animals/pen (n= 600) at the slaughter facility and were tested for *Salmonella* with previously-published qualitative and quantitative methods.

Results: *Salmonella* was recovered from 25% ($P < 0.01$) fewer lymph nodes for cattle fed NP51 when compared to controls. Quantitatively the NP51 cattle had significantly less ($P < 0.05$) *Salmonella* in lymph nodes (3.1 vs 4.2 log CFU/lymph node) and per gram of lymph nodes (1.9 vs. 4.2 log CFU/g). Control samples were more likely to have a higher concentration of *Salmonella* in lymph nodes with 10.4% vs. 11.7% between 3 and 4 log CFU/g; 13.7% vs. 6.4% between 4 and 5 log CFU/g, and 7.5% vs. 2.1% greater than 5 log CFU/g.

Significance: The results of this study indicate that supplementation with NP51 is an effective pre-harvest intervention to reduce the prevalence of *Salmonella* in cattle lymph nodes. This will potentially decrease the contamination of ground beef from lymph nodes and the associated tissues that are incorporated into this product.

P2-12 Confirmation and Typing of *Salmonella* by Genome Sequence Scanning in Presumptive Positive Food Samples

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Introduction: Safe production, distribution and storage of foods are a difficult responsibility of the food industry that requires rapid and accurate detection of foodborne pathogens. Pathogenetix has developed an automated instrument to type bacterial pathogens based on Genome Sequence Scanning (GSS) technology. GSS system automatically prepares long restriction DNA fragments (> 90 kb), which are fluorescently labeled with sequence-specific probes and a nonspecific intercalator. These DNA molecules are then stretched in a microfluidic device and passed through light spots that excite fluorescence of the probes. These optical patterns are defined by the underlying DNA sequence and are compared to a database for strain identification.

Purpose: The purpose of this study is to demonstrate typing of *Salmonella* in different food matrices following enrichment for a standard screening assay.

Methods: We spiked 25 g samples of ground beef or fresh spinach with < 5 CFU of one of the following *Salmonella* strains: 3 each of Enteritidis and Typhimurium and 1 each of Newport, Javiana, Montevideo, and Heidelberg. One set of experiments also included 10-fold excess of spiked microbes from cow feces as competing background flora. All samples were tested six times by enriching in BPW with *Salmonella* supplement (Biomerieux) for 18 h at 42°C and analyzed by GSS. Total aerobic counts and *Salmonella* were measured on TSA and XLD agars, respectively, for enrichment control.

Results: Of the 240 samples spiked with *Salmonella*, 235 were confirmed to be positive for the presence of the correct serovar by GSS analysis. *Salmonella* was not detected in five samples (4 in spinach, 1 in ground beef) due to poor enrichment. No false positives were detected from any of the 24 unspiked samples.

Significance: The data shows that GSS performed sub-typing *Salmonella* serovars in two different matrices using a commercial broth even in the presence of other bacteria.

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P2-13 CRISPR-MVLST Identifies Populations of *Salmonella* Typhimurium with Differences in Distribution and Antibiotic Resistance

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Developing Scientist Competitor

Introduction: *S.* Typhimurium is the second most common clinically reported serovar of *Salmonella,* but little is known about the ecology of this important pathogen within the food system and how it relates to the distribution and spread of antibiotic resistance.

Purpose: Previously, we developed a new subtyping method, CRISPR-MVLST, which is capable of discriminating between clonal populations of several common *Salmonella* serovars. Here, we hypothesize that CRISPR-MVLST will identify source associated populations within *S.* Typhimurium and separate populations within *S.* Typhimurium with distinct antibiotic resistance profiles.

Methods: A collection of 80 *S.* Typhimurium isolates from a variety of animal sources throughout central Pennsylvania was assembled and the CRISPR1, CRISPR2, *fimH*, and *sseL* alleles of each isolate were sequenced to determine a CRISPR-MVLST subtype. Each isolate was tested for resistance to a panel of 18 antibiotics, and associations between CRISPR-MVLST subtype and resistance to each antibiotic in the panel were tested using a Fisher's exact test ($P < 0.05$).

Results: CRISPR-MVLST identified 22 subtypes within the isolate collection, six of which were identified in more than two isolates. Two of these more frequently identified subtypes were identified in only a single animal source, while the other subtypes were all identified in several animal sources. Prevalence of resistance to seven antibiotics tested differed significantly among the six most frequently identified subtypes.

Significance: Therefore, CRISPR-MVLST is a promising subtyping method for monitoring the spread of antibiotic resistance in *S.* Typhimurium.

P2-14 Evaluation of the 3M™ Molecular Detection Assay (MDA) *Salmonella* for the Detection of *Salmonella* in a Variety of Foods: Collaborative Study

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Introduction: The 3M Molecular Detection Assay (MDA) *Salmonella* method, in conjunction with 3M™ Buffered Peptone Water ISO (BPW ISO), uses isothermal amplification of nucleic acid sequences to detect *Salmonella* in enriched food, feed and environmental samples. The MDA offers high specificity, efficiency, rapidity and bioluminescence to detect amplification of *Salmonella* spp. after 18 hours of enrichment.

Purpose: The purpose of this AOAC OMA Collaborative Study was to compare the assay to the FDA/BAM for wet pet food (375 g) and the USDA/FSIS-MLG for raw ground beef (25 g).

Methods: This new method was compared in a multilaboratory collaborative study to the FDA/BAM Chapter 5 method and the USDA MLG 4.05 method. Each matrix was artificially contaminated with *Salmonella* at 3 inoculation levels: a low-level inoculum of 0.2-2 CFU/test portion, a high-level inoculum 2-5 CFU/test portion and an un-inoculated control level 0 CFU/test portion. A total of 20 laboratories representing government, academia and industry, throughout the United States, participated.

Results: In this study, 1,512 samples were analyzed, of which 756 were analyzed by the assay and 756 were analyzed by either the USDA/FSIS-MLG or FDA/BAM reference method. Of the 1,512 unpaired replicates, 387 were presumptive positive by the MDA *Salmonella* method, with 383 confirmed positive samples. There were 389 confirmed positive replicates by the USDA/FSIS-MLG or FDA/BAM reference methods. Statistical analysis was conducted according to the Probability of Detection (POD) and the Relative Limit of Detection (RLOD) and no statistically significant difference was observed between the new and reference methods.

Significance: For all foods evaluated, the MDA *Salmonella* assay demonstrated comparable results to the reference methods for the rapid detection of *Salmonella* in as little as 18 hours of enrichment.

P2-15 A Comparative Evaluation of the 3M™ Petrifilm™ *Salmonella* Express System for the Detection of *Salmonella* Species in Food and Environmental Surfaces

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Introduction: The 3M™ Petrifilm™ *Salmonella* Express (SALX) System is designed for rapid detection of *Salmonella* in food, feed and food process environmental samples after 18-24 hours of enrichment. The assay plate is a chromogenic culture medium system that contains a cold water-soluble gelling agent and is selective and differential for *Salmonella*.

Purpose: The purpose of this internal evaluation was to compare the new method to the FDA/BAM for frozen uncooked shrimp, fresh spinach, dry dog food and stainless steel; to the USDA/FSIS-MLG method for raw ground chicken, pasteurized liquid whole egg, raw ground beef, raw ground pork and cooked chicken nuggets; and to the ISO 6579 method for raw ground chicken and liquid whole egg.

Methods: Each matrix was inoculated with a different strain of *Salmonella* and 20 replicates were analyzed at one inoculum level: 0.2-2 CFU/test portion. Five control replicates were analyzed at 0 CFU/test portion. For the new method, replicates were enriched in the *Salmonella* Enrichment Base containing *Salmonella* Enrichment Supplement for 18-24 hours at 41.5 ± 1°C. For foods with low microbial loads, test portions were streaked onto Petrifilm SALX after 18 and 24 hours broth incubation. For foods with high microbial loads, 0.1 mL of the primary enrichment was transferred into 9.9 mL of Rappaport-Vassiliadis R10 (RV[R10]) broth at both 18 and 24 hours of primary enrichment and incubated at 41.5 ± 1°C. Samples were streaked at 8 and 24 hours.

Results: No significant differences were observed between the new method and the reference methods as indicated by Mantel-Haenszel chi-square analysis and the probability of detection (POD) for all 9 matrixes.

Significance: For all foods evaluated, the Petrifilm *Salmonella* Express System demonstrated comparable results to the reference methods for the rapid detection and biochemical confirmation of *Salmonella*.

P2-16 Evaluation of the 3M™ Petrifilm™ *Salmonella* Express System for the Detection of *Salmonella* Species in Food, Feed, Environmental Surfaces and Strain Testing Results

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Introduction: The 3M™ Petrifilm™ *Salmonella* Express System is intended for the rapid detection of *Salmonella* in food, feed and food process environmental samples. The system includes an enrichment medium and Petrifilm plate with a biochemical confirmatory disk.

Purpose: The purpose of these internal studies was to evaluate the new system as compared to the FDA/BAM or USDA/FSIS-MLG methods for pet foods, raw ground meats, raw vegetables, processed meats, chicken rinses and environmental samples and with a diverse population of known *Salmonella* serotypes and non-*Salmonella* microorganisms.

Methods: Approximately 40 samples for each matrix group were evaluated with a strain of *Salmonella* at <5 CFU/25g or uninoculated with the new system and reference methods for recovery. In addition, raw ground beef and chicken were evaluated with 20 fractionally spiked replicates and 5 control replicates per food. Testing was also done with 105 *Salmonella* serotypes and 32 non-Salmonella strains. For the new system, samples and strains were enriched in *Salmonella* Enrichment Base containing *Salmonella* Enrichment Supplement at 41.5°C. For low microbial load foods, enrichments were sampled at 18 hours. For high microbial load foods and strains, 0.1 ml samples of the primary cultures were transferred into 10 ml of R-V R10 broth and incubated at 41.5°C for 8 hours. All enrichments were streaked onto the new plating medium and incubated 41.5°C for 22 hours. Plates were subsequently tested with the *Salmonella* Express Confirmation Disk for 4 hours at 41.5°C.

Results: No significant differences were observed between the new system and the reference methods as indicated by Mantel-Haenszel chi-square analysis or Student's t-test for all sample matrices. The new test system showed sensitivity and specificity of >98% among the pure culture strains.

Significance: For all matrices evaluated, the Petrifilm *Salmonella* Express System gave equivalent results to the reference methods for the rapid detection and biochemical confirmation of *Salmonella* within the procedures recommended time frame.

P2-17 Performance of the 3M™ Molecular Detection Assay *Salmonella* as Compared to the Canadian Reference Method MFHPB-20

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Introduction: The Public Health Agency of Canada estimates that about 11 million Canadians suffer from a food-related illness each year. As of 2011, *Salmonella* continued to be the most common pathogen reported to the National Enteric Surveillance Program (NESP), a total of 6809 *Salmonella* isolates (19.68 per 100 000 population). The 3M™ Molecular Detection Assay *Salmonella* is used with the 3M™ Molecular Detection System for the rapid and specific detection of *Salmonella* in enriched food and environmental samples. The 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 Molecular Detection Assay *Salmonella* against the Compendium of Analytical Method MFHPB-20 in a variety of food matrices for the inclusion in the Compendium of Analytical **Methods** as a Laboratory Procedure (MFLP).

Methods: The method and comparative reference method (MFHPB-20) were analyzed by testing 5 food categories with 3 matrices per category (ready-to-eat meat and poultry, fish and seafood products, dairy products, fruit and vegetable-based products, eggs and derivatives). 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 (POD) statistical model and exceeded the criteria outlined in the Health Canada MMC. Additionally, Level of Detection (LOD) results showed a range of 0.312 – 3.2 MPN/25g.

Significance: The Molecular Detection Assay *Salmonella* showed excellent performance and exceeded the Canadian requirements of the MMC. This new method offers the capability of detecting *Salmonella* species in foods after only 18-24 hours of incubation, thereby reducing presumptive reporting times over the reference method by almost 24 hours.

P2-18 Evaluation of the VIDAS® UP *Salmonella* Assay (SPT) for the Detection of *Salmonella* in a Variety of Foods and Environmental Surfaces: Collaborative Study

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Introduction: The VIDAS® UP *Salmonella* assay (SPT) utilizes recombinant phage proteins to detect *Salmonella* species in human and animal food products and production environmental samples after 18-26 hours of enrichment. This new method utilizes a single primary enrichment supplemented with a proprietary additive to eliminate the need for secondary enrichments.

Purpose: The purpose of this AOAC OMA Collaborative Study was to compare the assay to the USDA/FSIS-MLG for raw ground beef in 25-g and 375-g test portions.

Methods: This new method was evaluated in a multilaboratory collaborative study with 15 participating laboratories representing government, academia and industry. Each test portion size evaluated was artificially contaminated with *Salmonella* at 3 inoculation levels: a low-level inoculum of 0.2-2 CFU/test portion, a high-level inoculum 2-5 CFU/test portion and an un-inoculated control level 0 CFU/test portion. Assay samples were confirmed following the traditional confirmation procedures and an alternative confirmation procedure, by directing streaking the primary enrichments onto IBISA and ASAP chromogenic agar.

Results: In this study, 828 samples were analyzed by both the assay and the USDA/FSIS-MLG method for a total of 1,656 test portions tested. Of the 1,656 unpaired replicates, 475 were presumptive positive by the assay method, with 475 confirmed positive samples. Comparatively, there were 411 confirmed positive replicates by the USDA/FSIS-MLG reference method. Statistical analysis was conducted according to the Probability of Detection (POD) and the Relative Limit of Detection (RLOD) and no statistically significant difference was observed between the new and reference methods. There was no difference in the recovery of *Salmonella* on the chromogenic agars compared to the reference agars.

Significance: For both test portion sizes evaluated, the new method produced results comparable to the reference methods for the rapid detection of *Salmonella* in a variety of foods and environmental samples.

P2-19 A Comparative Evaluation of the BAX® System Real-time PCR Assay for *Salmonella* and the BAX® System PCR Assay for *Salmonella* 2 for Detecting *Salmonella* Enteritidis in Shell Eggs

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Introduction: The BAX® System Real-Time PCR Assay for *Salmonella* and BAX System PCR Assay for *Salmonella* 2 utilize Polymerase Chain Reaction (PCR) to amplify a specific fragment of bacterial DNA, which is stable and unaffected by growth environment and detected using the system software. These new methods simplify the PCR process by combining the requisite primers, polymerase and nucleotides into a stable, dry, manufactured tablet already packaged inside the PCR tubes. After amplification, these tubes remain sealed for the detection phase, thus significantly reducing the potential for contamination with one or more molecules of amplified PCR product. The Real-time *Salmonella* assay provides results in real-time reducing the time to final detection. The system *Salmonella* 2 assay utilizes a proprietary "hot-start" technology to help reduce the effects of human error.

Purpose: The purpose of the evaluation was to compare the BAX System methods to the FDA BAM for the detection of *Salmonella* Enteritidis in shell eggs.

Methods: For each new method, 20 replicates of approximately 1,000 g each (20-egg pool) were analyzed at one inoculum level: 0.2-2 CFU/test portion. Five control replicates were analyzed at 0 CFU/test portion. For each PCR Assay for *Salmonella,* aliquots of each enriched sample were analyzed with and without a 3 hour re-growth step in BHI.

Results: The results of a Mantel-Haenszel Chi-square analysis indicated no statistically significant difference observed between the two new methods and the FDA BAM reference method ($\chi^2 < 3.84$). For both the BAX System methods a χ^2 value of 1.21 was obtained. No false negatives or false positives were reported.

Significance: For the method comparison, these new methods demonstrated high specificity in the identification of *Salmonella* Enteritidis in shell eggs reducing the time of detection from 7 days to 2 days.

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P2-20 Habituation on Vegetable Surfaces Affects the Resistance of *Listeria monocytogenes* and *Salmonella* to Acidic, Osmotic and Thermal Stress

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Developing Scientist Competitor

Introduction: Foodborne pathogens colonize vegetable surfaces, where cells are exposed to desiccation, cold temperatures and nutrient limitations. This adaptation may affect their stress tolerance.

Purpose: To evaluate the effect of habituation of *Listeria monocytogenes* and *Salmonella* spp. on tomato or lettuce, on their subsequent resistance to lethal stresses.

Methods: Fresh cut lettuce or whole cherry tomatoes were inoculated (10^6 CFU/cm^2) with three strains of *L. monocytogenes* serotypes 1/2a, 4b or *Salmonella* spp. serovars Typhimurium, Enteritidis and incubated for 1 or 5 days at 5°C. Adapted cells were harvested and exposed in Tryptic Soy Broth to (i) pH 3.5 (lactate, acetate or HCl) and pH 1.5 (HCl) for 6 h; (ii) 20% NaCl for 14 days and (iii) 60°C for 150 s. *L. monocytogenes* grown overnight (TSBYE) at 30°C or *Salmonella* at 37°C (TSB) and at 5°C for 1 or 5 days were used as *in vitro* controls.

Results: Habituation on lettuce for 1 day sensitized *L. monocytogenes* to pH 3.5 compared to control (30°C); however after 5 days, resistance of cells to acetic acid and pH 1.5 increased ($P < 0.05$). Cells adapted on tomato surface survived at pH 1.5 for 5 h, while control cells only for 1 h. During osmotic stress, cells habituated on tomato surface for 5 days reduced to <0.7 log CFU/ml after 14 days, while those habituated for 1 day after 8 days; lettuce-adapted and control cells survived only for 4 days. Under thermal stress, tomato-adapted cells decreased below the limit of 0.7 log CFU/ml, while control cells and those adapted to lettuce remained at 2.5 log CFU/ml after 150 s. *Salmonella* adapted to lettuce or tomato surface, died-off after one day in 20% NaCl, whereas control cells (37°C) survived for 10 days.

Significance: Pathogen habituation on vegetables may enhance their stress tolerance and *in vitro* models are insufficient to assess such a risk.

P2-21 Diversity and Stability of Heat-stress Adaptation in 37 Strains of *Listeria monocytogenes*

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Introduction: The capacity of *Listeria monocytogenes* (*Lm*) to adapt to lethal heat-stress increases the likelihood of food safety associated risks.

Purpose: We have determined the heat-stress adaptation and heat tolerance response of 37 diverse strains of *Lm* representing all 13 serotypes and of 7 other *Listeria* spp**.**

Methods: A diverse set of 37 *Lm* strains were evaluated for heat tolerance at 60°C with 10^7 CFU/ml in TSBYE after pre-exposure to mild heat of 48°C for 0, 5, 15, 30, 60 and 90 min. Survival curves were plotted and D-values were calculated. Lag phase of mild heated cells was observed by measuring the optical density at 630 nm at regular intervals. The stability of heat-stress adaptation after pre-exposure to 48°C was determined by cooling *Lm* cells to 25°C or down to 4°C before 60°C lethal stress. Survivals were enumerated on TSA plates enriched with esculin and ferric ammonium citrate.

Results: The 37 strains of *Lm* were classified into three groups based on their survival at 60°C/10 min: low (0-2 log CFU/ml), medium (2-4 log) and high (4-6 log) heat tolerant strains. Heat-stress adaptation response of *Lm* was dependent on the time of pre-exposure to mild heat-stress. About 30-60 min pre-exposure to 48°C led to a maximum heat-stress adaptation in *Lm* strains where cells of some strains exhibited 5 log CFU/ml greater survival than non-stressed cells when subjected to 60°C lethal challenge. The heat-stress adaptation was stable in *Lm* after cooling cells from 48°C to 25°C for 2 h or for up to 24 h if cooled from 48°C to 4°C prior to challenging at 60°C.

Significance: Heat-stress adaptation of *Lm* differs with different strains. The stability of heat-stress adaptation in *Lm* after cooling cells to 4°C may create a risk for ready-to-eat foods.

P2-22 Formation of Alkali-stress Resistant Phenotypes of *Listeria monocytogenes*

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Introduction: *Listeria monocytogenes (Lm)* is capable of undergoing a phenomenon called stress hardening in which cells exposed to mild stress conditions develop increased resistance to lethal stress challenges.

Purpose: The purpose of this study is to determine the conditions inducing alkaline tolerance response in *Lm* 1/2a and 4b strains and the stability of such stress-resistant phenotypes.

Methods: *Lm* cells were pre-exposed to pH 9.0 for 1 h at 37°C followed by post-exposure to lethal pH 11.5 at 4°C, 22°C and 37°C in TSBYE. The time taken to induce alkali-stress adaptation was demonstrated by pre-exposing cells at pH 9.0 for 5, 15, 30 and 60 min. The stability assay was carried out by transferring adapted cells to neutral broth for 5, 15, 30, 60 and 75 min prior to lethal alkali stress in pH 11.5 using 4M KOH or NaOH.

Results: Mild alkali stressed *Lm* cells (pH 9/1 h) exhibited greater than 3 log CFU/ml survival as compared to non-stressed cells at lethal alkali pH 11.5 for 8 h. Only 15 min of mild alkali-stress was sufficient to induce alkali-stress resistant phenotype in *Lm* Bug600 and ScottA. Alkaline-stress adaptation in *Lm* was reversible within 1.5 h when cells were transferred to neutral pH. Similar patterns of alkaline-stress adaptation of *Lm* was observed at 37°C (greater than ~ 4 logs CFU/ml survival) when cells were pre-exposed to mild KOH instead of NaOH.

Significance: The alkaline-stress resistant phenotypes of *Lm* were easily formed when cells were exposed to sublethal alkali-stress at different temperatures.

P2-23 Filamentation of *Listeria monocytogenes* in the Presence of Sublethal Dose of Bacteriocins

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Developing Scientist Competitor

Introduction: Pathogenic foodborne bacteria can form filaments under environmental stresses such as cold temperature, acid and salt. These filaments are comprised of long-strings of undivided cells and can rapidly divide into daughter cells and start to multiply when placed in favorable growth conditions.

Purpose: The purpose of this study was to examine whether a sublethal dose of bacteriocins can cause filamentation in *Listeria monocytogenes*.

Methods: *Listeria monocytogenes* 08-5923 was grown in tryptic soy broth at 37°C and exposed to sublethal concentrations of the cell-free supernatant of *Clostridium maltaromaticum* UAL307. After 24 h, cells were fixed with 1% (v/v) formaldehyde for flow cytometry analysis to estimate the percentage of filamented cells in the population. For transmission electron microscopy (TEM) cells were fixed with 10% (v/v) formaldehyde and pelleted by centrifugation at 11,000 *x g* for 15 s prior to preparation for TEM.

Results: Treatment of *L. monocytogenes* 08-5923 resulted in a significant increase in the mean forward light scatter. Following 24 h exposure to the cell-free culture supernatant of *C. maltaromaticum* UAL307, nearly 90% of the *Listeria* population was filamented. TEM analysis of these cells showed that *L. monocytogenes* does not contain segments between the undivided daughter cells within the filaments and these filaments do not have flagella. In conclusion, the sublethal dose of the cell-free supernatant of *C. maltaromaticum* UAL307 induced filamentation in *L. monocytogenes* 08-5923.

Significance: This is the first evidence that a sublethal dose of bacteriocins caused filamentation in *L. monocytogenes*. The presence of filamented cells has the potential to underestimate the number of viable cells when determining counts of *L. monocytogenes* for regulatory compliance.

P2-24 Development and Characterization of Murinized *Listeria monocytogenes* Strains Carrying the Most Common Forms of Internalin A Premature Stop Codons

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Introduction: Internalin A (InlA) is used by *Listeria monocytogenes* (*Lm*) to invade host epithelial cells. InlA binds certain isoforms of E-cadherin (human and guinea pig) to cross the intestinal barrier; however, it shows limited interaction with murine E-cadherin.

Purpose: Engineer murinized *Lm* strains carrying the most common InlA premature stop codons (PMSCs) and characterize the invasion phenotype and virulence of resultant strains.

Methods: Site directed mutagenesis was used to introduce InlA PMSC3 and PMSC4 into a previously murinized strain (EGD-eInlA^{m*}). Growth and cell invasion capabilities were investigated using EGD-e, EGD-eInlA^{m*}, EGD-eInlA^{m*}:PMSC3, and EGD-eInlA^{m*}:PMSC4. For growth curves, BHI broth was inoculated with populations of the four strains (6.68 log (CFU/ml)) and was maintained at 37°C for 12 h. Invasion was assayed in mouse CT-26 and human Caco-2 cells to characterize invasiveness of all strains. BALB/c mice were intragastrically infected with EGD-e, EGD-eInlA^{m*}, or EGD-eInlA^{m*}:PMSC3 at 9.3 log CFU and recovery of strains from internal organs was used to define infection.

Results: All isolates grew similarly at 37°C and exceeded 9.7 log (CFU/ml) by 12 h. In CT-26 cells, invasion of EGD-eInlA^{m*} was significantly ($P < 0.05$) higher compared to all strains. As expected, invasion did not differ ($P > 0.05$) between EGD-e and EGD-eInlA^{m*} in Caco-2 cells, and both PMSC strains showed attenuated invasion ($P < 0.05$). After intragastric infection, EGD-eInlA^{m*} showed higher ($P < 0.05$) bacterial levels in the liver, spleen, mesenteric lymph nodes, and small intestines as compared to EGD-e and EGD-eInlA^{m*}:PMSC3. Importantly, EGD-eInlA^{m*}:PMSC3 displayed attenuated virulence as determined by lower bacterial loads in livers and spleens.

Significance: We engineered murinized *Lm* strains with the most common InlA PMSCs and demonstrated predicted invasion and virulence characteristics of these strains. Because immunological reagents are unavailable for the guinea pig model, it is critical to obtain a murine *Lm* oral infection model permitting investigation of immunological responses to InlA PMSC strains, which are commonly isolated from ready-to-eat foods (>45%).

P2-25 Ability of Virulence Attenuated *Listeria monocytogenes* Strains Secreting Truncated Forms of InlA of Different Lengths to Stimulate Cytokine and Chemokine Production by Intestinal Epithelial Cells

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Undergraduate Student Award Competitor

Introduction: *L. monocytogenes* utilizes Internalin A (InlA), a cell wall anchored protein to cross the intestinal barrier. Mutations leading to premature stop codons (PMSC) in *inlA* result in a truncated and secreted InlA. These mutations are causally associated with attenuated-virulence and are found in 45% of isolates from ready-to-eat foods. Types 3 and 4 are the most prevalent and represent the largest and smallest truncated forms of InlA, respectively.

Purpose: Compare the ability of *L. monocytogenes* strains producing full-length and truncated forms of InlA to induce cytokine/chemokine responses during invasion of human intestinal epithelial cells.

Methods: Caco-2 cells were used to assay cytokine/chemokine response after invasion with *L. monocytogenes* strains encoding a full-length, truncated InlA or *L. innocua* as an avirulent control. Caco-2 monolayers were infected with *Listeria* for 1h. Cells were harvested, counted, and lysed while stabilizing nucleic acids. QRT-PCR targeted interleukin-8 (IL-8), interleukin-15 (IL-15), and monocyte-chemotactic-protein-1 (MCP-1). The DDCt method was used for relative quantification of gene expression to the calibrator (uninfected Caco-2 cells), with Glyceraldehyde-3-phosphate-dehydrogenase as a normalizer.

Results: *L. monocytogenes* strains encoding either a full-length or truncated and secreted InlA significantly ($P < 0.05$) increased IL-8 levels as compared to *L. innocua* and uninfected Caco-2 cells. No strains elicited production of IL-15. Strains carrying PMSC types 3 and 4 produced similar ($P > 0.05$) levels of IL-8 and MCP-1, showing that the size of a truncated and secreted InlA does not affect cytokine/chemokine responses. All strains produced higher ($P < 0.05$) levels of MCP-1 compared to the calibrator. *L. monocytogenes* and *L. innocua* produced similar levels of MCP-1, indicating InlA does not stimulate MCP-1 production in Caco-2 cells.

Significance: InlA effects production of IL-8 in Caco-2 cells. Size of secreted InlA product has no effect on the cytokine/chemokines investigated. These results suggest that exposure to virulence-attenuated *L. monocytogenes* through contaminated foods may stimulate innate immunity.

P2-26 Transcriptome Based Comparison of *Listeria monocytogenes* Strains with Different Cold Adaptation Proficiencies

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Introduction: *Listeria monocytogenes* is an important pathogen that grows on refrigerated foods causing serious illness of individuals with impaired immunity consuming contaminated food. *L. monocytogenes* strains display variable cold adaptation and growth proficiencies at refrigeration

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temperatures. Strains possessing fast cold growth phenotypes than others might pose heightened food safety risks but molecular responses promoting such phenotypes remain unknown.

Purpose: To examine cold shock gene expression differences associated with fast and slow (cold growth lag phase period <70 h compared to >200 h in BHI at 4°C) cold adaptation phenotypes amongst *L. monocytogenes* strains.

Methods: Lm 60 and EGD-e, representing fast and slow cold adapting *L. monocytogenes* strains, respectively, were exposed to cold stress induced by transfer from 37 to 4°C, and their transcription responses were compared at 30, 60, and 120 min of cold acclimation.

Results: Lm 60 up regulated (≥ 2.5-fold) the expression of 200 genes compared to EGD-e. Up regulated genes were associated with carbohydrate and amino acid transport and metabolism, peptidoglycan synthesis, cell surface proteins, general stress responses, transcription regulation, flagella biosynthesis and motility. Comparative phenotypic analysis showed that cold adapted Lm 60 cells to be more tolerant to cell wall and bile stress exposure than EGD-e, as well as displaying enhanced flagellation, swarming motility, biofilm formation and cold growth in defined growth medium using fructose as a carbon source.

Significance: Genome wide insights into genetic response differences between a fast (Lm 60) and a slow (EGD-e) cold adapting *L. monocytogenes* strain have been provided by the present study. Our observations suggest more proficient cold adaptation in Lm 60 compared to EGD-e might be promoted through enhanced nutrient uptake and metabolism abilities as well as improved stress tolerance under cold stress.

P2-27 A Predictive Model to Determine the Combined Effects of Temperature, Sodium Chloride and Green Tea on Thermal Inactivation of Listeria monocytogenes in Ground Turkey

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Introduction: The use of heat is one of the most common intervention strategies to control pathogens in foods. Multiple food formulation factors can be assessed with an aim to render the contaminated pathogens more sensitive to the lethal effect of heat.

Purpose: The interactive effects of heating temperature (55 – 65°C), sodium chloride (NaCl, 0-2%), and green tea (GT, 0-3%) on heat resistance of a five-strain mixture of *Listeria monocytogenes* in ground turkey were determined.

Methods: Thermal death times were quantified in bags submerged in a circulating water bath set at temperatures 55, 57, 60, 63, and 65°C. The recovery medium was tryptic soy agar with added 0.6% yeast extract and 1% sodium pyruvate. The D-values (time for 10-fold reduction in viable cells) were determined by fitting the data to a survival equation with a curve fitting program. Thereafter, the D-values were transformed to the natural logarithm form and analyzed by second order response surface regression to develop a regression model for temperature, NaCl and GT.

Results: The multiple regression equation for the $\log_e$ D-values yielded an R^2 value of 0.97. The data indicated that all three factors interacted to affect the inactivation of the pathogen. Sodium chloride exhibited a protective effect and increased the heat resistance of *L. monocytogenes*. Green tea interacted with sodium chloride, and reduced the protective effect of NaCl on heat resistance of the pathogen. For example, increasing salt levels from 0.4% to 1.6% in turkey supplemented with 0.6% GT increased the D-value at 57.5°C from 15.42 min to 17.62 min. Conversely, increasing GT levels from 0.6% to 2.4% in turkey supplemented with 0.4% NaCl decreased the D-value at 57.5°C from 15.42 min to 10.52 min.

Significance: Food processors can use the predictive model to design an appropriate heat treatment for inactivating *L. monocytogenes* in cooked turkey products.

P2-28 Transposon Mutant Library Reveals Genes Involved in Biofilm Formation by *Listeria monocytogenes* 568 at a Simulated Food Plant Environmental Temperature of 15°C

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Developing Scientist Competitor

Introduction: *Listeria monocytogenes* is a pathogenic foodborne microorganism noted for its ability to persist in the food processing environment. This phenomenon is attributed to the bacterium's ability to form biofilms. However, much of the research on biofilm formation in this organism has been carried out at 37°C rather than temperatures more commonly associated with food processing.

Purpose: The purpose of this study was to identify the genes involved with biofilm formation of *L. monocytogenes* at 15°C.

Methods: A library of 11,000 *Listeria monocytogenes* 568 (serotype 1/2a) transposon mutants was created with plasmid pMC39. Biofilms were formed at 15°C and mutants with reduced or enhanced biofilm formation were selected after staining with crystal violet or safranin. The susceptibility of the mutants' pre-formed biofilm to benzalkonium chloride was also determined. Arbitrary PCR was used to generate sequence data and identify gene interruption sites.

Results: A total of 40 mutants were found to express altered phenotypes in two or three of the assays. Insertions were mapped into genes that encode for cell wall biosynthesis, motility, metabolism, and cell surface associated proteins. Benzalkonium chloride susceptible biofilm mutants contained interruptions in genes related to metabolism or cell surface proteins. In contrast, biofilm mutants selected in both the safranin and crystal violet assays were found to harbor the transposon in more varied targets, including three internalins and genes related to cell wall biosynthesis and motility.

Significance: This study carried out at a temperature common in food production environments revealed possible involvement of several genes not previously linked to biofilm formation as well as of genes also associated with biofilm formation at higher temperatures.

P2-29 Survival and Growth of Outbreak Strains of *Listeria monocytogenes* on Cantaloupe

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Introduction: In 2011 a multistate outbreak of listeriosis in the United States involved cantaloupe and resulted in 33 deaths and 147 illnesses (CDC 2012). This outbreak highlighted the need for further efforts to understand the ability of *Listeria monocytogenes* to survive and grow on cantaloupe and other produce.

Purpose: The objective of the study was to assess survival and growth of outbreak strains of *L. monocytogenes* on the outer surface of cantaloupe (rind) compared to inner surface (flesh) or in freshly extracted juice at various incubation temperatures.

Methods: Three *L. monocytogenes* strains implicated in the 2011 multistate outbreak in cantaloupe were employed in this study. Two strains were of serotype 1/2a and the third of serotype 1/2b. Cantaloupe was obtained at retail. Pre-wetted, freshly cut pieces of cantaloupe rind and flesh as well as cantaloupe extract were inoculated with *L. monocytogenes* (10^5 CFU /fragment or ml). Survival and growth were assessed by enumeration of cells over 21 days of incubation at 4 and 8°C and seven days of incubation at 25°C. Data were statistically analyzed with SAS using the linear mixed effects model

Results: *L. monocytogenes* populations increased by approximately 10 fold following 21 days incubation at 4 or 8°C, and by approximately 100 fold following 7 days incubation at 25°C. After 24 hours at 25°C *L. monocytogenes* populations increased by approximately 10 fold. Interestingly, increases were higher on the rind than on the flesh or in the juice, with statistically significant differences ($P < 0.0001$) after 7 days of incubation at 4°C and 72 h at 25°C. No significant differences were noted among the three different strains

Significance: The results of this study suggest that *L. monocytogenes* can not only survive on the surface of cantaloupe but that it also has temperature and time-dependent potential for growth. The data will be valuable in design of controls to limit persistence and growth of this pathogen on cantaloupe and other produce.

P2-30 Whole Genome Sequencing and Phenotypic Characterization of *Listeria monocytogenes* Isolates from the 2011 Cantaloupe Outbreak Reveals Three Distinct Genetic Clades with Different Phenotypic Traits

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Introduction: The 2011 listeriosis outbreak was the first instance where cantaloupe propagated a listeriosis outbreak. The outbreak involved five strains, belonging to two serotypes (1/2a and 1/2b).

Purpose: We characterized one cantaloupe isolate representing each of the outbreak strains with genomic and phenotypic methods to identify traits that may explain the *L.monocytogenes* strains' association with cantaloupe and processing equipment.

Methods: Whole genome sequencing (WGS) of five outbreak strains from cantaloupe was performed using the IonTorrent™ PGM. SNP-based phylogenetic methods were implemented to group these strains with previously sequenced *L. monocytogenes*. Outbreak strains were compared to 24 sequenced *L. monocytogenes* strains to find unique SNPs and genetic loci. Swarming in semi-soft BHI at 30°C and 37°C, adherence to polyvinyl chloride (PVC) at 30°C, sensitivity to chlorine sanitizer, and invasion in Caco-2 cells were also assessed.

Results: A phylogeny on 195 SNPs separated the outbreak strains into three clades. Four 1/2a strains separated into two clades of two strains each. One clade contained 1 unique SNP and 7 genetic loci (> 200 bp) not found in other sequenced *L. monocytogenes*, the other clade contained 1,059 SNPs and 14 loci. The 1/2b strain contained 4 unique SNPs and 10 loci. No differences in swarming were observed at 30°C ($P > 0.05$), however the 1/2b strain demonstrated higher swarming at 37°C than the other outbreak strains ($P < 0.05$). The 1/2b strain showed significantly lower attachment than three of the four 1/2a strains on PVC after five days ($P < 0.05$). Outbreak strains tolerated recommended concentrations of chlorine in TSB, yet were susceptible to sub-inhibitory concentrations of sanitizer in 1:10 dilute TSB. No differences in invasiveness were observed ($P > 0.05$).

Significance: WGS identified features unique to the cantaloupe outbreak strains, making it a useful tool in outbreak investigation. Interestingly, the 1/2b strain showed increased swarming at 37°C which may contribute to survival in certain niches.

P2-31 Inter-strain Competition Affects Growth and Detection of *Listeria monocytogenes* or *Salmonella* in Foods by ISO Methods

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Developing Scientist Competitor

Introduction: Classic pathogen detection methods in foods might not represent all strains initially present in samples, but those with competitive fitness, thereby introducing bias in detection.

Purpose: To investigate the competition among *L. monocytogenes* or *Salmonella enterica* strains during: (i) growth on laboratory media and foods, (ii) selective enrichment.

Methods: Growth of seven *L. monocytogenes* strains (serotypes 1/2a, 1/2b, 1/2c, 4b) was tested in single and two-strain cultures (1:1 strain ratio). The competitive potential of seven *Salmonella enterica* strains (serovars Typhimurium, Reading, Agona, Emek, Putten) was assessed in two and three-strain cultures. Strains were made resistant to different antibiotics for their selective enumeration on TSA+antibiotics. Pathogens were inoculated (3 log CFU/ml or CFU/cm^2) in TSB, on TSA, and vacuum packed sliced ham (5x5 cm^2) and stored at 10°C. According to ISO 11290:1:1996 and ISO 6579:1993 for *L. monocytogenes* and *Salmonella* spp. detection, respectively, two enrichment steps both followed by streaking on ALOA and XLD, were performed. Strain cultures were directly added in the enrichment broth or used to inoculate minced beef and sliced ham (3 log CFU/cm^2). 60-100 colonies were used to determine the relative percentage of each strain recovered on plates.

Results: *L. monocytogenes* strains had similar ($P > 0.05$) growth rates when cultured singly or in mixtures in TSB and TSA. Conversely, growth on ham resulted in cases where a strain did not manage to increase in the presence of another strain. In laboratory media, some *Salmonella* strains dominated over others, suppressing their maximum populations by 2 log lower than the 9 log CFU/ml or cm^2 observed in single cultures, or reducing their growth rate 2-fold ($P < 0.05$) compared to single cultures. During enrichment, 4b serotype outcompeted (80-100% of total colonies) other serotypes, regardless of the food. In BPW, the dominance (80-100%) of Reading and Putten serovars was reversed in the second enrichment in RVS (0-20% of total colonies).

Significance: Understanding how strain competition of pathogens may affect their survival and growth may contribute to the improvement of detection methods and assist in outbreak investigations.

P2-32 Characterization and Rapid Detection of Cantaloupe-associated *Listeria monocytogenes*

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Introduction: In 2011, an outbreak of listeriosis was associated with Colorado cantaloupes. Evaluations of the outbreak isolates indicated significant genetic diversity that was linked to divergent ecologies. Pulsed field gel electrophoresis (PFGE) served as the primary tool to characterize these isolates, but additional molecular analyses would enhance the genetic, epidemiological, and physiological understanding of these isolates.

Purpose: We utilized PFGE, riboprinting, and conventional sequencing to further characterize *Listeria monocytogenes* cantaloupe isolates and compared the identified patterns to previously reported genotypic and epidemiological data. Further, we evaluated the Phast Swab-PI-PLC-µPAD to detect cantaloupe-associated *L. monocytogenes* isolates.

Methods: Molecular characterizations of six cantaloupe-associated *L. monocytogenes* isolates were performed using PFGE (AscI and ApaI) and riboprinting (PvuII and EcoRI). Conventional sequencing was utilized to discern genetic differences in *inlA*, a virulence factor linked to host cell invasion. Further, a sampling and enrichment system (Phast Swab) was integrated with colorimetric paper-based analytical devices (µPAD) to sample, enrich, and detect *L. monocytogenes*.

Results: PFGE of the six *L. monocytogenes* isolates revealed five unique patterns matching those from the recent cantaloupe outbreak. These patterns were also observed, but to a lesser degree, in *L. monocytogenes* isolates from food and animals. Riboprinting differentiated the cantaloupe-associated isolates into four ribotypes. Two isolates shared the same PFGE pattern, but ribotyping was able to differentiate these isolates into two ribotypes, DUP_19169 and DUP_20238. Other ribotypes identified included DUP_1030 and DUP_1052 linked to human listeriosis and food/animals, respectively. Sequence analyses of *inlA*, further emphasized the genomic diversity of isolates. Finally, we demonstrated that the Phast Swab-PI-PLC-µPAD assay was able to detect all cantaloupe-associated *L. monocytogenes* isolates.

Significance: These results provide an enhanced molecular characterization of cantaloupe-associated *L. monocytogenes*, and subsequently link genetic differences to relevant epidemiological information. Additionally, we prove the effectiveness of a rapid assay to screen cantaloupes for *L. monocytogenes* contamination.

P2-33 Development and Application of a Rapid Lateral Flow Test Strip-based Method for the Detection of *Listeria monocytogenes* in Food

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Introduction: *Listeria monocytogenes* is the causative agent of listeriosis. The CDC estimates that 1,662 cases of listeriosis occur annually in the US, resulting in 1,520 hospitalizations and 266 deaths. USDA-FSIS monitoring programs at food processing establishments test ready-to-eat (RTE) foods and food contact surfaces for *L. monocytogenes*. Cultural methods require up to 3 days for results to be obtained while rapid instrument-based methods are less than 2 days. In order to simplify rapid testing, we have developed a test strip-based method for the detection of *L. monocytogenes* in food.

Purpose: The purpose of this study was to develop a new test strip-based method for the detection of *L. monocytogenes* in food.

Methods: Monoclonal antibodies (39) were screened against *L. monocytogenes* and *L. innocua* by sandwich ELISA (1521 combinations). Antibody pairs that gave the best specificity and sensitivity (84) were further pursued in the test strip format. Inclusivity/exclusivity studies evaluated 50 *Listeria spp.* strains, including 30 *L. monocytogenes* strains, and 35 non-*Listeria* bacterial strains. One test strip design was coupled to a 40 h enrichment and compared to the USDA-FSIS cultural reference method for the detection of *L. monocytogenes* in 125 g hot dog samples.

Results: The new method demonstrated 100% sensitivity and 100% specificity for *L. monocytogenes*. Out of 20 inoculated samples, the number of positive results reported for the new *L. monocytogenes* test strip method was 16 and the USDA-FSIS method was 19. The number of positive results obtained with the *L. monocytogenes* test strip method was equivalent to the number obtained with the USDA-FSIS method ($X^2 = 2.01$) for the detection of *L. monocytogenes* in 125 g hot dog samples.

Significance: The new test strip method should provide the food industry with a rapid and reliable diagnostic tool for monitoring and preventing *L. monocytogenes* contamination of food.

P2-34 Validation of a Test System to Detect Low Levels of *Listeria moncytogenes* in Composite Environmental Sponges and 125G Ready-to-Eat Meat Samples

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Introduction: The USDA's Food Safety Inspection Service (FSIS) provides the option of combining five environmental sponges for analysis of *Listeria monocytogenes*. In 2012, the agency also announced a change to the current procedures for product sampling and now allows the compositing of five 25-g samples; increasing the analytical sample size from 25 g to 125 g. Therefore, it is appropriate to ensure the RapidChek *Listeria* test system is validated with composite samples.

Purpose: The aim of the study is to validate the RapidChek *Listeria* test system for detection of low levels of *Listeria monocytogenes* in composite sponges and ready-to-eat meat samples

Methods: A method comparison study was conducted to determine accuracy, specificity, and sensitivity of the test system as compared to the USDA reference method (MLG 8.08). Fifty (50) samples of composite sponges and ready-to-eat meat samples were analyzed by both methods (n = 20 spiked samples plus 5 non-spiked negative controls).

Results: The RapidChek system reported eighteen (18) confirmed positive results, while the reference method reported ten (10) confirmed positive results in the composite sponge study. In the composite ready-to-eat meat study, the RapidChek system reported seventeen (17) confirmed positive results while the reference method reported nineteen (19) confirmed positive results. The relative sensitivity and relative specificity of the test method was 100%. The overall accuracy of the test method was 121%. According to Mantel-Haenszel Chi-Square analysis ($X^2 = 2.78$), there was no statistical significance between test and reference method.

Significance: Sample size does not adversely affect the performance of the test method. The target pathogen can be detected at very low levels of contamination in composite sponge samples and 125 g ready-to-eat meat samples. The study demonstrates the flexibility and robustness of the test method for use by food producers.

Poster

P2-35 A Comparative Evaluation of the ANSR™ *Listeria* Assay for the Detection of *Listeria* Species on Environmental Surfaces

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Introduction: ANSR™ *Listeria* is an isothermal, amplified nucleic acid assay for detection of *Listeria* spp. in a variety of food matrices and environmental samples. The method is based on the Nicking Enzyme Amplification Reaction (NEAR™) technology, preceded by reverse transcription of ribosomal RNA. Target cDNA is amplified through a mechanism of polymerization from the ends of nicks created in double-stranded DNA by the action of a specific endonuclease. Amplified target sequences are detected using fluorescent Molecular Beacon® probes. Amplification and detection require less than 20 minutes.

Purpose: The purpose of this evaluation was to compare the assay to the USDA/FSIS-MLG 8.07 reference method for detection of *Listeria* spp. from stainless steel, plastic, ceramic, sealed concrete and rubber environmental surfaces as part of the AOAC Research Institute™ validation process.

Methods: For both the ANSR and reference methods, 20 replicates were analyzed at an inoculation level intended to produce fractional positive results, along with a minimum of 5 high level samples and 5 uninoculated controls. Following enrichment of samples in ANSR *Listeria* Enrichment Broth, samples were assayed by the method after 16 and 24 hours of incubation.

Results: Probability of detection (POD) statistical analysis indicated that there were no significant differences between the ANSR and reference methods for the detection of *Listeria* species on ceramic, sealed concrete, plastic, and rubber surfaces at both 16 and 24 hours. For stainless steel, the method produced more positive results than the reference method in two internal trials, but results of the two methods were not statistically different in the independent laboratory trial.

Significance: ANSR *Listeria* is a rapid, reliable alternative method for detection of *Listeria* spp. in a variety of environmental samples.

P2-36 A Comparative Evaluation of the 3M™ Molecular Detection Assay *Listeria* for the Detection of *Listeria* species in Foods

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Introduction: *Listeria* is frequently associated with food and environmental surface contamination and is a significant food safety concern. The 3M™ Molecular Detection Assay *Listeria* is designed for rapid and specific detection of these adulterants in food samples after only 26 hours incubation. Using the 3M™ Modified Listeria Recovery Broth (mLRB) as the enrichment medium, the assay uses a combination of isothermal amplification of unique DNA target sequences and bioluminescence to analyze and detect the amplified sequences.

Purpose: The purpose of the study was to evaluate the ability of the MDA *Listeria* to detect *Listeria* species in a variety of foods in comparison to the FDA BAM Chapter 10, AOAC 993.12 and USDA/FSIS MLG 8.07 methods.

Methods: This new method and comparative reference methods were analyzed by testing 4 food matrices at 3 separate inoculum levels; 20 replicates were inoculated at a low inoculum level of 0.2-2.0 CFU/test portion, 5 replicates were inoculated at a high inoculum level of 2-5 CFU/test portion, and 5 replicates at an uninoculated control level of 0 CFU/test portion. The test portions were analyzed by the new method after 26 hours of incubation.

Results: Statistical analysis was conducted using the Probability of Detection (POD) statistical model and there were no significant differences between the candidate method and the reference method for each of the four matrices tested at the low inoculum level after 26 hours incubation.

Significance: The Molecular Detection Assay *Listeria* offers the capability of detecting *Listeria* species in foods after only 26 hours of incubation, thereby reducing the amount of time until presumptive detection in comparison to traditional reference methods.

P2-37 Effect of Storage Times and Temperatures on *Escherichia coli* Isolation

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Introduction: Beef released to commerce in the US and other countries must be tested and deemed free of *Escherichia coli* O157. Confirmation of the presence of *E. coli* O157 occurs at centralized laboratories and can often require the transport of enrichment broths for substantial distances and times. It is therefore important that the type of enrichment broth used, time between screening and confirmation, and storage temperatures of broths prior to confirmation do not negatively impact isolation of *E. coli* O157.

Purpose: Determine if storage times and temperatures affect the likelihood of isolating *E. coli* O157 from potential positive broths.

Methods: A cocktail of rifampicin resistant *E. coli* O157 were inoculated into three commercially available broths with no, low or high background beef microflora and enriched for 12 and 24 h. Enrichments were stored at 4, 10 or 20° C for 7 days and detection using IMS and counts of *E. coli* O157 were determined daily.

Results: The concentration and isolation of *E. coli* O157 from a potential positive enrichment broth stored at 4°C or 10° C was not significantly affected by the type of enrichment broth, concentration of background microflora, enrichment time, or the storage of the enrichment broth with maximum reductions of 0.59 log CFU/ml observed during the seven day storage period. Isolation of *E. coli* O157 from high background samples stored at the abuse temperature of 20°C was problematic.

Significance: Storage of enrichment broths at 4 or 10°C had minimal negative effect on the concentration or the likelihood of isolating *E. coli* O157 from potential positive enrichment broths during seven days storage. Whilst a 100% conversion of potential positives to confirmed positives may be difficult to achieve, it is clear that the transport and storage protocols currently used by Australian beef processors do not affect the likelihood of isolating *E. coli* O157.

P2-38 Investigation of Adherence Strategies of Environmental *Escherichia coli* to Food Samples and Human Epithelial Cells

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Introduction: Avian pathogenic *Escherichia coli* (APEC) are zoonotic members of the extraintestinal pathogenic *E. coli* pathotype (ExPEC). Although APEC are not normally associated with disease in humans like enterohemorraghic *E. coli*(EHEC), APEC isolates have been identified on foods and as carrying EHEC genes. Outbreak entero-aggregative (EAEC) strains have also been characterized as Shiga toxin positive. In this study, persistence on leafy greens, attachment to chicken breast and interaction with intestinal cells were assessed.

Purpose: The objective of this study was to evaluate the ability of 30 strains of environmental and clinical UPEC outbreak EAEC, and EHEC isolates to attach to chicken tissue and human intestinal cells.

Methods: Multiplex PCR was used to assess the presence of 12 EHEC and UPEC genes in *E. coli* strains (n = 30). Cells were inoculated on basil plants, retail chicken breast samples or human cells grown to confluency in 6-well plates. *E. coli*attachment to HCT-8 cells (human ileocecal) was compared to that of CaCo-2 cells (human colonic). Multivariate correlations were generated using JMP Software.

Results: Statistical analysis showed several strong correlations between the number of EHEC or UPEC virulence genes and attachment to various matrices. Moderate correlations (r = 0.51) indicated that attachment to chicken was higher for APEC and UPEC strains compared to EHEC strains. The UPEC isolate attached most strongly to chicken and APEC isolates persisted for longer periods on leafy greens indicating enhanced attachment. Attachment to human HCT-8 cells tended to be higher for strains containing low numbers of EHEC virulence genes (r = 0.98) but was weakly correlated with UPEC strains (r = 0.31). Strains with more EHEC genes strongly correlated with higher survival on basil, lettuce, and spinach (r = 0.98).

Significance: The number and type of EHEC and/or UPEC virulence genes present within the genome of *E. coli* may correlate to their ability to persist on food and human cell surfaces.

P2-39 Determination of Heat and Pressure Resistance of Verotoxin Positive and Negative *Escherichia coli*

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Developing Scientist Competitor

Introduction: Verotoxigenic *Escherichia coli* (VTEC) can cause severe illness and are particularly associated with beef and fresh produce. However, data on the resistance of VTEC to heat and pressure is essentially limited to *E. coli* O157 and thus does not reflect the phylogenetic and physiological diversity of food-associated VTEC.

Purpose: The aim of this study was to determine the heat and pressure resistance of a large group of VTEC and compare them to *E. coli* AW1.7, an extremely heat and pressure resistant beef isolate.

Methods: Eighty-seven strains of VTEC and fifteen verotoxin-negative *E. coli* representing 15 different serotypes were screened for survival of thermal treatment at 60°C for 5 min in LB broth. Nineteen heat resistant and sensitive strains were pressure treated at 600 MPa and 25°C for 3 min in LB broth. Pressure treatment was applied to ground beef inoculated with the 4 most pressure resistant strains of *E. coli*.

Results: With an initial concentration of 7 log CFU/ml, 77 of the 102 strains tested did not have survivors following 60°C for 5 min. Of the 25 heat resistant strains, 6 strains of VTEC were reduced by 4.2 log CFU/ml or less. Eight of the pressure treated strains were reduced by less than 2 log CFU/ml in LB broth. In ground beef the most pressure resistant VTEC was *E. coli* O26:H11 05-6544 with a reduction of 2.0 ± 0.1 log CFU/g; *E. coli* AW1.7 was reduced by 2.9 ± 0.8 log CFU/g.

Significance: The results of these experiments demonstrate that *E. coli* AW1.7 is more heat resistant than the VTEC strains tested. A number of the VTEC were resistant to pressure and survived treatment that is equivalent to current commercial processes (600 MPa at ambient temperature).

P2-40 Polynucleotide Phosphorylase is Required for *Escherichia coli* O157:H7 Growth at Low Temperature

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Developing Scientist Competitor

Introduction: The growth of *Escherichia coli* O157:H7 in contaminated dairy and other food products due to temperature fluctuation post a major food safety threat. To effectively control or inhibit the growth of *E. coli* O157:H7 depends on our understanding of mechanisms regulating its low temperature growth.

Purpose: Polynucleotide phosphorylase (PNPase) is needed for cold adaption of commensal *E. coli*. We hypothesized that PNPase has a critical role in the growth of *E. coli* O157:H7 at low temperature.

Methods: The PNPase gene deletion mutant (*Δpnp*) of *E. coli* O157:H7 was generated using l Red recombinase system. pBAD was used to construct the *pnp* complementation plasmid. Swimming motility was assayed using 0.3% agar plate. The growth curve was conducted in 96 well microplates. The growth and survival of *E. coli* O157:H7 during cold storage were analyzed every other day by enumeration.

Results: Growth of *Dpnp* and wild type (WT) strains in both LB broth and agar did not differ at 37°C, but *Dpnp* strain has impaired growth at 22°C and 10 °C. During 14 days of 10°C storage in both LB and milk, WT grew and reached >8 Log_{10}CFU/ml after 4 days of 10°C storage, while *Dpnp* was gradually dying off with a more pronounced effects in milk. In addition, *pnp* deletion impaired the swimming motility of *E. coli* O157:H7. The observed differences were completely or partially complemented by overexpression of *pnp* plasmid, indicating a *pnp* specific effect.

Significance: PNPase is required for the growth of *E. coli* O157:H7 at low temperature, which provides a molecular target to control the growth of *E. coli* O157:H7 at low temperature, having important practical applications to food industry. (USDA-AFRI2010-65201-20599, Agricultural Experiment Station at University of Wyoming)

P2-41 Thermal Resistance Parameters for Stationary Phase and Acid-adapted *Escherichia coli* O157:H7 in Apple and Carrot Juice Blends

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Developing Scientist Competitor

Introduction: Outbreaks involving juices contaminated with *Escherichia coli* O157:H7 have occurred and the safety of these products is a concern. However, only a few studies regarding the thermal resistance of this microorganism, in acidic juices with a wide range of pH values, have been published.

Purpose: This study aims to determine the effect of varying pH conditions on the thermal inactivation of stationary-phase and acid-adapted *Escherichia coli* O157:H7 (serogroup C7927).

Methods: *D*- and *z*-values were determined for stationary-phase *E. coli* in an apple-carrot juice blend (80:20) adjusted to three pHs (3.3, 3.5, and 3.7) with lactic, malic, and acetic acid, and at a pH of 4.5 adjusted with NaOH. Thermal parameters were also determined for acid-adapted cells in juices acidified with malic acid. Soluble solids content effect on the thermal resistance of *E. coli* was studied in samples with 9.4-11.5 °Brix range adjusted to pH of 3.7. *D*-values were determined at 54, 56, and 58°C, and thermal treatments were conducted in triplicate.

Results: *E. coli* exhibited the highest thermal resistance at pH 4.5, and resistance increased significantly ($P < 0.05$) with acid adapted cells. In acidified juices, samples adjusted to pH 3.7 with malic acid showed the highest resistance ($D_{54°C}$ = 6 ± 1 min, *z*-value of 9.1 °C), and the lowest was observed in samples adjusted to pH 3.3 with acetic acid ($D_{58°C}$ = 0.03 ± 0.01 min, *z*-value of 10.4 °C). For juices acidified to the same endpoint pH with different acids, *E. coli* was found more resistant in juices acidified with malic acid, followed by lactic, and acetic acid. Increasing the °Brix from 9.4 to 11.5 did not significantly alter the thermal resistance of *E. coli* ($P > 0.05$).

Significance: Data from this study will be useful for establishing the critical limits for safe thermal processing of pH controlled juices and similar products.

P2-42 Comparison and Correlation of *Escherichia coli* O157-associated Virulence Genes in Beef Trim and Variety Meats

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Introduction: The frequency of contamination of meat products by pathogenic microorganisms fluctuates because of many variables such as month and beef product type probably because of differences in temperature, rainfall, cattle source and plant processing practices.

Purpose: The association of various virulence genes associated with the enterohemorrhagic pathogen, *Escherichia coli* O157, was examined in beef trim and variety meats to determine if there were a regular relationship between their frequencies of occurrence in these products. Such information could be useful for risk assessments and for designing sampling and testing plans to maximize the frequency of detection of pathogens in beef-based meat products.

Methods: Samples (375g) were collected during 2012, enriched for at least 8 hours and screened by PCR for the virulence-associated genes *rfb, eae, stx*$_1$, and *stx*$_2$ and by lateral flow to detect the O157 antigen. Also, two *Salmonella*-specific genes were detected. From plants that tested both product forms, 301,786 and 14,648 samples of trim and variety meats, respectively, were analyzed.

Results: Enterohemorrhagic *E. coli* (*stx*$^+$ and *eae*$^+$) screen-positive sample frequencies for trim and variety meats were 12.9% and 19.1, respectively. Generally, contamination rates of variety meats were higher than rates for trim. However, while the frequency of occurrence of *E. coli* O157- and *Salmonella*-associated genes in beef samples was strongly correlated, the strength of association of O157-specific targets between trim and variety meats was highly variable during the year though tending to be higher during the warmer months.

Significance: Even though virulence-associated genes occur in samples more frequently during late spring and early summer months, there is no consistent relationship between their prevalence in trim and variety meats. Future analyses might consider differentiating between types of variety meats. However, both product types should be monitored and these observations might be considered in microbial hazard risk assessments.

P2-43 Correlation of *Escherichia coli* O157-associated Virulence Genes in Beef Trim with Precipitation Levels and Temperature

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Introduction: The frequency of contamination of meat products by pathogenic microorganisms fluctuates because of many variables such as month of testing and differences in temperature, rainfall, cattle source and plant processing practices.

Purpose: The association of various virulence genes associated with the enterohemorrhagic pathogen, *Escherichia coli* O157, was examined in beef trim samples to determine if there were a regular relationship between their frequency in these products and the environmental factors of precipitation and temperature. Such information could be useful for risk assessments and for designing sampling and testing plans to maximize the frequency of detection of pathogens in trim.

Methods: Samples (375 g) were collected throughout calendar year 2012, enriched for at least 8 hours and screened by PCR for the virulence-associated genes *rfb, eae, stx*$_1$, and *stx*$_2$ and by lateral flow (LF) to detect the O157 antigen. Also, two *Salmonella*-specific genes were detected. Two groups of four meat processing establishments each (upper Midwest [UM] and central Midwest [CM]; 99,067 and 122,869 samples, respectively) were compared using the frequency of virulence-associated genes and rainfall amount and temperature using data from the U.S. Climatological Divisions.

Results: For UM plants, annual average temperature and rainfall ranged from 49-52°F and 1.2-3.6"/month, respectively. CM plant temperature and rainfall was 63-68°F and 1.8-2.5"/month, respectively. The frequency of screen-positive samples for enterohemorrhagic *E. coli* (EHEC; *stx*$^+$ and *eae*$^+$) was 6.6% and 17.9% for UM and CM plants, respectively. The average number of signals per sample was 0.97 and 0.55 for UM and CM plants, respectively. Generally, plants in warmer weather tended to have a higher frequency of EHEC screen-positive samples and a higher average number of signals per sample. However, there was considerable variation between plants.

Significance: Beef trim samples in plants during warmer weather (seasonally and annually) tend to have higher percentages of samples screen-positive for *E. coli* O157 virulence-associated genes. More sophisticated statistical analyses may be able to more fully assess the relative contributions of temperature and rainfall to these findings. Such plants could consider these factors in designing their product sampling plans; future microbial hazard risk assessments might be informed by these observations.

P2-44 Dietary Effects on *Escherichia coli* O157:H7 Shedding in Beef Cattle during Transportation

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Introduction: Beef cattle production in Alabama is primarily based on a cow-calf production system;calves produced in Alabama are weaned and shipped to feedlots in the Midwest. During transportation, cattle will be under multiple stresses (such as limited spaces and feed) for up to 3 days, which could lead to body weight loss and *Escherichia coli* O157 cross-contamination. The dietary effects on stress as well as O157 shedding still need evaluation.

Purpose: The purpose of this study is to assess the effects of two diets, hull-based dry feed (DF) and corn silage based high moisture feed (HM) on body weight and *E. coli*O157 shedding in cattle during transportation.

Methods: Fifty four cattle were fed a DF diet while another 63 were fed a HM diet for 45 days before transportation. Twenty five DF-fed cattle and 33 HM-fed cattle were loaded on a truck and driven for 24 hours. The concentrations and prevalence of O157 were determined based on fecal samples collected from shipped cattle before (BT), right after (RAT) and 15-days after transport (15AT) and un-shipped cattle (BT and 15AT). The body weight was monitored everyday during the time.

Results: Transportation significantly reduced the cattle the body weight, and increased O157 shedding levels and prevalence regardless the diets. The HM-fed cattle shrink significantly at RAT and could not recover at 15AT. The O157 shedding levels increased at RAT (from 0.63 ± 1.26 to 1.26 ± 1.30 CFU/*g* feces, $P < 0.05$) but decreased back to the BT levels after 15 days. Cattle fed the HM diet had lower O157 shedding levels than DF-fed calves at 15AT ($P < 0.05$).

Significance: Diets play important role on O157 shedding. HM-fed calves had lower O157 shedding levels than DF-fed calves irrespectively of transportation status.

P2-45 Synergistic Interaction in Dual-species Biofilms Formation by *Escherichia coli* O157:H7 and *Ralstonia* spp

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Developing Scientist Competitor

Introduction: One strain of *Ralstonia* spp. isolated from produce processing environments, showed strong potentials for forming biofilms on various surfaces. It is hypothesized that the presence of strong biofilm forming microflora could potentially enhance the survival of *Escherichia coli* O157:H7 (EcO157) in a harsh environment. Previous results showed that EcO157 and *Ralstonia* spp. displayed a synergistic interaction in dual-species biofilms formation, as indicated by the increased biomass.

Purpose: This study was conducted to examine the potential factors that affected this synergistic interaction.

Methods: The ability of EcO157 attaching to solid surfaces was examined using tissue culture plates in the presence or absence of *Ralstonia* spp. A drip flow biofilm reactor was used to examine the development of dual-species biofilm over time. EcO157 strain EDL933 and mutants deficient in curli production were also tested for dual-species biofilm formation with *Ralstonia* spp. Cells in biofilms were enumerated by plating and biofilm structure was examined using confocal laser scanning microscopy (CLSM). Three replicates were conducted for each study.

Results: Within 4 h of inoculation, the presence of pre-formed *Ralstonia* spp. biofilms significantly enhanced the attachment of EcO157 for 0.83 log CFU/cm^2 ($P < 0.05$) compared to that on a plain plate. This enhanced initial attachment of EcO157 was not observed when it was co-inoculated with *Ralstonia* spp., indicating the importance of *Ralstonia* spp. biofilm in this interaction. While co-culture significantly increased ($P < 0.05$) the incorporation of EcO157 in dual-species biofilms after 24 h, this increase was not observed when curli deficient strains (ΔcsgA and ΔcsgD) were used. Microscopic examination of dual-species biofilms showed a unique arrangement of the two strains, where EcO157 microcolones were often encapsulated by *Ralstonia* spp. on the solid substrate.

Significance: This study provided evidence that biofilm formation by native microflora facilitates the survival of foodborne pathogens such as EcO157 through a synergistic interaction.

P2-46 Intra and Inter-strain Differences in Fitness of *Escherichia coli* O157:H7 to Protozoan Predation and Survival in Soil

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Introduction: *Escherichia coli* O157:H7 (EcO157) associated with 2006 spinach outbreak appears to have persisted as the organism was later isolated from environmental samples in the produce production areas of the central coast of California. Survival in harsh environments may be linked to the inherent fitness characteristics of EcO157.

Purpose: This study evaluated the comparative fitness of outbreak-related clinical and environmental strains to resist protozoan predation and survive in soil from a spinach field in the general vicinity of isolation of strains genetically indistinguishable from the 2006 outbreak strains.

Methods: The decreases in the populations of EcO157 strains during predation by *Vorticella microstoma* and *Colpoda aspera*, isolated previously from dairy wastewater, in decreased strength Sonneborn medium were measured. Strains with different proportions of curli-positive (C^+) and curli-negative (C^-) phenotypes were evaluated to determine whether protozoa are selective in grazing on them. We compared if specific phenotype subpopulations that evade predation are increased in fitness for survival in soil.

Results: Environmental strains from soil and feral pig feces survived longer (11 to 35 days for 90% decreases, D-value) with both protozoa; these D-values correlated ($P < 0.05$) negatively with protozoan growth. Similarly, strains from cow and pig feces survived significantly longer in soil compared to genetically indistinguishable clinical isolates. The C^+ phenotype, a fitness trait linked with attachment in ruminant and human gut, decreased after exposure to protozoa and in soils only C^- cells remained after 7 days. The C^+ phenotype correlated negatively with D-values of

EcO157 exposed to soil (r_s = -0.683; P = 0.036), *Vorticella* (r_s = -0.465; P = 0.05) or *Colpoda* (r_s = -0.750; P = 0.0001). In contrast, protozoan growth correlated positively with C^+ phenotype (*Vorticella*, r_s=0.730, P = 0.0004; *Colpoda*, r_s = 0.625, P = 0.006) suggesting a preference for C^+ cells.

Significance: We speculate that the C^- phenotype is a selective trait for survival and possibly transport of the pathogen in soil and water environments.

P2-47 Persistence of *Escherichia coli* and Attenuated *Escherichia coli* O157:H7 in Manure-enriched Soils in the Eastern Shore of Maryland

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Introduction: Delmarva Peninsula croplands are fertilized with poultry litter and other animal manures. Contamination of fresh produce by pathogens present in untreated animal manures is a major food safety risk concern.

Purpose: To investigate 1) survival of non-pathogenic *Escherichia coli* (*Ec*) and attenuated *E. coli* O157:H7 (attO157) in soils amended with poultry litter (PL), dairy manure (DM), dairy manure liquid (DL), or horse manure (HM) and 2) to compare the survival of *Ec* and attO157 in manure-amended soil.

Methods: Field plots (Othello soil) were amended with PL, DM, DL, or HM. Inocula containing equal numbers of three rifampicin-resistant (Rif^R) strains of *Ec* and two attO157-Rif^R strains grown in poultry-litter extract, were spray-inoculated (1-l per 2 m^2) at low, 4. 2 x 10^3 CFU/ml, or high, 1.29 x 10^6 CFU/ml, cell densities. Soil samples collected periodically 0-154 days post-inoculation (dpi) were analyzed for viable *Ec*and att0157 by direct plating and/or mini-MPN.

Results: Persistence of all strains was influenced by manure type, inocula concentration, and environmental factors. Populations declined more rapidly in DM compared to other treatments, except non-manured soils. By 7-dpi, all strains from both inoculum-level treatments declined to ≤1 CFU (MPN) gdry $soil^{-1}$ (gds^{-1}), except DL. *E. coli* populations in all manure treatments increased after rainfall except in DL. Populations at 28-dpi and thereafter, continued at ca. ≤1 CFU (MPN) gds^{-1}. No *Ec*or attO157 were found in uninoculated control soil.

Significance: Manure type and environmental factors influenced survival of *E. coli* in soil, with attO157 declining more rapidly than *Ec.* Both *Ec* and attO157 survived at higher populations in manure-amended soils compared to non-amended soils, indicating the effect manure has in extending survival of bacteria in soils. These findings should be taken into consideration when setting guidelines for fresh produce safety relative to manure application to soil as stated in proposed FDA standards.

P2-48 Comparison of Shiga Toxin Subtypes and Chromosomal Insertion Sites in *Escherichia coli* O157 Isolated from Australia and the USA

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Introduction: *Escherichia coli* O157 related disease in humans is primarily associated with the release of Shiga toxins encoded by genes associated with bacteriophages. Shiga toxin genes (*stx*) can be divided into two major types (stx_1 and stx_2) each of which comprises several subtypes. The diversity of *stx* subtypes carried by *E. coli* O157 has been shown to vary with respect to isolate source (cattle and human) and is believed to contribute to the variability in pathogenicity observed among genotypes.

Purpose: To determine the distribution of *stx* subtypes and *stx*-bacteriophage insertion sites in isolates derived from cattle and human sources from the USA (where large outbreaks have occurred) and Australia (very few, small outbreaks).

Methods: A total of 606 *E. coli* O157 isolates, including 284 from Australia (205 cattle and 79 human isolates) and 322 from the USA (143 cattle and 179 human isolates), were screened for *stx* subtypes (stx_1, stx_2 and stx_{2c}) and bilateral phage – chromosomal junctions of common *stx*-bacteriophage insertion sites (*argW*, *sbcB*, *wrbA* and *yehV*).

Results: Australian isolates were more likely to carry both stx_1 and stx_{2c} (62%) than those from the USA (12%) where the combination of stx_1 and stx_2 predominated (55%). The phage insertion site *argW* was occupied by the majority (67%) of Australian isolates while only 21% of isolates from the USA carried a phage in this insertion site. In contrast, the majority of isolates from the USA had an occupied *wrbA* (61%) while this site was rarely occupied in isolates from Australia (2%).

Significance: This study shows that *E. coli* O157 from different countries vary in *stx* subtypes and chromosomal insertion sites of *stx*-bacteriophages. Such differences may be one factor involved in the different human epidemiology observed in Australia and the USA.

P2-49 Exopolysaccharide and Attachment-related Protein Production by O157 and non-O157 Shiga Toxin-producing *Escherichia coli* Strains

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Introduction: Information related to attachment mechanisms of strains of the six major non-O157 Shiga toxin-producing *Escherichia coli* (STEC) serogroups is limited. However, understanding attachment is essential for effective decontamination of animal and processing equipment surfaces, as attachment may enhance survival when antimicrobial interventions are applied.

Purpose: The purpose of this study was to compare the production of cellulose, exopolysaccharide fractions, type 1-fimbriae and curli-fimbriae by STEC grown on solid media at 25°C or 37°C.

Methods: A total of 10 and 18 O157 and non-O157 STEC strains were evaluated spectrophotometrically (490 nm) for exopolysaccharide production using the phenol-sulfuric acid method with glucose as a standard. Cells were initially grown on Brain Heart Infusion Agar (BHIA) at 25°C for 48 h (n = 3). Data were expressed in µg/ml and compared using ANOVA (a = 0.05). Production of cellulose, type 1-fimbriae and curli-fimbriae was qualitatively evaluated for strains of same serotype after storage at 25°C for 48 h or 37°C for 24 h (n = 2). A modified Nutrient agar (MNA) base with added Congo Red dye or Calcofluor dye was used to examine curli or cellulose production, based on red color or fluorescence of colonies, respectively. Production of type 1-fimbriae by the same strains was determined with the yeast agglutination test.

Results: Exopolysaccharide (EP) production differed significantly (P < 0.05) among STEC strains, mainly due to one O26 strain, which produced the highest amount of EP. The second highest production was observed by one strain of serogroup O45 and one strain of serogroup O145. Type 1-fimbriae expression was generally higher for both O157:H7 and non-O157 STEC after incubation at 25°C than at 37°C. Specific strains

of serotypes O45:H2, O103:H2, O111:H8, O121:H19 and O145:H- exhibited a temperature-independent production of curli-fimbriae or type 1-fimbriae. In addition, phase-switching in production of curli-fimbriae was apparent for specific strains. Cellulose production was not observed for the majority of the strains tested.

Significance: This study shows that adherence of STEC to surfaces is mediated by expression of different polysaccharides and proteins. Non-O157 STEC may have the ability to outperform O157 STEC in attachment mechanisms and levels under different environmental conditions.

P2-50 Identification of Contamination Sources and Prevalence of *Escherichia coli* O157:H7 and *Salmonella* on Small-scale Cow/Calf Operations in Oklahoma, Texas and Louisiana

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Undergraduate Student Award Competitor

Introduction: Cattle are common reservoirs for pathogens such as *Escherichia coli* O157:H7 and *Salmonella*. On-farm practices can affect pathogen loads on cattle entering slaughter facilities. Most studies have concentrated on larger feedlots and ranches, with limited information on small-scale cow/calf operations. Understanding the factors affecting pathogen burden in these operations can be critical in developing risk management strategies along the farm-to-fork continuum.

Purpose: To determine contamination sources and the prevalence of *E. coli* O157:H7 and *Salmonella* on small-scale cow/calf operations in Oklahoma, Texas and Louisiana.

Methods: Studies were conducted in the states of Oklahoma, Texas and Louisiana during the summer and fall season over a period of two years. Fecal samples for *Salmonella* and fecal, water, sediments and trough-swab samples for detection of *E. coli* O157:H7 were collected from a total of 60 cow/calf farms in the three states. For *E. coli* O157:H7, samples were enriched in GNVCC or TSB, followed by immunomagnetic separation, plated on CT-SMAC and CHROMagar® and isolates confirmed using RT-PCR (*stx*$_1$, *stx*$_2$, *eae*, *fli*C, and *rfb* genes). For *Salmonella*, samples were enriched in RV and TT broth, plated on XLT-4 and confirmed by agglutination test.

Results: Of the 1,515 fecal, 238 water, 240 sediments, and 146 trough-swab samples collected, 11%, 15%, 7% and 3% were positive for *E. coli* O157:H7, respectively. Of the 610 fecal samples, 11.3% were positive for *Salmonella*. Cattle feces and water were the two major sources of contamination for *E. coli* O157:H7 followed by sediment samples. On the other hand, trough-swabs played a minor role in the prevalence of this pathogen in cattle operations. Fecal samples were also found to be a major source for *Salmonella* in the cow/calf operations.

Significance: These findings can be used for targeted educational and research efforts in the development of risk management strategies.

P2-51 Detection of Viable *Escherichia coli* O157:H7 in Apple Juice and Spinach Wash Water Using a Concentrating Pipette and Ethidium Monoazide-real-time PCR

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Introduction: *Escherichia coli* O157:H7 associated with food has caused many serious public health problems in recent years. Two significant limitations to the use of more sensitive and selective microbiological detection methods in the food industry include: (a) the need for a prolonged sample enrichment prior to analysis, and (b) the inability to differentiate between viable and dead cells.

Purpose: The objective of this study was to develop and optimize a method that combines a novel cell concentration step with real-time (RT)-PCR to detect only viable *E. coli* O157:H7 cells rapidly without the need for sample enrichment.

Methods: Apple juice and spinach wash water were artificially contaminated by different concentrations of viable and dead *E. coli* O157:H7 cells and concentrated using a Concentrating Pipette (CP) designed by InnovaPrep LLC. Samples were further purified by immunomagnetic separation and treated with ethidium monoazide (EMA), a dye that can penetrate dead cells and bind to cellular DNA. DNA was extracted and amplified by TaqMan® RT-PCR targeting the *uid*A gene to detect only viable *E. coli* O157:H7 cells.

Results: This assay could detect as low as 3 CFU ml^{-1} of viable *E. coli* O157:H7 in apple juice and 3000 CFU ml^{-1} in spinach wash water within 4 h. In addition, it completely prevented false-positive PCR results generated by 10^3 CFU ml^{-1} and 10 CFU ml^{-1} of dead *E. coli* O157:H7 cells in apple juice and spinach wash water, respectively.

Significance: In conclusion, the CP-EMA-RT-PCR assay can effectively detect viable *E. coli* O157:H7 cells in apple juice and spinach wash water within a short time without the need for sample enrichment, while preventing the amplification of DNA in dead cells.

P2-52 Microbiological Profile of Dough Systems during Pita Chips, Pretzels and Pretzel Products Production

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Introduction: Products with A_w > 0.91 and a pH > 4.5 and < 9.6 may permit growth of *Staphylococcus aureus* and/or *Bacillus cereus* and, therefore, potential heat stable enterotoxin formation. Dough systems (A_w > 0.91) used for pita chips, pretzels and pretzel products may have the potential to support the growth of *S. aureus* and/or *B. cereus* during routine production. Few dough formulations contain yeast; however, other formulations do not contain yeast. As a result, it is important to understand the effect of dough formulations on the proliferation of *S. aureus* and *B. cereus* during routine production conditions.

Purpose: The purpose of this study was to evaluate the microbiological profile of pita chips and pretzel dough systems during routine production conditions from a food safety standpoint.

Methods: Dough samples were collected from commercial pita chips and pretzel facilities. Dough samples were individually inoculated with strains of *S. aureus* and *B. cereus* to achieve a target level of 10^3 CFU/g. Following inoculation, samples were stored at 25°C and 30°C for pita chips dough samples and 25°C for pretzel dough samples. Samples were analyzed following storage at 0, 1, 2, 3, 4, and 8 days for pita chips and 0, 1, 2, 3, 4, 7, 14, 21, 28 and 35 days for pretzels. Baird-Parker agar, Bacara® agar, Potato Dextrose agar and Tryptic Soy agar were used as plating media for *S. aureus*, *B. cereus*, Yeasts and Total Plate counts, respectively. The experiment was performed in triplicate. Limit of food safety was defined as 10 bacterial multiplications.

Results: *The n*umber of bacterial multiplications did not exceed ($P < 0.05$) the limit of food safety for both *S. aureus*/ *B. cereus* in all the evaluated dough types. This was attributed to 'competitive inhibition' due to the presence of yeast populations or native flour microflora.

Significance: These findings indicate no significant food safety risk associated with the current production practices. The data generated in this study also provide scientific basis for the facility's HACCP plan in compliance with anticipated FSMA guidelines.

P2-53 Comparative Evaluation of the 3M™ Molecular Detection Assay *Escherichia coli* O157 (including H7) for the Detection of *Escherichia coli* O157 in Raw Ground Beef

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Introduction: *Escherichia coli* O157:H7 is a pathogen associated with foodborne diseases. The infection by *E. coli* O157:H7 can cause sporadic cases or outbreaks of diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS). The Argentine Food Code (CAA), Article 255 establishes the absence of this pathogen in five 65 g samples of ground beef.

Purpose: The purpose of this evaluation was to compare the 3M™ Molecular Detection Assay *E. coli* O157 (including H7) protocol to the USDA/FSIS-MLG method for raw ground beef.

Methods: A total of 77 samples were artificially inoculated at a level of 10 CFU/65 g with five strains of *E. coli* O157:H7 and one strain of *E. coli* O157: non-toxigenic HNT, isolated in Argentina from foods. Sixteen strains of non-*E. coli* were analyzed to determine the specificity of the method in levels of 10 CFU/65 g.

Results: All contaminated samples with *E. coli* O157 were detected by the MDS as positive in real time after 15 minutes. All contaminated samples with non-*E. coli* were detected as negative with the system in 75 minutes.

The results also indicate 100% inclusivity and 100% exclusivity with no false positives or false negatives. Additionally, this study demonstrated the usefulness of this molecular assay owing to its rapidity, sensitivity and specificity, and how these features can be applied directly in the field of the food industry for detecting prevalent pathogens in Argentina.

Significance: Statistically, there was no significant difference between the MDS *E. coli* O157 (including H7) when compared to the USDA/FSIS-MLG reference methods as indicated by McNemar's X^2 value of 0.0. The MDS *E. coli* O157 provides a rapid and accurate solution for the detection of *E. coli* O157 in raw ground beef.

P2-54 Use of a Rapid PCR Method to Detect *Escherichia coli* O157:H7 and Non-O157:H7 Shiga Toxin-producing *Escherichia coli* (STEC) in 25-gram Samples of Two Dry Pet Food Formulations

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Introduction: *Escherichia coli O157:H7* and non- O157:H7 Shiga toxin-producing *Escherichia coli* (STEC) are foodborne pathogens occasionally found in pet food that can cause serious, and sometimes fatal, illness when ingested. *Escherichia coli* has a very low infectious dose (as few as 15 organisms). Since culture-based methods can be complicated and time-consuming, and because the organism is difficult to isolate when in the presence of a complex and highly processed matrix, well-validated rapid methods for the detection of this pathogen are needed.

Purpose: The purpose of this study was to evaluate the DuPont™ BAX® System Real-Time PCR Assays for *E. coli* O157:H7 and Real-Time STEC Suite as rapid detection methods for two pet food formulations (one canine and one feline). Samples used in this study were artificially inoculated with target organisms to ensure matrix inhibition would not cause false results.

Methods: Seven target strains of *Escherichia coli*(O157:H7, O26, O45, O103, O111, O121, O145) were separately inoculated onto three samples each of pelleted ground pet food at levels determined to return positive results based on previous studies. All samples were stressed for a period of 72 hours at room temperature, then combined with pre-warmed (42°C) System MP Media and incubated for 22 hours at 42°C. Samples were tested both with and without a re-growth step (1:50 dilution of primary enrichment into BHI incubated for 3 hours at 37°C), which was performed to eliminate potential PCR inhibitory compounds. All samples were tested with each of the PCR assays, followed by culture confirmation protocols outlined in the FDA-BAM.

Results: The PCR assays returned positive results for all inoculated samples (canine and feline foods) after the 22-hour primary enrichment, and identical results were returned after re-growth was performed. Therefore, the additional re-growth step is not necessary for this matrix. All positive enrichments were confirmed using the procedures included in the FDA-BAM.

Significance: Overall, the PCR Assay for *E. coli* O157:H7 and the Real-Time STEC Suite were found to give equivalent results to the reference method using the proprietary enrichment media. Furthermore, the BAX® System method returned results faster than the culture method, significantly decreasing the overall time to result.

P2-55 Development and Validation of Predictive Models for Growth of Non-O157 Shiga-toxigenic *Escherichia coli* (STEC) and *Salmonella* spp. in Ground Beef, Lettuce, and Non-fat Dry Milk

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Introduction: Microbial predictive models are food safety tools that can be used to evaluate potential risk of pathogen growth in foods to facilitate effective decision making. Research is limited regarding growth characteristics of non-O157 Shiga-toxigenic *Escherichia coli* (STEC) in food and on *Salmonella*spp. in lettuce and reconstituted non-fat dry milk.

Purpose: Develop and validate predictive models for growth of *Salmonella*spp. and non-O157 STEC in targeted food matrices.

Methods: Ground beef and shredded iceberg lettuce were inoculated with a six-serotype STEC cocktail (O26, O45, O103, O111, O121 and O145). A five-serovar *Salmonella*spp. cocktail was used to inoculate reconstituted non-fat dry milk (NDM) and lettuce. Isothermal growth data was collected for each of the pathogen cocktails at various temperatures (5.0-47.5°C). A Baranyi model was used to fit the primary model. A modified Ratkowsky model was used to generate the secondary model. Two sinusoidal temperature profiles (5-15°C and 10-40°C) were used to validate the dynamic models. Mean absolute relative error (MARE) was used to judge the accuracy of the models, with 0% MARE indicating best fit.

Results: MARE values for the ground beef model for low and high temperature non-O157 STEC profiles were 8.5 and 1.7%, respectively. MARE values for growth of non-O157 STEC in lettuce were 26.7 and 4.8% for the low and high temperature profiles, respectively. Similarly, the

dynamic model predicted the growth of *Salmonella* spp. on lettuce with MARE values of 5.4 and 6.3%, respectively, for high and low temperatures. MARE values for *Salmonella*in NDM were 5.8 and 6.6% for high and low temperature, respectively.

Significance: The dynamic models for both pathogens and the foods they were evaluated in resulted in low MARE% values (1.7-8.5%) at both storage temperature ranges, indicating acceptable model accuracy. MARE% for non-O157 STEC in iceberg lettuce stored at low temperatures (26.7% MARE) indicated a lack of fit of the model and a need for additional research.

P2-56 Cold-tolerance of Individual or Combined Non-O157 and O157 Shiga Toxin-producing *Escherichia coli* Strains in Ground Veal at 10°C

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Introduction: Recent studies have shown that the prevalence of Shiga toxin-producing *Escherichia coli* (STEC) O157 in veal reaches 20%, far higher than the 3-5% noted in beef. Research is needed to investigate whether processing interventions developed for beef systems against O157 and non-O157 STEC meet the level of intervention required for veal.

Purpose: This study compared the survival and/or growth of STEC of the six major non-O157 serogroups to that of O157:H7 STEC in ground veal stored at 10°C for 96 h or 120 h.

Methods: Two strains of each non-O157 serogroup (all clinical isolates from the CDC) and five O157:H7 strains (strain ATCC 43895 and four cold-tolerant beef-trim isolates), were grown individually in Brain Heart Infusion Broth (BHIB) at 37°C for 24 h. Inocula, each strain separately or combined as multi-strain cocktails of non-O157 or O157:H7, were prepared, 1 ml of inoculum was transferred to packages of fresh ground veal (25 g), resulting to an initial population of ca. 4.00 log CFU/g, and incubated at 10°C for up to 120 h. At each sampling point, one package of inoculated ground veal was withdrawn from storage. Serial dilutions were made in BPD and 0.1 ml samples were spread-plated on modified Eosin Methylene Blue agar (MEMB) with added sorbitol and bile salts. Samples were taken at 0 h, 12 h, 24 h, 36 h, 48 h, 72 h, 96 h or 120 h. Control samples of uninoculated ground veal with 1ml of BHIB added were spread-plated on Brain Heart Infusion agar (BHIA) in order to enumerate native microflora (n = 3). Data analysis was done using ANOVA (a = 0.05).

Results: For individual-strain studies, growth did not exceed 0.33 log CFU/g after 120 h of storage at 10°C and no statistical differences ($P > 0.05$) were observed in growth patterns of all the STEC strains tested. When STEC were combined into multi-strain cocktails of O157:H7 or non-O157, strains grew, with growth of the O157:H7 > non-O157 STEC, but not significantly ($P > 0.05$). Native microflora exhibited exponential growth over 120 h at 10°C, reaching a final population of ca. 8.00 log CFU/g.

Significance: The results of this study show that individual STEC may behave differently than combined strains in ground veal stored at 10°C. Further research is needed to validate these results under different environmental conditions.

P2-57 Pathogenicity Islands in Shiga Toxin-producing *Escherichia coli* O26, O103 and O111 Isolates from Humans and Animals

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Developing Scientist Competitor

Introduction: Non-O157 Shiga toxin-producing *Escherichia coli* (STEC) are emerging foodborne pathogens. Serogroups O26, O111 and O103 cause most known severe diseases and outbreaks related to non-O157 STEC. Pathogenicity islands (PAIs) play a major role in the evolution of STEC pathogenicity.

Purpose: To determine the distribution of pathogenicity islands among STEC O26, O103, and O111.

Methods: A collection of STEC O26 (n = 45), O103 (n = 48), and O111 (n = 52) from humans and animals were included in this study. Pulsed-field gel electrophoresis (PFGE) with *XbaI* digestion was used to characterize the clonal relationship of the strains. In addition, a PCR-RFLP assay was used to determine *eae* subtypes. Additional virulence genes on PAIs were identified using specific PCR assays, including OI-122: *pagC, sen, efa-1, efa-2,* and *nleB* ; OI-43/48: t*erC, ureC, iha,* and *aidA-1*; OI-57: *nleG2-3, nleG5-2,* and *nleG6-2*; and HPI: *fyuA*, and *irp2*.

Results: The strains of the same serotypes tend to have identical PAI patterns regardless of source except OI-43/48 in O103:H2 ($P < 0.05$). PFGE dendrogram demonstrated that instead of clustering together with strains from same O type (O111:H8), the O111:H11 (n=16) strains tended to be clustered together with strains of the same H type (O26:H11, n=45). In addition, O26:H11 and O111:H11 strains carried *eae* subtype beta, whereas O111:H8 strains had *eae* gamma2/theta. The O26:H11 and O111:H11 stains contained an incomplete OI-122 lacking *pagC* and a complete HPI. However, a complete OI-122 but no HPI was found in the O111:H8 strains. Additionally, *aidA-1* of OI-43/48 and *nleG6-2* of OI-57 were significantly associated with O26:H11 and O111:H11 strains but were almost missing in O111:H8 strains ($P< 0.001$).

Significance: This study demonstrated that H11 (O111:H11 and O26:H11) strains were closely related and may have come from the same ancestor. H antigen might be used as a valuable marker to predict virulence potential and evolutional relationship of non-O157 STEC in combination with other markers.

P2-58 Differential Induction of Shiga Toxin 2-encoding Bacteriophages in Shiga-toxin Producing *Escherichia coli*

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) is an important foodborne pathogen that causes severe intestinal and systemic diseases. The main virulence factor is the Shiga toxin, whose genes are encoded in the genome of temperate phages (Stx phages). Stx phages activate their lytic cycle by various induction mechanisms. This increases the Stx expression and as a consequence the severity of the STEC infections. The process concludes with the lysis of the bacterial cell. The critical question is what is the advantage for STEC to harbor a Stx phage, if this could cause its final lysis and destruction? We speculate that differential rates of induction should happen within a single STEC population.

Purpose: To evaluate if there is a differential induction of Stx phages from a population of *E. coli* lysogenic for Stx phages.

Methods: A recombinant phage harboring a Green fluorescent protein inserted in the *stx* was constructed. Activation of phage lytic cycle results in an increase of fluorescence in the cells after activation and before its lysis. We evaluated this fluorescence through epifluorescence microscopy and flow cytometry.

Poster

Results: When inducing the lytic cycle of Stx phages in a STEC population, epifluorescence microscopy showed that not all the cells were fluorescent. Flow cytometry showed a fraction of the population that induced after 4 hours. This fraction increased after 6 and 24 hours. However, there was always a part of the STEC population that remained uninduced and never lysed.

Significance: A fraction of the STEC population lysed after Stx phage induction, causing higher Stx expression, thus higher virulence of the infection. This is the advantage conferred by the Stx phage. However, a small fraction of the population remained uninduced, and this will guarantee the continuity of the population.

P2-59 Heat Resistance and Biofilm Formation of *Escherichia coli* Serotypes at Various NaCl Concentrations

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Introduction: Many studies have indicated that stress response and growth of foodborne pathogens depend on serotype as well as strain.

Purpose: Therefore, the objective of this study to compare the heat resistance and biofilm formation among *Escherichia coli* serotypes.

Methods: *E. coli* O157:H7 NCCP11142, *E. coli* O111 ATCC12795, and *E. coli* O26 ATCC43887 exposed to 0, 2, and 4% NaCl supplemented in tryptic soy broth (TSB), and *E. coli* cells were harvested at early stationary and late stationary phases. The *E. coli* cells were washed twice with phosphate buffer solution. Inocula of *E. coli* O157:H7, *E. coli* O111, and *E. coli* O26 were inoculated into TSB plus 0, 2, and 4% NaCl, followed by exposure to 50°C for 2 h. The survivals of *E. coli* serotypes were then enumerated on tryptic soy agar. In addition, the NaCl-exposed cells were inoculated into 96-well microtiter plate, followed by incubation at 35°C for 24 h. Biofilms were then measured by crystal violet.

Results: *E. coli* O157:H7 exposed to 4% NaCl had higher ($P < 0.05$) heat resistance than other *E. coli* serotypes, regardless of growth stage. Comparing biofilm formation, biofilms of three *E. coli* serotypes generally decreased ($P < 0.05$) for early and late stationary stage cells, except for the early stationary stage cells of *E. coli* O26. In addition, late stationary cells formed more biofilms ($P < 0.05$) than early stationary cells.

Significance: This result indicates that different serotypes of *E. coli* have different stress responses and biofilm formation.

P2-60 Attachment of Shiga Toxigenic *Escherichia coli* (STEC) on Stainless Steel Hex Nuts in Minimal and Full Nutrient Broth

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Introduction: Formation of biofilms on processing equipment within food production facilities can have a detrimental effect on both quality and safety of the products by harboring pathogens and spoilage bacteria. Recent regulations identifying six additional serogroups (O26, O45, O103, O111, O121, and O145) of Shiga toxigenic *Escherichia coli* (STEC) plus the original designation of *E. coli* O157 as adulterants in non-intact beef product underscores the importance of understanding how these strains grow and interact within the food processing environment.

Purpose: The objective of this research is to determine if strains of each STEC serogroup can attach to stainless steel and potentially form biofilms.

Methods: Four strains for each serogroup with variation in presence of *stx1* and *stx2* and origin of strain were examined. Stainless steel hex nuts, with a rough or smooth surface, were incubated in either minimal or full nutrient broth inoculated with a single strain of STEC at 25°C up to 48 h. At the time interval (4, 8, 12, 24, or 48 h), the nut was removed, rinsed, stained with crystal violet, and the crystal violet was removed from the nut to determine solution absorbance at 590 nm to measure resultant biomass.

Results: Significant differences ($P < 0.05$) between strains within serogroup were found within the rough surface/minimal media combination for O45, O103 and O111 with some evidence of differences ($P < 0.08$) for O157 with no differences found over time. Significant differences between strains within serogroup were also found within the smooth surface/minimal media combination for O26, O45, O103, and O121 and no differences over time except for STEC O45 serogroup.

Significance: These data indicate differences in attachment ability between strains within serogroup. Further research utilizing strains with greater attachment abilities is needed to examine the effectiveness of commonly used detergents and sanitizing agents on STEC biofilm renewals as well as improvements of current methodologies to determine formation of STEC biofilms.

P2-61 Free Shiga Toxin 2-encoding Bacteriophages from Food to Feces and Beyond

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Introduction: Shiga toxin-producing bacteria (STEC) are important foodborne pathogens implicated in food outbreaks and causing severe intestinal and systemic diseases. The main virulence factor is the production of Shiga toxin. The genes of Shiga toxin are encoded in the genome of temperate bacteriophages (Stx phages). Infectious Stx phages have been found free in wastewater and in food samples, suggesting they could be directly excreted through feces.

Purpose: Detection of free Stx phages in 80 fecal samples from healthy individuals to confirm if humans could excrete Stx phages independently of the presence of STEC strains.

Methods: Eighty human fecal samples were collected from humans that were not involved in a foodborne outbreak and did not show any severe gastrointestinal pathology. The DNA from the bacteriophage fraction of the samples was extracted and the *stx* gene was detected and quantified by real-time quantitative PCR. Positive amplicons were sequenced.

Results: A 55 % of the samples showed the presence of stx_2 in phage DNA. The densities of Stx phages were of 5.2×10^4 Stx phages/g of feces (from 4.5×10^1 to 1.3×10^6 stx/g). Bacteriological cultures for enteropathogens in these samples were negative. Sequencing suggested that the phages could carry various Stx variants (stx_2, stx_{2v}, stx_{2c} and stx_{2d}).

Significance: Free Stx phages circulate among the healthy population. They could be ingested through some foods and are consequently excreted by feces. Detection of *stx* using PCR approaches is commonly used for STEC detection in clinic and food. However, free Stx phages can also show a positive *stx* result in a molecular analysis, resulting in a positive STEC diagnostic, without a real presence of the pathogenic strain in the sample.

Poster

P2-62 The Effect of Weaning Stress on the Levels of Shiga Toxin-producing *Escherichia coli* and Fecal Butyrate-producing Bacteria in Young Calves

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Introduction: To ensure better beef safety, pre-harvest control strategies targeting Shiga toxin-producing *Escherichia coli* (STEC) have attracted a lot of attention. Our previous study showed that higher levels of certain butyrate-producing bacteria (BPB) corresponded with lower STEC shedding levels in young un-weaned calves and the amount of BPB changed as the calf age increased.

Purpose: The purpose of this study was to evaluate the effects of weaning stresses on BPB and the STEC shedding levels.

Methods: One hundred and nineteen calves were used in this study. The calf body weight, fecal STEC and O157 were determined 2-days before and 10-days after the weaning time. The gene copy numbers of butyryl CoA:acetate CoA transferase (BCCT) in feces was used to determine the abundance of BPB via a SYBR Green Real-time PCR.

Results: Weaning reduced the calf body weight (ANOVA, $P < 0.05$), increased the STEC shedding level (from 2.35 ± 1.60 to 3.05 ± 1.04 log CFU/g fecal sample, Wilcoxon signed ranks test, $P < 0.05$) but had no effects on *E. coli* O157 levels ($P > 0.05$). High STEC shedders (calves that shed more than 4 log CFU/g of STEC) had significantly lower BPB than none-STEC calves before and after weaning (Mann-Whitney U test, $P < 0.05$). Weaning procedure lead to an increase in BPB levels (Wilcoxon signed ranks test, $P < 0.05$).

Significance: The data confirmed that correlations exist between STEC shedding and BPB levels. Weaning is a step that can change both the BPB and STEC and can serve as an additional potential STEC control opportunity during pre-harvest stages.

P2-63 Adherence to and Invasion of Bovine and Human Colonic Epithelial Cells by Non-O157 Shiga Toxin-producing *Escherichia coli*

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Introduction: Serogroup O26, O45, O103, O111, O121, and O145 Shiga toxin-producing *Escherichia coli* (STEC) have been declared adulterants in non-intact, raw beef by the USDA-FSIS, and a foodborne enteroaggregative STEC O104:H4 strain that emerged and caused an epidemic in Europe is an additional concern.

Purpose: The objectives of this study were to compare strains of varying intimin and flagellar composition from each of these seven serogroups for adherence and attaching-effacing (A/E) lesions in bovine and human colonic mucosal epithelial cells.

Methods: Bovine colonic mucosal explants and human Caco-2 cells were inoculated with strains, and adherence and invasion were evaluated by immunohistochemistry (IHC), fluorescent actin staining (FAS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and a standard invasion assay.

Results: Based on IHC, all strains adhered to mucosal epithelium in explants. By SEM, adherent O104:H4 bacteria had structures resembling aggregative adherence fimbria and some, e.g., O45:H2, O103:H2, and O103:[H11] adhered to intestinal mucus and epithelial cells via structures morphologically consistent with flagella. All intimin-positive strains induced A/E lesions on Caco-2 cells by FAS. Invasion in explants was detected by IHC and TEM with one O103:H2 and one O104:[H21] strain, with the O103:H2 strain also invasive in Caco-2 cells. Non-O157 STEC expressing different flagellar types, viz., H2, H10, H11, H16, H19, H21, and H28, and variants of intimin, viz., β1, ε, θ, and γ1, adhered to bovine colonic epithelium and induced the formation of A/E lesions. Non-O157 STEC strains lacking intimin were also shown to adhere to bovine colonic epithelium; hence, must utilize other mechanisms for attachment.

Significance: These studies suggest that most non-O157 STEC adulterant strains and potentially O104:H4 have the capacity to colonize bovine intestinal epithelium. More studies are needed to address these adherence and colonization mechanisms to provide a basis for effective pre-harvest interventions.

P2-64 Prevalence of Non-O157 Shiga Toxin-producing *Escherichia coli* Shed by Beef Calves before and after Weaning

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Developing Scientist Competitor

Introduction: Cattle are primary reservoirs of Shiga toxin-producing *Escherichia coli* (STEC) and are considered to be "super-shedders" if they shed more than 10^4 CFU/g of STEC. In addition to *E. coli* O157:H7, six non-O157 STEC serogroups were added to the zero-tolerance adulterant list in June 2012. Limited information is available about the prevalence of non-O157 serogroups in beef cattle during the pre-harvest stages.

Purpose: The purpose of this study was to determine the prevalence of non-O157 STEC in beef calves and identify the main serogroups associated with the "super-shedders" before and after weaning.

Methods: At 6 and 9.5 months of age, fecal samples were taken from 110 calves and the concentrations of STEC were enumerated by plating samples onto CHROMagar™ STEC agar. Three suspect non-O157 colonies were picked from the plates of "super-shedder" samples, confirmed to be non-O157 by using CHROMagar™ O157 plates, and then were further tested by a multiplex PCR assay targeting *stx*1, *stx*2, and *eae*A genes. Isolates that were positive for at least one gene were sent for serotyping and an antibiotic resistance study was completed for the O26 isolates obtained from the farm.

Results: Based on the CHROMagar™ STEC enumeration results, 9 out of 110 calves were super-shedders; together with the super-shedding calves, the shedding levels of an additional 20 calves which had approximately 10^2 CFU/g STEC did not change before and after weaning while two calves changed from high STEC shedders to non-shedders. Among the suspect non-O157 "super-shedder" isolates, 56% of them were positive for at least one target gene. Serotyping results showed that O26 was the dominant serogroup.

Significance: The results from this study show that calves can start STEC shedding early in their lives and the weaning step does not generate a big impact on STEC shedding levels.

P2-65 2012 Voluntary Non-O157:H7 STEC Proficiency Testing Study

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Introduction: For U.S. clinical laboratories, proficiency testing (PT) has evolved into an essential component of quality assurance programs. These laboratories are regulated by CLIA 1988, which mandates participation in PT. Food laboratories may soon be subject to similar requirements with passage of the Food Safety Modernization Act, which may require laboratory accreditation for foods regulated by the FDA. On September 14, 2011, the USDA announced that the following "Big 6" non-O157:H7 Shiga Toxin-producing *Escherichia coli*'s (STECs) are considered adulterants: O26, O45, O103, O111, O121, and O145. Mandatory testing of beef trim for these STECs began June 4, 2012.

Purpose: This pilot PT study includes data from three test events in 2012, where participants volunteered to submit results for detection and identification of an unknown STEC. Our objective was to assess whether laboratories have the ability to identify the presence of one of the above STECs.

Methods: False (-) rates for STECs were recorded from 101 cumulative proficiency testing results submitted in three test events in the year 2012. No false (+) data was collected as participants were informed that an STEC was present in the sample.

Results: Performance accuracy for STECs appears problematic with results indicating a false negative/misidentification rate of 6%. In addition, a significant number of results (21%) indicated that an STEC was present, but participants were unable to identify the strain.

Significance: As laboratories begin to routinely test for STECs, and as laboratory test methodologies improve, it is anticipated that the ability of laboratories to correctly identify STECs will increase. However, this false (-) rate is similar to the 14 year API average of false (-) *E. coli* O157:H7, 6.6%.

P2-66 Animal Age is a Factor to Target Developing Pre-harvest Intervention to Reduce Shiga Toxin-producing *Escherichia coli*

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Introduction: Controlling the prevalence of Shiga toxin-producing *Escherichia coli* (STEC) in cattle at the pre-harvest level is critical to reducing outbreaks of this pathogen in humans. A multilayer of factors, including environmental and bacterial factors, modulates the colonization and persistence of *E. coli* O157 in cattle, which serve as a reservoir of this pathogen. However, understanding the animal factors, which affect the prevalence of this pathogen remains unknown.

Purpose: The purpose of this study was to identify animal factors that affect the prevalence of STEC in cattle to provide insights for the development of mitigation strategies at the pre-harvest level.

Methods: Cattle fecal swab samples were collected at the rectal anal junction (RAJ) from 90 animals, which were used twice in this project over the course of two years. Swab samples were plated on MacConkey agar, and incubated at 37°C for 24 hours to isolate lactose fermenting colonies. Twenty pink colonies per plate were analyzed for STEC using multiplex PCR amplifying *stx1* and *stx2* genes. Pearson's Chi-squared test was used to compare the prevalence of *stx1*, *stx2*, or *stx1/2* positive microorganisms in cows and heifers.

Results: The total number of STEC from the RAJ varied between animals, ranging from 0 to more than 10^6. The prevalence of STEC (positive with either *stx1* or *stx2*) was 47% and 50% in the 1st and 2nd year, respectively, and the majority of positive samples contained this pathogen at 10^2-10^5CFU/swab. The prevalence of STEC was significantly lower ($P < 0.01$) in heifers compared to cows, indicating animal age plays a key role in the prevalence of STEC.

Significance: Our data reveal that animal age affects the prevalence of STEC in cattle, providing a potential mitigation strategy to reduce STEC at the pre-harvest level.

P2-67 Evaluation of NP51 Feed Additive in a Research Feedlot Facility and Its Effectiveness in Reducing Shiga Toxin-producing *Escherichia coli* (STEC) in Cattle Feces

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Introduction: Due to harmful and sometimes deadly effects to consumers, Shiga toxin-producing *Escherichia coli* (STEC) are a growing threat to public health. Various beef products are associated with STEC illnesses and cattle harbor these pathogens in their gastrointestinal tract. When fed, selected strains of *Lactobacillus acidophilus* (NP51) are proven to reduce the presence of some pathogens, including *E. coli* O157:H7, in the digestive track of cattle; thus, this intervention warrants investigation into the control of non-O157 STECs.

Purpose: Evaluate the effectiveness of *Lactobacillus acidophilus* (NP51) at a rate of 10^9/head/day as a feed additive in cattle rations to reduce STEC in fecal samples.

Methods: One hundred and twelve head of cattle were separated into twenty-eight pens and fed either a treatment (NP51) or control feed ration. Fecal samples were collected from cattle and evaluated for the presence of *Escherichia coli* O26, O45, O103, O111, O121, O145 and O157:H7 by enriching ten grams of fecal sample in ninety ml of gram negative broth. Enrichments were then screened for the non-O157 STEC serogroups using the Dupont Qualicon BAX®system and O157:H7 immunomagnetic bead were used to plate enrichments on R&F *E. coli* O157:H7 agar to test for O156.

Results: A 60% ($P < 0.10$) reduction in overall STEC prevalence was observed among cattle fed NP51 in comparison to control cattle. Similarly, cattle fed NP51 demonstrated reduced prevalence rates of 20%, 15%, 15% and 8% for serogroups O26, O45, O121 and O103, respectively. Conversely, an increase in prevalence was observed for serogroups O111 and O145 among cattle fed NP51.

Significance: These data indicate a significant reduction in most STEC fecal populations among cattle fed the pre-harvest intervention *Lactobacillus acidophilus* NP51 at 10^9/head/day. Decreasing STEC in cattle before harvest is an important step to improving food safety and this study demonstrates the efficacy of NP51 as a pre-harvest intervention.

P2-68 Efficacy of Lactic Acid, Hot Water, and Acidified Sodium Chlorite for the Reduction of Non-O157 Shiga Toxin-producing *Escherichia coli* (STEC) Utilizing a Hot Carcass Model and *Escherichia coli* O157:H7 as an Indicator

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Introduction: To address the emergent concern of non-O157 STEC impact on public health, USDA-FSIS regulations recently declared six non-O157 STECs as adulterants in ground beef and non-intact beef products. Testing for individual non-O157 STECs in addition to *Escherichia coli* O157:H7 may result in substantial increases in production costs for beef producers.

Purpose: To measure reductions of each non-O157 STEC serogroup included as an adulterant by applying lactic acid (LA; 5±0.2%), hot water (HW; 180 ±5° F) and acidified sodium chlorite (ASC; ≤1100 PPM) in parallel with *E. coli* O157:H7 in a hot carcass model (Brisket).

Methods: Briskets (n=14) were heated to 37°C, cut into halves and four brisket halves randomly assigned to each of seven serogroups (O157:H7, O26, O121, O45, O145, O111, O103). Four per each serogroup, brisket halves were inoculated by submersion into a 3-strain inoculum cocktail for 1 minute, allowed 30-minute bacterial attachment time, followed by simultaneous application of antimicrobial interventions (LA, HW and ASC). Pre-intervention and post-intervention swabs were obtained and plated onto MacConkey agar.

Results: Lactic acid significantly reduced the non-O157 STECs and the reductions are not statistically different compared to *E. coli* O157:H7, with the exception of STEC O111 ($P = 0.049$). When treated with HW, a 0.57-log reduction of *E. coli* O157 was achieved, which is not statistically different from serogroups O26, O121, O145, and O45. This study showed a log reduction of 0.63 for STEC O157 when treated with acidified sodium chlorite, which is significantly not different from serogroups O26, O121, O145, and O45.

Significance: These data indicate *E. coli* O157 is an effective indicator of intervention efficacy for the non-O157 STEC serogroups O26, O121, O145, and O45 and O103 when 5% LA is utilized, but may not be justified for serogroups O111 and O103 when HW or ASC are utilized.

P2-69 Efficacy of Lactic Acid, Hot Water, and Acidified Sodium Chlorite for the Reduction of Non-O157 Shiga Toxin-producing *Escherichia coli* (STEC) Utilizing Chilled Beef Subprimals and *Escherichia coli* O157:H7 as an Indicator

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Introduction: Testing for the presence of *Escherichia coli* O157:H7 and the six non-O157 STECs in ground beef and non-intact beef products will impose greater costs for the beef processing industries. This would also pose a challenge for them to test for O157 and non-O157 STECs in the beef products.

Purpose: To quantify reductions of each non-O157 STEC serogroup achieved by lactic acid (LA), hot water (HW) and acidified sodium chlorite (ASC) simultaneously with *E. coli* O157:H7 on chilled beef subprimals.

Methods: Chilled subprimals (n = 86) are utilized and four subprimals are randomly assigned to each serogroup and antimicrobial intervention (2 control + 2 interventions). For each serogroup and intervention, four subprimals were inoculated by submersion (1 minute) into a 3-strain cocktail, subjected to a 30 minute attachment period and placed in a chad cabinet for intervention application. Sample swabs were collected both pre- and post-intervention and plated onto MacConkey agar.

Results: When treated with LA, O157 showed 0.65-log reduction while the non- O157 STEC serogroups showed 0.24 to 0.58-log reduction except O103, which showed no numerical reduction. STEC O157 when treated with HW and ASC exhibited 0.61 and 0.44-log reductions, respectively. HW and ASC achieved reductions in all the six non-O157 serogroups ranging from 0.07 to 0.43 and 0.26 to 0.83-log reductions, respectively. When treated with LA, non-O157 STEC serogroup reductions were not statistically different from *E. coli* O157 reductions, with the exception of serogroup O103 ($P = 0.011$), but are all significantly different from *E. coli* O157 reductions, with the exception of O121, when treated with HW. Reductions observed for non-O157 STEC serogroups treated with ASC were not significantly different from *E. coli* O157.

Significance: Although not universal among all serogroups and interventions (i.e., O103 and LA), *E. coli* O157 may act as an effective indicator for detecting reductions among the 6 non-O157 STEC serogroups.

P2-70 Fate of Shiga Toxin-producing *Escherichia coli* during Storage at Different Temperatures of Gamma Irradiated Spinach (*Tetragonia expansa*)

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Introduction: In recent years, vegetables, including spinach, have been associated with many foodborne outbreaks. Among the pathogenic microorganisms that can be transmitted by these foods, Shiga toxin-producing *Escherichia coli* deserve attention. As the use of sanitizers does not significantly reduce the population of microorganisms present in vegetables, the application of more efficient techniques, such as irradiation, is necessary.

Purpose: To evaluate the behavior of Shiga toxin-producing *E. coli* in spinach after exposition to gamma radiation, during storage at different temperatures.

Methods: Samples of spinach (*Tetragonia expansa*) were inoculated with a cocktail of three strains of Shiga toxin-producing *E. coli* O157:H7 ($10^5 – 10^6$ CFU/g) and exposed to 0.85 kGy. The samples were stored at 4°C and at abuse temperature (10°C) for up to 12 days. Population was enumerated on days 0, 3, 6, 9 and 12, using tryptone soya agar (TSA) with an overlay of sorbitol MacConkey agar plus cefixime and potassium tellurite. Plates were incubated at 37°C/24 h.

Results: The applied dose (0.85 kGy) reduced the population of Shiga toxin-producing *E. coli* in approximately 6 log on day zero. Populations of *E. coli* remained below 1 log when samples were stored at 4°C and at 10°C for up to 12 days.

Significance: The results showed that gamma radiation combined with storage at cold temperatures can reduce the risk posed by Shiga toxin-producing *E. coli* to consumers when it is contaminating minimally processed vegetables.

P2-71 Gamma Radiation Inactivation of Non-O157:H7 Shiga Toxin-producing *Escherichia coli* in Foods

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Introduction: Non-O157:H7 serovars of shiga toxin-producing *Escherichia coli* are emerging foodborne pathogens that have been associated with illness outbreaks and food product recalls on a global basis. Ionizing (gamma) radiation is a nonthermal food safety intervention technology that has been approved for use in over fifty countries to improve the safety and shelf-life of foods.

Purpose: In this study, the use of gamma radiation to inactivate pathogenic *E. coli* in foods was investigated.

Methods: A cocktail of six non-O157:H7 serovars, O26:H11, O45:H2, O103:H2, O111:H11, O121:H19, and O145, or six O157:H7 serovars was inoculated (10^8 CFU/g) into ground beef (GB), ground turkey (GT), catfish fillets (CF), or shrimp (SH). Food samples (10 g) were placed in sterile poly-nylon sample bags, sealed under vacuum and then frozen for 3 min at -59°C using a Cryo-Test Chamber by exposing the samples to controlled liquid nitrogen vapor. The samples were then irradiated (0, 0.6, 1.2, 1.8, 2.4, and 3.0 kGy) using a self-contained 137-Cs gamma radiation source (0.074 kGy/min at -20°C). Duplicate samples were processed per experiment at each radiation dose, and each experiment was conducted 3 times.

Results: Radiation D-values (the radiation dose needed to inactivate 1 log of pathogen) for *E. coli* O157:H7 were 0.47, 0.46, 0.52, and 0.42 kGy in GB, GT, SH, and CF, respectively. The D-values for the non-O157:H7 serovars were 0.40, 0.34, 0.41, and 0.38 kGy in GB, GT, SH, and CF, respectively. With the exception of catfish, the D-values for the non-O157:H7 serovars were significantly lower ($P < 0.05$) than those for O157:H7.

Significance: These results indicate that the radiation doses used to inactivate *E. coli* O157:H7 in these foods can also be used for the non-O157:H7 serovars.

P2-72 Differentiating Non-O157:H7 STEC Colonies from Competing Background Microflora in Ground Beef Broth by Hyperspectral Imaging

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Introduction: Detection and recovery of non-O157 Shiga toxin-producing *Escherichia coli* (STEC) in high background food matrices, such as ground beef, is difficult due to the competing background microflora. The use of acidification, immunomagnetic separation procedures and plating on modified Rainbow agar has increased the recovery. However, in some cases it is still problematic to differentiate and select a well isolated target STEC colony for confirmation.

Purpose: To investigate the ability of hyperspectral imaging to detect STEC serogroups (O26, O45, O103, O111, O121, and O145) artificially inoculated in ground beef broth from background microflora plated on Rainbow agar.

Methods: Ground beef (65 + 2 g) was enriched in 585 +15 ml of modified tryptic soy broth (m-TSB, 20 mg / ml novobiocin) overnight at 42°C. Four ten-fold dilutions of the enriched ground beef sample were prepared in sterile saline. Then for each serogroup, 1000 CFU (10 ml of a 10^5 CFU/ml cell suspension) of STEC was spiked into 990 ml of each of the enriched ground beef sample dilutions. The STEC spiked ground beef sample dilutions were spread onto Rainbow agar plates. The Themis Vision Systems' hyperspectral imaging system was used to acquire images of agar plates. Regions of interest associated with STEC target colonies and competing background microflora colonies were created for validation using a previously developed Mahalanobis distance classifier.

Results: Principal component (PC) score plots revealed separability of the target STEC colonies from each other and the background microflora colonies with the exception of O111. Scores of the competing background microflora were mixed into target O111 PC space. As a result, 33% of the background flora was misclassified as serogroup O111. No other serogroups were misclassified as background microflora. A new Mahalanobis distance classifier was developed to differentiate only O111 from the background microflora which reduced the false positive rate to 5.3%. However, the classifier had a false negative rate of 13% for O111.

Significance: Hyperspectral imaging can improve the speed and accuracy of selecting well isolated STEC colonies from enriched samples plated on Rainbow agar for confirmation.

P2-73 Shiga Toxin-producing *Escherichia coli* H Antigen Clustering Evidenced by the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) Array

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Developing Scientist Competitor

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) is a leading foodborne pathogen in the U.S., causing serious disease and other health complications. In response, several STEC serogroups are considered adulterants and are banned from meat products. The development of new detection and subtyping approaches for foodborne pathogens depends on the identification of novel molecular markers. A recently discovered genetic element –CRISPR array— is present and variable in *Escherichia coli*. In this study, we are exploring the utility of CRISPR as genetic marker.

Purpose: To characterize the CRISPR array in Shiga toxin-producing *E. coli*.

Methods: The CRISPR arrays of 195 *E. coli* isolates were characterized by PCR amplification and DNA sequencing. The CRISPR elements were extracted using a combination of the "CRISPRFinder" online utility and an Excel macro, and then graphically displayed using the described macro. Along with the CRISPR data we also screened for select pathogenicity genes –*eae*, *stx*1 and *stx*2, and *hly*A.

Results: CRISPR1 and CRISPR2 arrays of 195 strains show minimal variations within a serotype, but more than one type of sequence within a serogroup exist. Interestingly, almost identical arrays are shared by strains from different serogroups; these strains have the same H type (O26:H11, O103:H11 and O111:H11). Serotypes O45:H2 and O103:H2 share similar arrays as well. Finally, CRISPR 3 and CRISPR 4 were detected in only one of the 195 isolates, but a residual CRISPR3-4 array was detected in 10 strains.

Significance: This characterization of CRISPR array in STEC could lead to the development of molecular methodologies for the detection and subtyping of this pathogen.

Poster

P2-74 Rapid and Sensitive Detection of Shiga Toxin-producing *Escherichia coli* in Environmental Samples by Multiplex PCR

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) has been associated with numerous foodborne illness outbreaks. A rapid and sensitive detection method is essential for environmental monitoring and outbreak investigations.

Purpose: The objective of this study was to develop a simple and sensitive method for rapid detection of STEC in challenging environmental samples.

Methods: Multiplex PCR targeting *eae, stx*1 and 2 genes was developed and its sensitivity was evaluated using pure overnight culture of STEC O157:H7 and O26:H11, respectively, and those spiked with background microflora from enriched soil and pecan samples. The effect of additives, Bovine serum albumin (BSA), Polyvinylpyrrolidone (PVP), Polyethylene glycol (PEG) and Gelatin, on PCR sensitivity was also evaluated.

Results: The developed multiplex PCR method, pre-spin sample matrices in combination with the addition of BSA and PVP in PCR reaction mix, can detect up to 4 CFU/reaction of STEC O157:H7 and 40 CFU/reaction of O26:H11 either as pure culture or in the presence of background microflora from overnight enriched environmental samples of soil or pecan.

Significance: The developed multiplex PCR assay is simple and sensitive with broad applicability for rapid detection of STEC in intricate environmental samples.

P2-75 Reductions of *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus* on Seasoned Dried File Fish Using UV and COP as Light Sterilization

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Introduction: Seafood is known as the best natural food including various beneficial nutrients to promote and maintain the health of human body. Processed seafood products are mainly distributed at traditional markets with inadequate hygiene. Also, most processed seafood products can be manufactured without thermal processing. Based on above reasons, the hygiene for processed seafood products is the main concern in the food industry and distribution. *Staphylococcus aureus* and *Bacillus cereus* are the main potential pathogenic contaminants in seasoned dried seafood products including file fish. Ultraviolet (UV) light irradiation and cold oxygen plasma (COP) light can be used for application of surface sterilization on during the manufacturing process of seafood dried products

Purpose: The objective of the current study was to examine the UV light and COP in reducing *Escherichia coli*, a hygiene indicator and *S. aureus* and *B. cereus*.

Methods: *E. coli* ATCC 10536, *S. aureus* ATCC 6538 or *B. cereus* F4810/72 were inoculated with approximately 8-9 log CFU/sample on the surface of seasoned dried file fish. The prepared samples were exposed to 396, 660, 1,320, 2,640 and 3,960 mW·s/cm^2 of COP irradiation that TiO_2 content was 100% or exposed to UV irradiation at 180, 300, 600, 1,200 and 1,800 mW·s/cm^2. All treated samples were rinsed with 90 ml of 0.1% peptone and inoculated selective media.

Results: In case of UV at 1,800mW·s/cm^2, the maximum reduction values of total bacteria and *E. coli, S. aureus* and *B. cereus* are 1.01, 1.65, 2.42, 2.63 log CFU/g, respectively. In case of COP at 2,640 mW·s/cm^2, the maximum reduction value of total bacteria and *E. coli*, *S. aureus* and *B. cereus* are 1.38, 2.41, 2.54, 2.93 log CFU/g, respectively. The reduction effect of total bacteria is smaller than food poisoning bacteria. The result indicated that UV and COP treatment have an effect to reduce food poisoning bacteria on seasoned dried file fish. Especially, COP has effect to reduce food poisoning bacteria than UV on seasoned dried file fish.

Significance: According to this study, COP treatment is more suitable for reducing food poisoning bacteria on seafood processing industry.

P2-76 Optimization for Synergistic Effects of Combined Chlorine and Sonication on *Bacillus cereus* in Dried Laver Using a Predictive Reduction Model

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Introduction: Dried laver (*Porphyra tenera*) is widely consumed in Korea and Japan. It is an important seaweed that is high in minerals and vitamins and has considered nutritional value and a pleasing taste to the people in two countries. However, 12.0% of *Bacillus cereus* was detected in Korean dried laver and thus this pathogen is an emerging seafood pathogen with public significance. Combined treatment of chlorine and sonication can be potentially used in the seafood industry to reduce *B. cereus* for food preparation and manufacturing.

Purpose: This study was conducted to investigate the optimization for synergistic effects of combined chlorine and sonication for the reduction of *B. cereus* in dried laver using a mathematical predictive reduction model.

Methods: The combination treatment of chlorine concentrations (0-200 ppm) and sonication time (0-100 min) at 1,200 W and 36 kHz was arranged by central composite experiment design (CCD). The Polynomial models were developed for predicting reduction values of *B. cereus* in dried laver by combined chlorine disinfection and sonication. Hunter "L" was measured by color difference meter.

Results: The polynomial equation predicting the inactivation of *B. cereus* was as follows: $Y = 0.001 + 0.006\ X_1 - 0.013\ X_2 - 1.8E\text{-}05 X_1X_2 + 4.8E\text{-}05 X_1^2 + 1.1E\text{-}05 X_2^2$ (X_1: sonication time, X_2: chlorine concentration). The predictive reduction models by response surface methodology were fit well ($R^2 = 0.9998$) and expressed as adequate models by Prob > F-value ($P < 0.0001$). The optimized effect of combined chlorine and sonication for the reduction of *B. cereus* in dried laver was predicted at 57 ppm of chlorine and 10 min of sonication. The reduction value was 1.8 log CFU/g. There was no significant difference of Hunter "L" (lightness) between no treatment dried laver and combined chlorine and sonicated dried laver.

Significance: Using a mathematical prediction model, combined treatment of 57 ppm of chlorine and 10 min of sonication provides an effective reduction of *B. cereus* in dried laver without changes in color.

P2-77 Virulence Testing of Multi-drug Resistant *Staphylococcus aureus* Isolated from Meat and Clinical Samples

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Introduction: Over 200 known diseases are transmitted via food, and the causes include bacteria, viruses, parasites, toxins, metals and prions. It is estimated that foodborne diseases are attributable to 76 million illnesses, resulting in 325,000 hospitalizations and 5,000 deaths annually in the United States. Multi-drug resistant *Staphylococcus aureus* is one of the leading causes of foodborne disease outbreaks, and the incidence

Poster

of hospital- and community-acquired staphylococcal infections are also increasing which have shown to exhibit multiple virulence factors, such as, production of clumping factor A, Panton-Valentine leukocidin and γ-hemolysin.

Purpose: Therefore, the aim of the study was to determine the virulence properties of multi-drug resistant *Staphylococcus aureus* isolated from clinical and meat sources.

Methods: Fifty-five strains of multi-drug resistant *Staphylococcus aureus* isolated from 34 meat and 21 clinical samples were characterized using polymerase chain reaction (PCR) to detect the presence 16S rRNA gene for *Staphylococcus* spp. and clumping factor A (*Clf-A*); Panton-Valentine leukocidin (PVL) and hemolysin (γ-*hly*) virulence genes. To determine strain diversity, pulse field gel electrophoresis (PFGE) cluster analysis for the *Clf-A* and 16SrRNA positive isolates was compared.

Results: Results of this study indicated that 18 (32.7%) of the clinical and 6 (11.0%) of meat samples were positive for both Clf-A and 16Sr-RNA genes. All Isolates (100%) were negative for the detection of the Panton-Valentine Leukocidin and hemolysin gene by polymerase chain reaction. When PFGE patterns were compared, 6 (11%) of the SmaI-digested plugs had distinct PFGE cluster patterns.

Significance: Our study demonstrated that higher percentage of virulent strains of multi-drug resistant *Staphylococcus aureus* could be detected from clinical samples compared to meat samples, which can be attributed to higher incidence of virulent multi-drug resistant *S. aureus* detected from human samples.

P2-78 Effects of NaCl on Antibiotic Resistance and Biofilm Formation of *Staphylococcus aureus*

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Introduction: *Staphylococcus aureus*could be exposed to NaCl concentration in food, and the pathogen has caused critical problems such as antibiotic resistance and biofilm formation.

Purpose: The objective of this study is to evaluate the effect of NaCl on stress resistance and biofilm production of *S. aureus*.

Methods: *S. aureus* ATCC13565 was exposed to 0, 2, 4 and 6% NaCl supplemented in tryptic soy broth (TSB) for 24 h at 35°C, followed by plating 0.1 ml of the culture on tryptic soy agar plus NaCl 0, 2, 4 and 6%. After incubation at 35°C for 24 h, the colonies on plates were collected and diluted to OD_{600} = 0.1. The diluents were spread on mueller hinton agar plus NaCl 0, 2, 4 and 6%. Twenty-two antibiotic disks (aminoglycoside, penicillin, macrolide, tetracycline, lincosamide, nitrofuran, aminocoumarin, chloramphenicol, fluoroquinoline, peptide, and rifampin group) were placed on the plates and then incubated at 35°C for 24 h. The inocula were inoculated in TSB adding NaCl 0, 2, 4 and 6% in 96-well microtiter plate and incubated at 35°C for 18 h. Biofilm formation was measured by crystal violet.

Results: In antibiotic disk diffusion assay, the antibiotic resistance of *S. aureus* to aminoglycoside antibiotics such as streptomycin, kanamycin, gentamicin and neomycin increased significantly ($P < 0.05$), but the pathogen did not have increased resistance to other antibiotics as NaCl concentration increased. Moreover, biofilm formation of *S. aureus* also increased ($P < 0.05$) as NaCl concentration increased.

Significance: Theses results indicate that NaCl in foods may increase antibiotic resistance and biofilm formation of *S. aureus*.

P2-79 The Effect of Temperature and Water Activity on Kinetic Behavior of *Staphylococcus aureus* on Cheese

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Developing Scientist Competitor

Introduction: Cheese has been consistently modified to improve taste and microbiological safety, and thus physicochemical properties of cheese have become diversity, especially for water activity.

Purpose: Therefore, the objective of this study was to develop a mathematical model to describe the kinetic behavior of *Staphylococcus aureus* on the cheese formulated at different water activities.

Methods: Low sodium (0.3% NaCl) Cheddar cheese was ground, and NaCl was added and well-mixed to obtain 0.970, 0.975, 0.983, and 0.991 of water activities. The mixture was restructured in 6-well microtiter plates. A mixture (0.1 ml) of *S. aureus* strains (ATCC13565, ATCC14458, ATCC23235, ATCC27664, and NCCP10826) was inoculated on the surface of the restructured cheese. The samples were then aerobically stored at 7, 15, 25, and 30°C for up to 30 days. The cell counts of total bacteria and *S. aureus* were enumerated on tryptic soy agar and mannitol salt agar, respectively. The growth data of *S. aureus* were fitted to the Baranyi model to calculate maximum specific growth rate (μ_{max}; log CFU/g/h) and lag phase duration (LPD; h). The kinetic parameters were fitted to a secondary model. The model performance was evaluated with observed data, and root mean square error (RMSE) was calculated.

Results: In general, total bacterial and *S. aureus* growth increased ($P < 0.05$) as storage temperature increased depending on water activity of cheese. For a primary model, μ_{max} values were increased, but LPD values were decreased as storage temperature and water activity increased. The secondary model also well-described the effect of storage temperature and water activity on the kinetic parameters. Moreover, the *S. aureus* cell counts predicted by the model simulation were very close to the observed *S. aureus* cell counts on cheese.

Significance: The result indicates that the developed predictive model in this study should be useful in predicting *S. aureus* on cheese at various water activities.

P2-80 Predictive Models to Describe the Kinetic Behavior of NaCl-habituated and Non-habituated *Staphylococcus aureus* in Sweet Pumpkin Salad

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Introduction: In food-related environments, *Staphylococcus aureus* is habituated to NaCl, followed by contamination in ready-to-eat foods. Exposure of *S. aureus* cells to NaCl may influence the bacterial growth.

Purpose: This study developed mathematical models to describe the kinetic behavior of NaCl-habituated and non-habituated *S. aureus* in sweet pumpkin salad.

Methods: The mixture of *S. aureus* strains (KACC10768, KACC10778, KACC11596, KACC13236, and NCCP10862) was habituated up to 9% NaCl and non-habituated. The inocula of NaCl-habituated and non-habituated *S. aureus* were inoculated in 5 g portions of sweet pumpkin

salad. The samples were then aerobically stored at 10 (360 h), 15 (360 h), 20 (240 h), 25 (120 h), and 30°C (120 h). Total bacterial and *S. aureus* cell counts were enumerated on tryptic soy agar and mannitol salt agar, respectively. The growth data were fitted to the modified Gompertz model and the Baranyi model to calculate lag phase duration (LPD; h), maximum specific growth rate (μ_{max}; log CFU/g/h), N_0 (log CFU/g), and N_{max} (log CFU/g). The kinetic parameters were then fitted to the Davey equation, a polynomial equation, and the square root model as a function of storage temperature. The model performance was also evaluated under constant (20 and 27°C) and dynamic condition, and root mean square error (RMSE) values were calculated.

Results: NaCl-habituated *S. aureus* cell counts were higher ($P < 0.05$) than those of non-habituated *S. aureus* at 15°C. *S. aureus* growth was observed at 15-30°C. For both primary models, LPDs were decreased, but μ_{max} were increased as temperature increased. The developed secondary models were acceptable (R^2: 0.898-0.981). The RMSEs were 0.55 and 0.88 for 20°C and 27°C, respectively. In addition, the prediction of *S. aureus* cell counts were close to the observed *S. aureus* cell counts under dynamic temperature.

Significance: The results indicate that the developed mathematical models should be useful in describing kinetic behavior of NaCl-habituated and non-habituated *S. aureus* cells in sweet pumpkin salad.

P2-81 A Comparative Analysis of Early Transcriptional Responses in Human Keratinocytes (HEK001), Intestinal Epithelial (Caco-2), and Pulmonary (HBE4) Cells after Infection with the Spores of Avirulent Strain of *Bacillus anthracis*

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Introduction: Anthrax is caused by *Bacillus anthracis*and could be acquired via contact with farm animals, intentionally contaminated food supply or bioterrorist attack. It can manifest itself in the form of cutaneous, gastrointestinal, and inhalational anthrax with mortality rates of < 1%, 25-60%, and > 90%, respectively. The reasons for the differences in mortality rates of three forms of anthrax are still unknown.

Purpose: The purpose of this study was to understand the genetic basis of differences in three forms of anthrax by studying the early transcriptomic gene expression profiling in human cutaneous (HEK001), gastrointestinal (Caco-2) and pulmonary (HBE4) cell lines 3 h after infection with the spores of an avirulent strain of *B. anthracis*.

Methods: Three independent RNA samples from spore-challenged and control groups were used for whole genome microarray analysis for each cell line. The raw data were normalized using 75% scaling and Student t-test was used to compute the significant gene list ($P < 0.05$ and fold change > 1.5).

Results: Analysis of the test vs control samples revealed cell-specific and common gene responses. Significantly up- (↑) and down-regulated (↓) cell-specific genes included 1164 genes (569↑/595↓) in HBE4, 985 genes (777↑/208↓) in HEK001, and 540 genes (191↑/259↓) in Caco-2 cells. There were 59 genes (55↑/4↓) that were commonly affected in all cell types. Pathway analyses showed that the most significantly affected functional groups were related to cell growth control, immune responses, genetic disorder, and apoptosis. Both cell-specific and common biomarkers were identified. Common biomarkers may account for the onset of anthrax and the cell-specific markers may account for differences in mortality rates of the three forms of anthrax.

Significance: The data has yielded important insights into the disease process and could potentially be used for developing improved and effective treatment options for anthrax, and in developing counter-terror measures.

P2-82 Inactivation of Nonproteolytics Strains of *Clostridium botulinum* Spores by High Pressure and Thermal Processing

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Introduction: The impact of High Pressure Processing (HPP) on the survival of nonproteolytic spores of *Clostridium botulinum* is important in extended shelf life refrigerated (ESL) foods.

Purpose: Study the resistance of nonproteolytic *C. botulinum* types B, E and F spores exposed to combined high pressure and thermal processing.

Methods: Spores of 17 nonproteolytic strains of *C. botulinum* (8 type B, 7 type E, and 2 type F) were prepared using biphasic media. Individual strains spores were 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 80°C and high pressures (600, 650 and 700 MPa) for up to 15 min in a laboratory scale high pressure test system (Model PT-100). Survivors were determined by plating using PYGS agar medium.

Results: Nonproteolytic type E strain spores demonstrated much less resistant than type B or F strains processed at 80°C and 650 to 700 MPa for up to 15 min. All type E strains were reduced by > 6 logs within 5 minutes under these conditions.

Among type B strains, Kap9-B appears to be the most resistant type B strain, resulting in reductions of 2.7, 5.3 and 5.5 logs, coinciding with D-values 7.7, 3.4, and 1.8 min at 80°C and 600, 650 and 700 MPa, respectively. Of the two type F strains, 610-F was the most resistant of the two F strains, showing 2.6, 4.5 and 5.3 log reductions with D values of 8.9, 4.3 and 1.8 min at 80°C and 600, 650 and 700 MPa, respectively.

Significance: *C. botulinum* types B and F appear to be the most resistant of the nonproteolytic strains of *C. botulinum* to high pressure and thermal processing with strains 610-F and Kap9-B appearing to be the most resistant.

P2-83 Prevalence of *Clostridium difficile* in Korean Ground Meat Products

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Introduction: *Clostridium difficile* is a spore-forming, rod-shaped bacterium and is one of the most frequent causes of severe antibiotic-associated diarrhea (AAD), leading to pseudomembranous colitis, which is a severe inflammation of the colon. *C. difficile* is one of the most common nosocomial pathogens. Ground meat products have been reported as the vehicle of *C. difficile*, and many countries have reported widely varying results on the prevalence of contamination.

Purpose: We monitored the prevalence of *C. difficile* contamination in ground meat products purchased in Korea, Seoul.

Methods: The purchased sample was aseptically weighed and 5 g of the sample was inoculated in 20 ml brain heart infusion (BHI) broth, and incubated anaerobically at 37°C for 10-15 days. From the enriched culture, 1 ml was added to 1 ml of 95% ethanol and allowed to stand for 30 min. After centrifugation (3800 x g, 10 min), the sediment was streaked onto *Clostridium difficile* selective agar (CDSA) plates and incubated at 37°C

for 2 days. After incubation, the plates were examined under long-wave ultraviolet light for the green/yellow fluorescence of *C. difficile* colonies. The presumptive *C. difficile* colonies were tested for indole production, L-proline-aminopeptidase activity using the PRO Kit, and checked for the presence of *tpi*, *tcdA, tcdB* genes by using PCR.

Results: *C. difficile* was isolated from 1 ground beef of 400 retail ground meat purchased over a 15-month period from 2011 to 2012 in Korea. The isolated strain had a toxin A gene but not the toxin B gene.

Significance: Prevalence of *C. difficile* in meat, including pork and beef, has been widely reported to range from 0% to 42%, worldwide. Our study showed that 0.25% of Korean ground meat products were likely contaminated with *C. difficile*.

P2-84 Determining the Fate of *Clostridium difficile* in Animal Manure-based Compost

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Introduction: *Clostridium difficile* is one of the most important nosocomial human pathogens causing antibiotic-associated diarrhea and pseudomembranous colitis. Studies have reported the prevalence of this pathogen in a variety of animal manure. However, there is a lack of research on the fate of *C. difficile* during composting.

Purpose: The purpose of this study was to develop a sensitive method for detecting *C difficile*from animal manure-based compost.

Methods: Vegetative cell and spore suspensions of *C. difficile* ATCC 43593 were artificially inoculated into the autoclaved and unautoclaved dairy compost. Inoculated cells were recovered on cycloserine cefoxitin fructose agar (CCFA) enriched with 7% horse blood and 1% sodium taurocholate (HT-CCFA), CCFA enriched with 7% horse blood (H-CCFA), brain hear infusion agar with yeast extract (BHIA-YE), BHIA-YE with antibiotic supplement (BHIA-YE-CC), CCFA enriched with 1% sodium taurocholate (T-CCFA), and *Clostridium difficile* agar with horse blood (H-CDA). Inoculated spores were recovered on HT-CCFA and BHIA-YE with taurocolate (BHIA-YE-T). The recovery studies were performed at selected intervals after anaerobic incubation at 37 °C for 2~3 days. Commercial compost samples were enriched in CCFB for 7 days and analyzed for *C. difficile* spores on HT-CCFA supplemented with cycloheximide (HTC-CCFA). Suspected colonies were isolated as pure cultures and tested for Gram's reaction, endospores, PRO disc method, PCR for housekeeping gene *tpi*, and latex agglutination test.

Results: Our plate count data revealed that HT-CCFA was the best recovery medium among 7 media tested for both *C. difficile* cells and spores. The recovery rate of cell suspension (containing 1~5% of spores) for day 0 was 105.2% and 102.1% for autoclaved and unautoclaved compost, respectively, as compared with 76.4% and 69.1% for spore suspension, respectively. There was a slight reduction of spore population in both types of compost during room temperature storage up to 3 days. Using the optimized *C. difficile* detection method, we analyzed 29 commercial compost samples and 3 samples were positive for the presence of *C.difficile*.

Significance: Our data suggests that CCFA supplemented with horse blood and taurocholate enhances the recovery of *C. difficile* spores, which can be used for analyzing commercial composts for the presence of *C. difficile*.

P2-85 Field Trial Study on BAX® System Real-time PCR Assay for the Detection of *Shigella* in China FDA Testing Foods

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Introduction: A scorpion probe-based real-time PCR assay followed by culture confirmation method was developed and validated in-house for the detection of *Shigella* species in various foods including raw and ready-to-eat meats, jelly and produce. The specificity of the assay for *Shigella* was demonstrated with a diverse range of *Shigella* species, related organisms, and unrelated genera. The assay was sensitive enough to detect less than 2 log colony-forming units (CFU)/ml of the target pathogen in both pure broth culture and food samples. Our in-house validation studies demonstrated BAX® RT-*Shigella* assay performed as well as the three reference methods (FDA-BAM Chapter 6, ISO21567 and China National Standard GB4789.5-2010) for the detection of *Shigella* in 8-20 hour enrichment in the selected foods.

Purpose: The objective of this study was to perform pre-commercial field trial study to detect *Shigella* in various local FDA testing foods by assay and reference culture method.

Methods: The 25 g of various food samples including ready-to-eat meats, poultry, sea foods, mushrooms, soy products, vegetable and fruit salads, granulated sugar, drinking water, and juice etc. were weighed and enriched in 225 ml of *Shigella* broth as adopted by China National Standard GB4789.5-2010. After 20 h enrichment in *Shigella* broth, samples were either lysed, detected by the test method, or following the reference methods for the detection of *Shigella*.

Results: For in-house validation study with spiking *Shigella* in similar foods, the test method showed 100% sensitivity and specificity, and Chi-square analysis indicates it performed equivalent to the reference methods. For the total 74 samples tested in the field trial studies, 6 samples inhibited PCR reaction, while all others were in agreement with the reference method. The PCR inhibition can be relieved by growing back the enrichments in BHI broth for additional 3 hours and test the lysate by the test method.

Significance: Method comparison studies showed that the test method performed as well as the reference method for detecting *Shigella* in 20 hour enrichment in the testing foods. The test method allows for detection *Shigella* in 1 day instead of 2-3 days by culture method. It is rapid and convenient to screen large varieties of foods with complexity of background microflora.

P2-86 Withdrawn

P2-87 Identification and Subtyping of *Cronobacter sakazakii* in Food Commodities

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Introduction: *Cronobacter sakazakii* has been associated with bacterial infections in infants. Although reconstituted powdered infant formula has been the most common vehicles implicated in *C. sakazakii* infections, *C. sakazakii* can be found in a wide range of foods and environments. Due to the very low levels of *C. sakazakii* in food commodities, improvement of molecular identification and standardized subtyping methods on different *Cronobacter* species in foods are required.

Purpose: The purposes of this study were to evaluate the effectiveness of conventional polymerase chain reaction (PCR) and pulsed field gel electrophoresis (PFGE) in the identification and characterization of *Cronobacter* species isolated from various food products and to determine antibiotic susceptibility of isolated strains.

Methods: A total of fifteen *Cronobacter* species were isolated from various food commodities using the Bacteriology Analytical Manual (BAM). Each isolate was identified using bioMérieux's API 20E identification system and VITEK 2 system. Traditional PCR protocol was evaluated to detect fifteen *Cronobacter* isolates. Fifteen *Cronobacter* isolates were subtyped using PFGE.

Results: API 20E biochemical test has been shown to be a reliable identification tool for *Enterobacter sakazakii*; however, it was unsuccessful for identification of *Cronobacter* spp. VITEK 2 system identified nine *Cronobacter* isolates as *Cronobacter sakazakii* subsp. *sakazakii.* The six remaining isolates were identified as *C. sakazakii* subsp. *malonaticus*. The primer pair of Esakf/Esakr and EsAgf/EsAgr was proved to be reliable PCR identification tools for *Cronobacter* spp. PFGE testing using two different enzymes (XbaI and SpeI) was an excellent tool for characterization and subtyping of *Cronobacter* spp. All isolates were susceptible to a wide range of the tested antibiotics exception of azithromycin and sulfisoxazole.

Significance: PCR method using two primers, Esakf/Esakr and EsAgf/EsAgr was a reliable identification tool for *Cronobacter* species. Fifteen *Cronobacter* isolates were subtyped successfully by PFGE using PulseNet standardized protocol from *Escherichia coli* O157:H7.

P2-88 Seroprevalence and Risk Factors of *Toxoplasma gondii* Infection in Meat Products Destined for Human Consumption

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Introduction: *Toxoplasma gondii* is a parasite that is responsible for approximately 24% of all estimated deaths attributed to foodborne pathogens in the U.S. The main transmission route for human infection is through consumption of raw or undercooked meat products that contain *T. gondii* tissue cysts.

Purpose: The objective of this study was to summarize seroprevalence and risk factors of human *T. gondii* infection through ingesting meat products, both conventional and organic, in the past twenty years.

Methods: Relevant studies in literature were searched in Pubmed and Google Scholar database by key words '*Toxoplasma gondii*' and in combination with 'pig', 'pork', 'sheep', 'lamb', 'chicken', 'poultry', 'cattle', 'meat' and 'organic meat'. This structured review was focused on studies of *T. gondii* infection through meat-consumption route. Other studies for the transmission through ingesting oocysts shed by felid were excluded.

Results: At least half of the human *T. gondii* infections are through consumption of raw or undercooked meat products containing tissue cysts. Seroprevalence of *T. gondii* is more frequent in conventional pig and sheep compared to cattle and poultry. Seroprevalence of *T. gondii* is greater in organic compared to conventional meat products because of outdoor access that have substantially greater opportunities for exposure to infected rodents, wildlife, and oocyst contaminated feed, water, or environmental surfaces. Several different risk factors related to *T. gondii* infection identified are farm type, feeding, presence of cats, rodent control, bird control, farm management, carcasses handling and water quality.

Significance: This review provided a summary of *T. gondii* infection through consumption of both conventional and organic meat products that contained tissue cysts. This study would serve as a useful resource and information repository for informing quantitative risk assessment studies for *T. gondii* infection in humans through meat consumption.

P2-89 Identification of Zoonotic Foodborne *Cryptosporidium* and *Giardia* Domestic and International: Species and Risk Factors

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Introduction: *Cryptosporidium* and *Giardia* are two ubiquitous protozoan parasites that cause zoonotic foodborne and waterborne infection. Multiple species and genotypes of the two parasites infect humans and food animals. Socioeconomic factors associated to human infection while management and environmental factors associated to animal infection.

Purpose: The purpose of the present work was to investigate the prevalence of foodborne *Cryptosporidium* and *Giardia* in young children and in food animals and determine risk factors associated to the infection of the two parasites and discuss strategies to prevent zoonotic foodborne transmission domestic and international.

Methods: Fecal samples were collected from dairy calves in California and young children with diarrhea in Guangzhou, China. Samples were screened for *Cryptosporidium* oocysts and *Giardia* cysts using fluorescent microscopy. DNA was extracted from microscopic positive samples and the SSU rRNA genes were amplified for both *Cryptosporidium* and *Giardia*. PCR products were purified and sequenced. Sequences were analyzed using the Vector NTI software and the NCBI online Blast tool. Species/genotypes of *Cryptosporidium* and *Giardia* were determined by comparison to existing sequences in GenBank and by phylogenetic analysis. Questionnaires were administered to determine socio-economic factors associated to *Cryptosporidium*/*Giardia* infection in children and environmental factors and management practices associated to the prevalence of the parasites in food animals.

Results: Total 300 fecal samples were collected from young children in Guangzhou, China and 360 fecal samples were collected from dairy calves in California. *Cryptosporidium* spp. was detected in approximately 10% of samples from children and 22% of samples from dairy calves. *Giardia* spp. was detected in approximately 7% of samples from children and 17% from dairy calves. Infections of the two parasites in children were associated to consumption of raw local produce and unpasteurized water and that in animals associated to age and facility sanitation practice. Molecular characterizations are being carried out to determine the unique species and genotypes cause zoonotic foodborne infection.

Significance: Data from the present work suggest that infection of *Cryptosporidium* and *Giardia* in children can be reduced through changing eating habits and that in food animals through improved management practice. Strategies are discussed to reduce the transmission of these two pathogens in domestic and international food chain and to prevent zoonotic infection.

P2-90 Comparison of Gel Electrophoresis and Microfluidic Separation of PCR Products for Identification of Pathogenic *Vibrio* spp

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Introduction: *Vibrio cholerae*, *V. parahaemolyticus*, and *V. vulnificus* are well-documented human pathogens. The U.S. FDA Bacteriological Analytical Manual (BAM) recommends conventional PCR procedures for identification and characterization of pathogenic *Vibrio* isolates which requires agarose gel electrophoresis for visualization of the amplified products.

Purpose: The current study compares microfluidic separation by the Agilent 2100 Bioanalyzer to gel electrophoresis as an alternative for vibrio confirmation.

Methods: DNA templates were prepared from overnight broth cultures as described in the BAM. The organisms tested were previously characterized for the species specific and virulence markers listed below. PCR was performed as described in the BAM for *V. cholerae* cholera toxin (777 bp fragment of *ctx*AB), *V. vulnificus* species (519 bp fragment of *vvh*) and *V. parahaemolyticus* (triplex assay for 450 bp fragment of *tlh* species specific marker, 500 bp fragment of *trh* virulence marker and 270 bp fragment of *tdh* virulence marker). Product from each PCR was visualized by gel electrophoresis as described in the BAM and by microfluidic separation on "DNA chips" with the Agilent 2100.

Results: Of 51 *V. cholera*e isolates tested, *ctx* was detected after PCR in the same 16 isolates by both gel electrophoresis and by the Agilent. The *vvh* gene was detected in 52/52 isolates by gel electrophoresis and in all 53 *V. vulnificus* isolates with microfluidic separation. The *V. parahaemolyticus* species specific marker, *tlh*, was detected in 52/53 isolates by Agilent and gel electrophoresis. The *trh* marker was identified in 26/53 *V. parahaemolyticus* isolates with the Agilent and in 25/53 with gel electrophoresis. The *tdh* gene was detected in 41/53 *V. parahaemolyticus* isolates by the microfluidic separation technique and 40/53 by gel electrophoresis.

Significance: These results indicate that microfluidic separation is a suitable alternative to gel electrophoresis for sizing and visualization of PCR fragments for the identification and characterization of pathogenic *Vibrio* spp.

P2-91 Ecology of *Vibrio cholerae* in Florida Bays

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Developing Scientist Competitor

Introduction: *Vibrio cholerae* (Vc), the etiological agent of cholera, has been responsible for seven pandemics since 1817. U.S. outbreaks are rare, and only 8 sporadic cases were reported to CDC between 2003 and 2007. Recent epidemics in Haiti and Cuba confirmed cases due to the 7th pandemic strain of Asiatic Vc. A Florida outbreak in 2011 was associated with raw oyster consumption and indicated emergence of a serotype (O75) that differed from pandemic Vc.

Purpose: This study investigated the genetic structure of Vc populations in Florida relative to toxigenic 7th pandemic Vc.

Methods: Water, sediment, oyster, fish and various plant samples were collected seasonally from 2011 to 2012 at different sites in Tampa and Apalachicola Bays. Environmental parameters, including temperature, salinity, pH, and dissolved oxygen were recorded. Presumptive Vc was isolated from CHROMagar™ and TCBS, and confirmed by rRNA intergenic spacer region-based PCR. Confirmed isolates were compared to clinical Vc by multilocus sequence typing (MLST) of five housekeeping genes (*recA*, *gyrB, pyrH, gapA,* and *topA*) and screened for cholera toxin gene, *ctxA,* by PCR.

Results: Vc was isolated from only water samples in Apalachicola and from both water and oysters in Tampa Bay. Unlike other *Vibrio* species, Vc was not widely distributed throughout the bay, but was mostly associated with near-shore sites with lower salinity. Most strains from Apalachicola (92%) and Tampa (63%) closely associated with a genetic clade that included Vc O75 and classical Vc 395 but were distinct from the 7th pandemic Vc clade that included only one isolate from Apalachicola. Other strains formed unique clades. No environmental strain was positive for *ctxA.*

Significance: Vc is endemic to Florida waters but is divergent from the current 7th pandemic strain, and virulence potential appears limited due to the absence of genes for cholera toxin.

P2-92 Inactivation of *Vibrio vulnificus* in Shucked Oysters Using Natural Antimicrobials

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Introduction: *Vibrio vulnificus* is a gram-negative bacterium, occurring naturally in the Gulf Coast water where the majority of U.S. oysters are harvested. It is the leading cause of foodborne illness associated with the consumption of raw oysters. It has the highest fatality rate (40–50%) among foodborne pathogens in the United States. The CDC estimates 47 foodborne illnesses of *V. vulnificus* yearly in the U.S., resulting in 18 deaths.

Purpose: The purpose of this investigation was to study the efficacy of citric acid (CA), grape seed extract (GE) and lactic acid (LA) on the inactivation of *V. vulnificus* to achieve a 5.0 log reduction.

Methods: The minimum inhibitory concentration (MIC) for CA, GE or LA against *V. vulnificus* was determined. Furthermore, the shucked oysters were artificially inoculated with *V. vulnificus*. The inoculated shucked oysters (25 g) were then dipped in 250 ml CA, GE or LA solutions (according to the MIC results) for 10 min. The population of *V. vulnificus* in shucked oysters was determined. The surviving cell population was expressed as log CFU/g. The effects of the treatment with GE, CA, and LA on the inherent microbiota on oysters during storage at 5°C for 20 days were also studied.

Results: The MICs for CA, GE or LA against *V. vulnificus* were 5.0, 10.0 or 1.0 mg/ml, respectively. The concentration of 300, 500, or 150 mg/ml CA, GE or LA solutions were needed to reduce the population of *V. vulnificus* to below the detection level (1.0 log/g). Treatment with 500, 300, 150 mg/ml GE, CA, and LA significantly reduced the initial inherent microbiota on oysters and inherent levels were significantly ($P < 0.05$) lower than the control sample throughout refrigerated storage for 20 days.

Significance: The results of investigation indicated that LA is a strong natural antimicrobial to eliminate *V. vulnificus* or spoilage bacteria in shucked oysters.

P2-93 Fourteen-day Natural Relay to High Salinity Seawater Decreases the Presence of *Vibrio parahaemolyticus* in Oysters (*Crassostrea virginica*)

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Introduction: The shellfish aquaculture industry has suffered increasingly more frequent *vibrio*-associated disease outbreaks linked to shellfish consumption. *Vibrio* diseases are an important emerging issue to account for when shellfish harvesting and processing to ensure safety for consumers and economic viability for the shellfish aquaculture industry in New England.

Purpose: Within different harvesting regions there are naturally occurring dynamics and varying population levels of *Vibrio parahaemolyticus* (Vp) in oysters. The objective of this study is to investigate the reduction levels for Vp in oysters relayed within the seasonally changing Great Bay estuary of New Hampshire and Maine.

Methods: The effectiveness of the relocation (relay) of oysters from the Piscataqua River to Spinney Creek, a salt pond near Spinney Creek Shellfish Company (Eliot, ME), was assessed by sampling oysters on days 0, 7, 10, and 14. Vp concentrations in the oysters were determined using

the 3-tube MPN enrichment method coupled to a cultured based confirmation assay in conjunction with genetic marker-based qPCR. Log concentrations of Vp were plotted against the relay sampling days for each month (June to September).

Results: Averaged concentrations of triplicate sample data for the four sampling days from day 0 to day 14 of the June, July, and August relays showed 1.08, 2.01, and 2.25 log reductions, respectively. A separate sample of oysters collected in August, that were temperature abused to increase initial Vp levels, showed a 4.5 log decrease from day 0 to 14.

Significance: Vp reduction in relayed oysters suggests the surrounding waters may have an effect on the extinguish of Vp from the oyster. Further investigation to discover the conditions that affect the Vp concentrations may aid in the treatment of oysters in the summer months prior to consumption.

P2-94 Development and Evaluation of an Immunochromatographic Rapid Assay for the Detection of Pathogenic *Vibrio parahaemolyticus* in Food

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Introduction: *Vibrio parahaemolyticus* is a Gram-negative, halophilic bacterium of the estuarine environment and a major cause of foodborne illness from consumption of raw or undercooked seafood, particularly in Asia. Standard detection methods are mainly cultural based, labor-intensive and time-consuming. Rapid deterioration of raw seafood in particular, requires faster detection methods. Lateral Flow technology offers a reliable, fast, user-friendly, alternative detection method.

Purpose: To develop and evaluate an immunochromatographic assay for detection of pathogenic *Vibrio parahaemolyticus* from food, as a rapid alternative to standard reference methods.

Methods: A sandwich Lateral Flow assay was developed, using gold-labeled specific antibodies for detection of thermostable direct hemolysin (TDH) of *Vibrio parahaemolyticus*. Evaluation was by determination of pure culture detection limit, sensitivity and specificity in fresh and frozen seafood samples artificially contaminated with *V. parahaemolyticus* at 10^1 to 10^4 CFU/g, inclusivity testing of *tdh*-positive *V. parahaemolyticus* strains and exclusivity testing of *tdh*-negative *V. parahaemolyticus* strains, other Vibrios and non-Vibrios. Reference method comparison was ISO/TS 21872-1:2007.

Results: Pure culture detection limit of 4 strains of *V. parahaemolyticus* was 125 pg/ml. 81% Inclusivity demonstrated from testing 23 *tdh*-positive *V. parahaemolyticus* strains. 100% Exclusivity demonstrated from testing 69 *tdh*-negative *V. parahaemolyticus* strains, other Vibrios and non-Vibrios. *V. parahaemolyticus* was detected in fresh food samples artificially contaminated with 10^1 to 10^2 CFU/g and in frozen samples at 10^3 to 10^4 CFU/g. After 24 h sample enrichment and implementation of a pre-treatment step (centrifugation), sensitivity and specificity of 100% was attained. Performance was equivalent to the culture-based ISO reference method. The LFA reduced time-to-result to a 24 h enrichment plus 1 h sample pre-treatment and assay performance.

Significance: The developed Lateral Flow Assay provides a unique, alternative, fast and simple method for detection of pathogenic *V. parahaemolyticus* from food.

P2-95 Thermal Inactivation of Human Norovirus Surrogates in Blue Mussels (*Mytilus edilus*)

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Introduction: Mussels are able to filter large quantities of water in order to obtain food present in water. In addition to water, they also retain bacteria and viruses. Thus, mussels are a food that is commonly involved in foodborne norovirus outbreaks. It is therefore advisable to eat mussels only after a heat treatment. However, little is known about the thermal inactivation kinetics of viruses in mussels. Due to the absence of human norovirus infectivity assays, cultivable surrogates such as feline calicivirus (FCV-F9) and murine norovirus (MNV-1) have been used to begin to understand thermal inactivation behavior.

Purpose: The purposes of this study were (i) to determine thermal inactivation kinetics of MNV-1 at 60°C and FCV-F9 at 56°C in blue mussels, and (ii) to compare First-order and Weibull models in describing the data in terms of selected statistical parameters.

Methods: Thermal inactivation was performed using 2 ml vials on the surrogate viruses at titers of 2.5×10^6 (MNV-1) and 7.3×10^5 (FCV-F9) plaque forming units (PFU)/ml. Each treatment was replicated thrice. Inactivation data were fitted to Weibull and First-order models and statistical evaluation and linear and non-linear regression analyses were performed using SPSS Statistical package.

Results: The D-value for MNV-1 at 60°C was 2.48 ± 0.41 for Weibull model and 3.87 ± 1.07 for First-order model. For FCV-F9, the D-values were 3.68 ± 0.67 and 3.59 ± 0.75 for Weibull model and First-order model, respectively. No difference was found in the D-values using either model ($P > 0.05$). In contrast, the Weibull model ($R^2 = 0.93$-0.99) had higher regression coefficients than the First-order model ($R^2 = 0.84$-0.91) for both norovirus surrogates. Thus, the model chosen for calculation of thermal inactivation parameters of norovirus surrogates during thermal inactivation is important.

Significance: Knowledge of the thermal inactivation kinetics of norovirus surrogates will allow development of processes that produce safer shellfish products and improving consumer safety.

P2-96 Inactivation of Norovirus Surrogate and Hepatitis A Virus in Suspension and in Dried Mussels Using Thermal Treatment

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Introduction: Norovirus (NoV), as the most serious foodborne virus, is a cause of epidemic gastroenteritis worldwide. Murine norovirus (MNV-1) has emerged as a more suitable model for NoV studies. Together with NoV, hepatitis A virus (HAV) infection is one of the major causes of foodborne diseases, particularly in developing countries. These two viruses are regarded as the potential foodborne virus present in dried mussels (*Mytilus edulis*) commonly manufactured by thermal treatment and followed by drying.

Purpose: This study was conducted to investigate the effectiveness of thermal treatment for the inactivation of MNV-1 and HAV in suspension and dried mussels.

Methods: Viral suspensions of about 7 log $TCID_{50}$/ml were exposed to 60, 85, or 100°C of water bath for certain reaction time. Ground dried mussels containing viral suspensions were also exposed to 60, 85, or 100°C of water bath. After reaction, each virus was recovered and calculated as 50% tissue culture infectious dose /ml (log $TCID_{50}$/ml).

Poster

Results: When MNV-1 in suspension was treated with 60°C for 5, 10, and 15 min, the reduction was 3.03, 3.69, and 4.35 log $TCID_{50}$/ml, respectively. MNV-1 was also reduced to 5.88 log $TCID_{50}$/ml by of 85°C for 6 min. When HAV in suspension was treated with 60°C for 5, 10, and 15 min, the reduction was 3.61, 4.48, and 5.06 log $TCID_{50}$/ml, respectively. HAV was greatly reduced to 5.66 log $TCID_{50}$/ml by 85°C for 10 min. When MNV-1 in dried mussels was treated with 60°C for 5, 10, and 15 min, the reduction was 0.78, 2.00, and 3.35 log $TCID_{50}$/ml, respectively. When MNV-1 in dried mussels was treated with 85°C for 3, 6, and 10 min, the reduction was 1.95, 2.40, and 3.68 log $TCID_{50}$/ml, respectively. When HAV in dried mussels was treated with 60°C for 5, 10, and 15 min, the reduction was 1.34, 1.94, and 3.16 log $TCID_{50}$/ml, respectively. When HAV in dried mussels was treated with 85°C for 3, 6, and 10 min, the reduction was 2.36, 3.43, and 4.38 log $TCID_{50}$/ml, respectively. Both MNV-1 and HAV in dried mussels were completely inactivated by 85°C for 15 min and over 85°C.

Significance: This study demonstrates that MNV-1 and HAV in dried mussels was greatly reduced up to 3 log $TCID_{50}$/ml by over 85°C for 10 min.

P2-97 Modeling Norovirus Transmission from an Episode of Vomiting

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Developing Scientist Competitor

Introduction: The importance of vomiting incidents in the transmission of human noroviruses (NoV) is increasingly recognized and there are examples of food becoming contaminated because of such events. Effective cleaning and sanitation after vomiting in public places like food service establishments remains challenging. Unfortunately, little is known about the role of vomiting on NoV transmission.

Purpose: As part of a broad project to model the impact of vomiting on foodborne NoV transmission, the purpose of this work was to characterize the radius of impact of a simulated vomiting event.

Methods: To model vomit "splatter," or the distance traveled by vomitus upon its deposition on a solid surface, a "Tipping Bucket" experiment was designed. This consisted of rigging a bucket to a ladder, and pouring colored simulated vomitus onto a tarp upon which a target was drawn, allowing the measurement of the distance from the center of splatter to the farthest droplet. Data were also recorded by camera. Two simulated vomitus matrices were used: reconstituted instant oatmeal (to simulate vomitus having high solids content) and artificial saliva (a dilute solution porcine mucin in saline). Volumes ranging from 50-800 ml were dropped 3.5 ft. off the ladder. The ImageJ program was used to analyze the data.

Results: For oatmeal, the furthest distance traveled by a droplet was highly dependent upon volume, with the mean distance traveled ranging from 3-3.5 ft. for higher volumes (> 600 ml). On the other hand, regardless of volume, artificial saliva experiments yielded a mean distance of 8-12 ft.; the greatest distance traveled in any one experiment was 14.5 ft.

Significance: Taken together, these measurements suggest that vomitus splatter can be deposited between 3 and 15 ft. away from the initial vomiting contact area. This has implications relative to recommended clean up zones after a public vomiting event. Further work to characterize aerosolization of vomitus is in progress.

P2-98 Characterization of Nucleic Acid Aptamers with Broad Reactivity to Human Norovirus Strains

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Introduction: To date, no single antibody has been identified with broad reactivity to all human norovirus (NoV) strains. Aptamers are single-stranded (ss) DNA or RNA molecules that naturally fold into complex three-dimensional shapes that demonstrate target binding affinity. They have been proposed as alternatives to antibodies in pathogen capture and detection assays.

Purpose: Using aptamers previously selected for binding specificity to Snow Mountain virus (SMV, a GII.2 strain), the purpose of this study was to characterize aptamer binding specificity to a variety of human NoV strains using virus-like particles (VLPs).

Methods: Five aptamer candidates (13, 19, 21, 22, and 24) were chosen for characterization based on previous studies demonstrating their high binding affinity to SMV and their low free energy (dG) values. Screening was done using an aptamer-linked enzyme assay, which was developed as a modification of a previously reported enzyme immunoassay. The aptamers were screened for binding to Norwalk (GI.1), Houston (GII.4) and Snow Mountain (GII.2) VLPs.

Results: Three aptamer candidates (19, 22 and 24) showed high binding affinity to SMV and Houston VLPs, and reduced yet still significant binding affinity to Norwalk VLPs ($P \leq 0.05$). The binding affinity of these aptamers for SMV and Houston VLPs was as high as that observed for commercial antibodies. Interestingly, two aptamers (13 and 21) showed high binding affinity to all three VLPs tested. This affinity was equal to that observed for commercial antibodies specific to the GII strains (SMV and Houston), and better than that for Norwalk antibody.

Significance: Several aptamer candidates may be promising broadly reactive reagents for use in human NoV capture and detection assays. Studies now focus on further characterization of these aptamers using a broader group of viruses and VLPs, and scale-up for their use in virus capture and detection in foods, environmental, and clinical samples.

P2-99 Evaluation of a Novel Surface Sampling Wipe for Recovery of Human Noroviruses Prior to Detection Using RT-QPCR

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Introduction: Human noroviruses (NoV) are a significant cause of foodborne disease. Lab-based and epidemiological studies demonstrate their high degree of environmental persistence and their resistance to commonly used surface sanitizers. Environmental sampling is an important tool for assessing the effectiveness of cleaning and sanitizing regimens.

Purpose: To test the efficacy of a novel surface sampling material ("wipes") relative to human NoV recovery.

Methods: Dilutions of a representative human NoV GII.4 outbreak strain were applied to frequently used hard surfaces (stainless steel and ceramic) and the surface of representative foods (green pepper, apple, tomato, and cheese). After inoculum drying, the sampler was used to recover viruses by wiping the surface in a circular, back and forth motion. The wipes were then directly processed for nucleic acid extraction using the automated EasyMag system, and the viral RNA quantified by RT-qPCR targeting the *orf* 1-*orf* 2 junction. The efficiency of recovery of virus by the wipes was determined by direct comparison of RT-qPCR signals to a standard curve produced from the NoV GII.4 inoculum.

Results: Recovery efficiency of the wipes ranged from 37-100% when used for sampling fresh fruits, vegetables, and hard surfaces artificially inoculated with high concentrations (3.4-5.4 log genome equivalent copies, GEC) of GII.4 human NoV. At lower inoculum concentrations (1.4-2.4

log GEC), the wipes also yielded positive RT-qPCR signals for NoV. Less efficient recovery was observed for cheese, ranging from 18-52% at high inoculum concentration, and failure to detect the GII.4 strain at lower concentrations.

Significance: The wipes are a user-friendly means by which to sample hard surfaces and foods that also provide high recovery efficiency for human NoV. They are being field tested in environmental surveys designed to evaluate NoV prevalence in public restrooms during the typical epidemic (winter) season.

P2-100 Comparison of Human Norovirus Recovery using Magnetic Beads Coated with Porcine Gastric Mucins or Monoclonal Antibodies

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Developing Scientist Competitor

Introduction: Recovery of human noroviruses from food and water using Porcine Gastric Mucin (PGM)-functionalized superparamagnetic beads has recently been described. However, the assay has not been directly compared to immune-magnetic separation and the lower limit of detection is not known.

Purpose: To assess the applicability of this method, capture human noroviruses by PGM- and monoclonal antibody (mAB)-coated magnetic beads was compared in the context of elution buffers that are commonly used in food virology.

Methods: Amine- and tosyl-functionalized superparamagnetic beads were coated with PGM and mAB (NV3901), respectively, or left uncoated to determine non-specific binding of virus to beads. The beads were re-suspended in glycine (pH 7.0 and 9.5), 1M NaCl (pH 7.4), or citrate (pH 3.6) buffers containing 10-fold serial dilutions of Norwalk (GI.1) virus. After attachment and washing, viral RNA was detected by realtime RT-qPCR. The lower detection limit (LDL) for each bead/buffer combination was reported by expressing the lowest viral inoculum yielding positive RT-PCR results.

Results: For PGM-coated beads, LDLs were similar in the context of all buffers tested; 3.3, 3.3, 3.6 and 3.7 log genome copy numbers for glycine (pH 7.0), citrate, glycine (pH 9.5) and 1M NaCl buffers, respectively. With the mAB-coated beads, LDLs were generally higher; 4.5, 4.3 and 4.9 log genome copies for glycine (pH 7.0), citrate and 1M NaCl buffers, respectively. The mAB-coated beads in glycine buffer (pH 9.5) yielded the lowest LDL of the group (3.5 log genome copy numbers). Non-specific binding was problematic, especially for uncoated tosyl beads in 1M NaCl buffer and for all cases when virus input approached 5 log genome copies or more.

Significance: PGM-coated beads were the most versatile, out-performing the mAB-coated beads in nearly all buffer matrices. However, because LDLs were higher than desired, further optimization is needed before implementation in a foodborne outbreak investigation.

P2-101 Withdrawn

P2-102 Characterization of *Campylobacter coli* and *jejuni* Strains Isolated from Turkeys

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Introduction: Campylobacters are zoonotic bacteria frequently colonizing intestinal tracts of turkeys and associated with significant foodborne disease. *Campylobacter jejuni* and *C. coli* are the two most prevalent species contributing to human diarrheal disease.

Purpose: The objective of this study was to determine the routes of transmission for *Campylobacter* throughout turkey production and processing.

Methods: A flock of 140 turkey breeder poults was placed in a growout house after the housing environment was sanitized and tested for *Campylobacter* presence. Poults were separated by sex, then separated further into Treatment (82 females and 22 males) and Control (28 females and 8 males) groups and placed in different sides of the house. Treatment birds were inoculated via gavage at 10 days old and 12 weeks old with a marker *Campylobacter* strain; *C. coli* 12456 (resistant to gentamicin and kanamycin) was administered to females, and *C. jejuni* 10882 (resistant to tetracycline, streptomycin, kanamycin and quinolones) was given to males. Fecal droppings were analyzed weekly.

Results: Marker strain *C. jejuni* from inoculated males was isolated from feces in 100% of female pens 3 weeks after inoculation, and the female marker strain *C. coli* was isolated from 100% male pens at the same time. Both marker strains were isolated in 100% of control bird pens, both males and females. Marker strains persisted for only 1 to 6 weeks before descending below detection levels. Wild (non-marker) strains of *Campylobacter* were isolated in 100% control and treatment bird pens 9 weeks after inoculation. The most frequent wild isolates found in Treatment and Control groups, in both females and males, from fecal droppings were *C. jejuni (*tetracycline resistant) and *C. coli (*kanamycin resistant). Wild *Campylobacter* strains persisted throughout the remainder of the study.

Significance: Results indicate *Campylobacter* spreads rapidly and cross-contaminates turkeys throughout the growout house and wild strains may outcompete marker strains.

P2-103 Differential Protein Expression between Poor and Robust Colonizing *Campylobacter jejuni* Isolates

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Introduction: *Campylobacter jejuni* is a leading cause of bacterial gastroenteritis in humans. Poultry is considered a major source of *C. jejuni* but colonization mechanisms in the chicken remain unclear.

Purpose: The purpose of this study was to determine colonization-associated factors of *C. jejuni* at the proteome level.

Methods: The proteomes of *C. jejuni* were quantitatively analyzed using trypsin catalyzed $^{16}O/^{18}O$ labeling in conjunction with two-dimensional liquid chromatography separation and tandem mass spectrometry analysis (2DLC-MS/MS).

Results: After oral challenge with 10^5 CFU/ml of *C. jejuni* per chick, a poor colonizing isolate (A74/O) showed a 3 log reduction in the chick ceca relative to a robust colonizer (A74/C). *C. jejuni* recovered from birds were determined to be the same *flaA* SVR genotype as the original isolates. A total of 776 proteins were identified; 72 proteins (approximately 9.28% of the total proteins identified), were significantly changed (over 1.4-fold, $P < 0.05$) in the good colonizer. Surprisingly, only 4 of these proteins (RplL, CjaA, GlyS, and putative oxidoreductase subunit) were up-regulated, whereas the majority (68 proteins) were down-regulated. The differentially expressed proteins are primarily involved in cell motility, amino acid and lipid transport, post-translational modification, protein turnover, and chaperones. In addition, the robust colonizing isolate (A74/C)

attached and invaded Caco-2 cells at significantly higher numbers than the poor colonizer (A74/O). Similarly, A74/C isolate rapidly translocated through differentiated Caco-2 monolayers compared to A74/O.

Significance: The present study clearly demonstrates the usefulness of proteomic profiles in the investigation of mechanisms that *C. jejuni* uses to occupy the poultry intestine and it could potentially be used for the vaccine development to reduce the threat of *Campylobacter* infection in chickens.

P2-104 Novel Plasmid Conferring Kanamycin and Tetracycline Resistance in Turkey-derived *Campylobacter jejuni* 11601MD

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Developing Scientist Competitor

Introduction: In *Campylobacter spp.* resistance to the antibiotics kanamycin and tetracycline is frequently associated with plasmid-borne genes. In spite of the important role of plasmids in drug resistance of *Campylobacter* spp., relatively few plasmids of *Campylobacter jejuni* have been fully characterized to date.

Purpose: The objective of this project was to analyze the genome of the recently sequenced strain *C. jejuni* 11601MD isolated from turkeys in order to identify plasmid-associated sequences and to characterize possible conjugative plasmids harboring antibiotic resistance determinants.

Methods: Total genomic DNA of *C. jejuni* 11601MD was sequenced at the Genome Core Facility at Duke University. Annotation of the genome sequence was done using GAMOLA (Altermann and Klaenhammer, 2003), taking into account all *Campylobacter* genomes sequenced to date. compACTor (Altermann, 2012) and the ARTEMIS Comparison Tool (ACT, Carver et al., 2005) were used to determine presence and comparative genomic location of sequences homologous to the tetracycline resistance gene *tet(O)* in the different genomes.

Results: A novel plasmid (44,095 nts) harboring *tet(O)* was identified. Further analysis of the sequence revealed the presence of a putative aminoglycoside transferase gene, highly similar to kanamycin resistance genes in other organisms and located downstream of *tet(O)*. Several genes putatively involved in conjugative transfer were identified on the plasmid. A functional genome distribution (FGD) analysis, based on the plasmid ORFeomes was performed with the newly identified *C. jejuni* plasmid and 27 other published *Campylobacter* plasmids using compACTor, MEGA, and ACT. The FGD results suggested that the plasmids clustered in two well differentiated groups but were highly conserved within each group. The plasmid of *C. jejuni* 11601MD was clustered together with other *tet(O)*-harboring plasmids in one of these groups. Complementarily, antibiotic susceptibility tests performed on *C. jejuni* 11601MD confirmed the resistance to both kanamycin and tetracycline.

Significance: These findings will contribute to a better understanding of the distribution of potentially self-mobilizing plasmids harboring antibiotic resistance determinants in *Campylobacter* spp. Strain specific sequences of the *C. jejuni* 11601MD plasmid can be utilized to develop tools for detection of similar plasmids in *C. jejuni* from turkeys and other sources.

P2-105 Development of a Selective Enrichment Broth Supplemented with Bacteriological Charcoal and a High Concentration of Polymyxin B for the Detection of *Campylobacter jejuni* and *Campylobacter coli* in Chicken Carcass Rinse

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Developing Scientist Competitor

Introduction: A new *Campylobacter*-selective enrichment broth supplemented with bacteriological charcoal and a high concentration of polymyxin B was developed (charcoal-cefoperazone-polymyxin B-deoxycholate broth; CCPD broth).

Purpose: The ability of CCPD broth to be used for the detection of *Campylobacter jejuni* and *Campylobacter coli* in chicken carcass rinse was compared to that of modified Bolton (mBolton) broth.

Methods: A total of 80 whole chickens purchased from retailers were rinsed with 400 ml buffered peptone water. The rinse samples were enriched with 2 × blood-free mBolton enrichment broth and 2 × CCPD broth at 42°C for 48 h and then streaked onto modified charcoal-cefoperazone-deoxycholate agar (mCCDA). Suspected colonies were confirmed by colony PCR.

Results: The *Campylobacter* isolation rate was significantly ($P < 0.05$) higher in the CCPD broth than in the mBolton broth (CCPD broth, 61 out of 80; mBolton broth, 34 out of 80). Moreover, the selectivity of CCPD broth agar was also superior ($P < 0.05$) to that of the mBolton broth when comparing the number of contaminated mCCDA plates (CCPD broth, 16 out of 80; mBolton broth, 58 out of 80) and the growth index of competing flora (CCPD broth, 1.4; mBolton broth, 2.9).

Significance: Significant elimination of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* in CCPD broth was also observed.

P2-106 A Comparative Evaluation of the Invisible Sentinel *Campylobacter* Assay for the Detection of *Campylobacter* Species in Chicken Carcass Rinsates

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Introduction: The Invisible Sentinel (IS) *Campylobacter* assay is a molecular-based test designed to detect the presence of *Campylobacter jejuni* and *Campylobacter coli* from chicken carcass rinsates, after 24 hours of enrichment, without the need for growth in a specialized anaerobic chamber. The assay utilizes a PCR-based detection method coupled with a rapid vertical flow-based assay and eliminates the need for gel electrophoresis or fluorophore-based analysis of target amplification. This new method provides the specificity and sensitivity of PCR-based amplification in a cost-efficient and easy-to-use format with presumptive results in as little as 26 hours.

Purpose: The purpose of this independent evaluation was to compare the new method to the USDA/FSIS-MLG 41.01 for chicken carcass rinsates and conduct Inclusivity and Exclusivity testing as part of the AOAC Research Institute™ validation process.

Methods: The method comparison analyzed two lots of chicken carcass rinsate for the presence of naturally occurring *Campylobacter* species. For the new method, chicken carcasses were rinsed and enriched with IS Bolton's Broth. After incubation, samples were lysed and the target DNA was amplified using a thermocycler. The amplified DNA was mixed with a proprietary buffer, transferred to a cassette and results were

obtained. Samples were confirmed following procedures outlined in the USDA/FSIS-MLG. For the inclusivity and exclusivity evaluation, 50 *Campylobacter* isolates and 30 closely related non-*Campylobacter* isolates were evaluated.

Results: A POD statistical analysis indicated no significant differences observed between the new method and the reference method. For inclusivity, 50 out of 50 strains of *Campylobacter* species (38 *Campylobacter jejuni* and 12 *Campylobacter coli*) were correctly identified. All 30 exclusivity organisms were correctly excluded.

Significance: This new method demonstrated reliability as a rapid qualitative method for the detection of *Campylobacter* species in chicken carcass rinse.

P2-107 Improvement of Karmali Agar by Addition of Polymyxin B for the Detection of *Campylobacter jejuni* and *Campylobacter coli* in Whole-Chicken Carcass Rinse

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Introduction: The Karmali agar was modified by supplementation with a high concentration of polymyxin B.

Purpose: The goal of the study was to evaluate the effect of a high concentration of polymyxin B on the ability and selectivity of the modified Karmali agar to isolate *Campylobacter jejuni* and *C. coli* from whole chicken carcass rinse.

Methods: A total of 80 whole chickens were rinsed with 400 ml of buffer peptone water. The rinsed samples were incubated with 2 × blood-free modified Bolton enrichment broth for 48 h and then streaked onto unmodified Karmali agar and modified Karmali agar [polymyxin B (100,000 IU/l) supplemented Karmali agar; P-Karmali agar]. The suspected colonies were finally confirmed by colony PCR.

Results: The P-Karmali agar exhibited a significantly better ($P < 0.05$) isolation rate than the unmodified Karmali agar (P-Karmali agar, 73.8%; unmodified Karmali agar, 33.8%). Moreover, the selectivity of the P-Karmali agar was also better ($P < 0.05$) than that of the other selective agar when comparing the number of contaminated plates (P-Karmali agar, 68.8%; unmodified Karmali agar, 87.5%) and growth index (P-Karmali agar, 1.4; unmodified Karmali agar, 2.7) of competing flora.

Significance: The improved selective agar excluded competing flora resistant to antibiotic agents in unmodified Karmali agar, increasing isolation rate and selectivity for *C. jejuni* and *C. coli*.

P2-108 Rapid Detection of *Campylobacter jejuni* in Poultry Products Using Quantum Dots and Nanobeads Based Fluorescent Immunoassay

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Introduction: *Campylobacter jejuni* causes 2.1 to 2.4 million cases of foodborne illnesses in the United States each year with some of the cases linked to eating undercooked poultry or handling raw poultry and poultry products. Thus, a rapid, specific method is needed to detect *C. jejuni* on poultry and poultry products.

Purpose: The objective of this research was to develop a sensitive immunoassay method for rapid detection of *C. jejuni* by using both magnetic nanobeads to separate and concentrate the target bacteria and quantum dots (QDs) as fluorescent markers.

Methods: In this research, both streptavidin conjugated QDs 620 (8 nm diameter) and magnetic nanobeads (150 nm diameter) were separately coated with the specific biotin conjugated anti- *C. jejuni* antibody. The conjugated magnetic nanobeads then were mixed with a sample containing *C. jejuni*. After immunomagnetic separation, the magnetic nanobeads- *C. jejuni* conjugates were mixed with the conjugated QDs. Unattached conjugated QDs were removed using immunomagnetic separation. A spectrometer was used to measure the fluorescence of the complexes of magnetic beads- *C. jejuni* –QDs.

Results: The results showed that this method could detect *C. jejuni* in pure culture, ground turkey, chicken juice or chicken carcass wash solution at a concentration of 2-3 cells/0.1 ml sample (20-30 CFU/ml). The total detection time was less than 2 hrs.

Significance: This study would provide the poultry industry a more effective rapid method for detection of major foodborne pathogens on products to ensure food safety.

P2-109 A Comparative Evaluation of the bioMérieux™ TEMPO® AC for the Enumeration of Total Viable Count in a Variety of Foods

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Introduction: The TEMPO AC (Aerobic Count) is an automated method for the enumeration of viable aerobic mesophilic flora in food products and environmental samples in a minimum of 22 hours. The test system consists of a vial of culture medium and a test card. The culture medium is inoculated with the sample to be analyzed and transferred into the test card containing 48 wells across 3 different volumes. The microorganisms in the card reduce the substrate in the culture medium during incubation causing a fluorescent signal to appear, which is detected by the TEMPO reader. The system enumerates the number of microorganisms present according to a calculation based on the Most Probable Number (MPN) method.

Purpose: The purpose of the study was to compare the candidate method to the AOAC 966.23 method for the enumeration of total viable aerobic microorganisms in raw ground beef, fresh tomatoes, and stainless steel environmental surfaces. The method was also compared to the Standard Method for the Examination of Dairy Products (SMEDP) for pasteurized whole milk.

Methods: The new method and reference methods were evaluated by analyzing 3 food matrices and 1 environmental surface at 3 levels of natural contamination. The test portions were analyzed by the candidate method after 22 hours of incubation and by the reference methods after 48 hours of incubation.

Results: Statistical analysis was conducted using the Probability of Detection (POD) statistical model with no significant difference between candidate method and the AOAC 966.23 method observed in 7 of the 9 lots tested. There was no significant difference between the candidate method and the SMEDP for all 3 lots analyzed.

Significance: The product demonstrated reliability as a rapid, automated enumeration method for total viable count in foods and environmental surfaces by providing results in 22 hours compared to 48 hours for traditional methods.

P2-110 100K Foodborne Pathogen Genome Project

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Introduction: A consortium of government, academic and private organizations are partnering to sequence the genomes of foodborne pathogens with the ultimate goal of making the data and culture bank publically available to improve food security. In five years the consortium is collecting and sequencing 100,000 pathogen isolates. These isolates are to comprise diverse foods, environments, world-wide coverage, and single serovars collected across time.

Purpose: Currently, it takes weeks to identify an organism, such as those listed in Table 1, using serotyping methods, a challenge during a foodborne pathogen outbreak. Robust molecular identification methods cannot be developed to industry standards with available sequences.

Methods: The genome sequence diversity of the top 5 foodborne pathogens will be produced using next generation sequencing (NGS) with BGI@UCDavis). The facility at Davis will have ~10 HiSeq 2000 instruments for use by this project. Isolate priority list includes *Salmonella, Campylobacter, Escherichia coli, Vibrio, Listeria,* then *Yersinia, Shigella, Clostridium, Enterococcus, Cronobacter*, followed by toxigenic bacilli, norovirus, hepatitis A & E, rotavirus and enteroviruses. A small number of whole genomes will be "finished" to completion. The majority of isolates will be used to produce draft genomes and assembled using genomes in the public domain. This approach will enable a systematic analysis of the minimal set of genes associated with persistence, serotype diversity, location, antibiotic resistance, pathogenesis, and host association. Isolates will be grown, serotyped, banked, sequenced, and then metadata will be stored with the genomic sequences, either in finished or in draft form.

Results: This extraordinary collaboration is resulting in the public availability of matched genomic sequences and isolates. As the food network is increasingly global, faster approaches for use in surveillance and outbreak investigation are imperative.

Significance: This microbial ecology-based information will lead to the use of new molecular methods for diagnostic development.

P2-111 Effect of Matrix on Food Microbiology Proficiency Testing Samples

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Introduction: For U.S. clinical laboratories, proficiency testing (PT) has evolved into an essential component of quality assurance programs. These laboratories are regulated by CLIA 1988, which mandates participation in an approved PT program. Food laboratories may soon be subject to similar requirements with passage of the Food Safety Modernization Act, which may require laboratory accreditation for foods regulated by the FDA. American Proficiency Institute, a federally approved clinical PT provider, and an ISO/IEC 17043 accredited Food Microbiology and Chemistry PT provider via A2LA, offers PT programs for the food industry.

Purpose: Our objective was to determine if a select matrix of either dehydrated meat or non-fat dry milk, will influence the false negative or false positive rates on qualitative analysis for three food pathogens. This PT study includes data from 2011 and 2012 for *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium.

Methods: A total of 7,789 proficiency testing results were submitted over three test events for each year. Participants tested the proficiency samples using the method of their choice; methods used included cultural techniques as well as immunoassay and PCR. The false negative and false positive rates were then determined.

Results: A null hypothesis test was conducted for the false negative and false positive rate for each matrix, per food pathogen. Using a 95% confidence limit, it was determined that no significant statistical difference exists in the rate of false positive results or the rate of false negative results when comparing data for the dehydrated meat matrix to data for the non-fat dry milk matrix, for all three food pathogens.

Significance: Selection of matrix in this study did not influence false negative or false positive detection rates. No statistically significant difference in rates was found between the matrices.

P2-112 Identification of a Second Bacteriocin Produced by *Enterococcus mundtii* CUGF08 may be Involved in the Self-lethality Phenotype

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Developing Scientist Competitor

Introduction: *Enterococcus mundtii* CUGF08 is a lactic acid bacterium (LAB) isolated from alfalfa sprouts that produces mundticin L (munL), a class IIa bacteriocin. Similar to other class IIa bacteriocins, it is active against closely related bacteria and *Listeria* spp., including *L. monocytogenes*. However, unlike other bacteriocin-producing strains, this bacterium exhibits self-lethality, resulting in a halo-shaped inhibition zone surrounding the producer strain when using the deferred inhibition assay.

Purpose: This self-lethality phenotype is rarely observed in bacteriocin-producers because immunity proteins are generally co-transcribed with the bacteriocin structural gene. Additionally, no mutations or atypical gene arrangements were observed within the gene cluster for munL. Therefore, the objective of this study was to identify the component(s) causing the self-lethality.

Methods: The supernatant from an 18-hour culture of *E. mundtii* CUFG08 was filter-sterilized and applied to a SepPak C18 solid phase extraction cartridge. The fraction eluted via 50% isopropanol was applied to a cation-exchange column, and the non-retained portion was applied to a 250 mm x 4.6 mm Jupiter 5 µ C5 300 Å column connected to an Agilent 1100 HPLC. The peak that contained the bacteriocin activity was collected and sequenced using trypsin digestion and MS/MS for amino acid sequencing.

Results: The isolated compound was found to be a 50-residue peptide, designated mundticin K (munK). This mature peptide is identical to mundticin KS (munKS), except that munKS is cleaved after the double-glycine motif, resembling other class IIa bacteriocins. MunK contains an additional 7-residue N-terminal sequence including the double-glycine motif, which is longer than the mature 43-residue munKS.

Significance: This data may be used to discover more information on the mechanism of action of class IIa bacteriocins, the maturation process, and the mechanism of the immunity protein. It may also help increase the understanding of how sensitive strains, such as *L. monocytogenes*, develop resistance to class IIa bacteriocins.

P2-113 Biological Control of Hydrogen Sulfide-producing Bacteria in Raw Poultry By-Products and on Surfaces of Rendering Plant

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Developing Scientist Competitor

Introduction: Raw animal by-products destined for rendering process may contain high population of H_2S producing bacteria (SPB) that can multiply very quickly and produce harmful gas - H_2S. Additionally, SPB can form biofilm on the surfaces of the rendering plants and serve as the sources of recontamination.

Purpose: This study was to apply bacteriophages to reduce H_2S production from raw poultry products and decontaminate SPB biofilm on surfaces of rendering processing environment.

Methods: A mixture of 9 selected SPB-specific bacteriophages was inoculated to raw poultry by-products such as chicken meat (fresh and spoiled), offal and feather, and SPB biofilm on stainless steel and plastic surfaces. A greenhouse study was performed to simulate the conditions for transporting raw materials to rendering plant. Multiplicity of infection (MOI) of 100 or 1000 was used for phage treatment. The population of SPB was enumerated using a trypticase soy agar (TSA) - H_2S selective medium and the amount of H_2S production was determined using either test strips impregnated with lead acetate or a H_2S monitor.

Results: H_2S production by SPB in various raw poultry by-products including fresh chicken meat artificially inoculated with SPB, spoiled chicken meat, offal and feather was reduced ranging from 25 to 71% by bacteriophages under laboratory condition. MOI of 1,000 was more effective than MOI of 100 in reducing H_2S production by SPB for all treatments. Phage treatment also reduced H_2S production by 30~85% in raw poultry by-products in a greenhouse study. Furthermore, bacteriophages was able to reduce SPB biofilm population on surfaces of steel and plastic by 1.5~2 log and 0.4~1.4 log CFU/cm^2, respectively, under both laboratory and rendering plant conditions in spring and summer.

Significance: Control of H_2S production from raw poultry by-products and in rendering environment can not only provide a safer working environment for workers but also produce high quality rendered animal meals for feed industry.

P2-114 Inhibition of Foodborne Spoilage Organisms in Low pH Food Segment Using Cultured Sugar

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Introduction: Yeasts and molds represent most common spoilage problem in low pH food matrices like beverages, sauces and dressings. *Candida albicans*, *Fusarium oxysporum* and *Aspergillus niger* are representative spoilage species for this segment. Currently the preservation system of benzoate and sorbate is the most common used. Due to concerns like decarboxylation of benzoate to benzene and their chemical image, the food industry is looking for alternative natural preservatives. Verdad F95, a fermentation-based natural antimicrobial contains a mixture of natural organic acid salts, peptides and sugars and has excellent antimicrobial properties.

Purpose: The objective is to evaluate the antimicrobial efficacy of fermentation-based cultured sugar product Verdad F95 against in low pH model beverage system.

Methods: Model low pH broth was prepared using water, sucrose, apple juice concentrate, and an acidifier. *Candida albicans* cells, *Fusarium oxysporum* and *Aspergillus niger* spores were independently inoculated to have ca. 2-3 log CFU/ml counts in the model system with pH of 3.2. Treatments with Verdad F95 from 0-2% in 10 equal concentration steps were prepared. The inoculated samples were stored at room temperature ca. 20 °C for 20 days, respectively, for optical density measurement. All the treatments were done multiple samples (n = 5). The yeast growth was measured using OD measurement at 595 nm and mold growth was evaluated using visual examination.

Results: The *C. albicans* experimental data indicate that at minimum use level of 1.0% cultured sugar, the yeast growth was inhibited throughout the incubation period, keeping the OD value less than 0.1. The *F. oxysporum* and *A. niger* growth data indicates that 1.0% cultured sugar inhibited the mold growth throughout the incubation period ($P < 0.05$).

Significance: This research substantiates the excellent antimicrobial efficacy of natural antimicrobial, cultured sugar, against spoilage micro-organisms in model system. This research also provides food industry with a potential antimicrobial to replace current chemical preservatives.

P2-115 Reduction of Artificial *Salmonella* Typhimurium Contamination on Stainless Steel by Application of Bacteriophage

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Introduction: Bacteriophages have been studied as natural bactericidal agents for decontamination of fruits, vegetables, and animal hides. The effectiveness of bacteriophage to reduce bacterial contamination on food contact surfaces such as stainless steel remains to be extensively studied.

Purpose: The objective of this study is to test the effectiveness of a phage cocktail for reducing *S.* Typhimurium on artificially contaminated stainless steel surface at 4° and 22°C.

Methods: Bacteriophages that cause lysis of *Salmonella* were isolated from raw sewage samples collected from four local water treatment plants. Five isolates with different host ranges were selected and combined into a phage cocktail. Sterile stainless steel spatulas were artificially contaminated by soaking a 14-cm^2 area in *S.* Typhimurium cell suspension prepared in phosphate buffer saline (PBS) for 1 h, and dried for 2 h at 22°C. The contaminated areas were then soaked in PBS or the phage cocktail at 4 or 22°C for 2 or 24 h.

Results: The mean initial *Salmonella* count was 7.3 log CFU/sample Soaking contaminated stainless steel surface in a phage cocktail for 2 h resulted in about 2 log reduction in *Salmonella* counts at both 4 and 22°C. Soaking additional 22 h in the phage cocktail did not cause significant more reduction in *Salmonella* counts.

Significance: Bacteriophages have been proven to reduce bacterial contamination on many food surfaces. This study shows that treating a stainless steel surface with a bacteriophage cocktail for 2 h could reduce *Salmonella* contamination by 2 logs at both 4 and 22°C.

P2-116 Isolation and Identification of Bacteriocinogenic Strain of *Lactobacillus plantarum* with Potential Beneficial Properties from Donkey Milk

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Introduction: Bacteriocins are ribosomally-synthesized peptides or proteins with antimicrobial activity, produced by different groups of bacteria. Many lactic acid bacteria (LAB) produce bacteriocins with rather broad spectra of inhibition and they often have potential applications in food preservation.

Purpose: The goal of this study was to isolate and characterize a bacteriocinogenic LAB from donkey milk with potential beneficial properties.

Methods: LAB were isolated from donkey milk and identified based on physiological, biochemical and molecular methods. The promising isolate (*Lact. plantarum* LP08AD) was evaluated for production of bacteriocin, including stability in presence of various enzymes, surfactants, salts, pH and temperatures. Presence of bacteriocin genes in strain LP08AD was investigated. Growth of strain LP08AD in different pH values and presence of ox bile, adhesion to non- cancerogenic cell lines was explored.

Results: Based on the preliminary test, selected isolate LP08AD was identified as *Lact. plantarum*. Bactericidal effect of bacteriocin LP08AD on *Listeria monocytogenes, Ent. faecium* and *Lactobacillus curvatus* was shown for actively growing and stationary cells. Similar growth and bacteriocin production were observed when strain LP08AD was cultured in MRS at 30°C or 37°C. Bacteriocin LP08AD adhere at low levels on the producer cells (200 AU/ml). Presence of plantaricin W gene on the genomic DNA was recorded based on PCR reaction. Good growth for strain LP08AD was recorded in MRS broth with pH from 5.0 to 9.0 and in the absence of ox bile or concentration below 0.8%. *Lact. plantarum* LP08AD was applied to the small intestinal epithelial polarised monolayers of H4, PSIc1 and CLAB and demonstrated low attachment ability on cell lines from human and pig origin.

Significance: To the best of our knowledge, this is the first report on detection and characterization of bacteriocinogenic *Lact. plantarum* from donkey milk. The strain LP08AD shows to have potential beneficial properties, as demonstrated by use of non-cancerogenic cell lines.

P2-117 Characterization of Bacteriocin Production, Safety and Technological Potential of Two *Enterococcus faecium* Strains Isolated from Brazilian Artisanal Cheeses

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Introduction: Many lactic acid bacteria produce bacteriocins with a broad spectra of inhibition and may be applied in food preservation or as potential probiotic candidates.

Purpose: This study was on the characterization of bacteriocins produced by *Enterococcus faecium* EM485 and EM925, isolated from Coalho cheeses, and check for its safety and technological properties.

Methods: Isolates EM485 and EM925, differentiated by RAPD-PCR, have been tested for production of bacteriocins against foodborne pathogenic microorganisms and been investigated for their probiotic and technological potential, including aggregation, hydrophobicity, deconjugation of taurocholic acid (TC), taurodeoxycholic acid (TDC), glycocholic acid (GC), and glycodeoxycholic acid (GDC), survival rates in the conditions simulating the GIT, resistance to antibiotics and presence of virulence genes.

Results: Isolates EM485 and EM925 were selected based on their effective inhibition against *Listeria monocytogenes,* and classified as *E. faecium* based on 16s rDNA analysis. In MRS at 37°C, bacteriocins produced by both strains were detected as 3200AU/ml. These peptides were inactivated by proteolytic enzymes, but not by a-amylase, catalase and lipase. The two bacteriocins remained stable at pH from 2.0 to 10.0 and after exposure at 100°C for 120 min and in presence of surfactants and salts. DNA from both strains generated positive PCR results for enterocin A and enterocin B genes. High levels of co-aggregation have been observed for both strains with *Escherichia coli* (78.35 ± 2.16% and 74.31 ± 3.64%) or *Clostridium* spp. (81.13 ± 1.92% and 84.26 ± 3.44%). Both strains presented low levels of hydrophobicity (8.18% and 11.33%). *E. faecium* EM485 and EM925 were able to grow in presence of 0.5% of TC, TDC, GC and GDC, however, only being able to deconjugate GDC and TDC. Both strains showed good survival when exposed to the conditions simulating the GIT. When tested for presence of virulence genes, only tyrosine decarboxylase and vancomycin B generated positive PCR results.

Significance: This is the first report on detection and characterization of bacteriocinogenic *E. faecium* from Coalho cheeses with potential beneficial and technological properties with low virulence profile.

P2-118 Control of *Listeria monocytogenes* by Bacteriocins Produced by *Lactobacillus curvatus* MBSa2 during Ripening of Salami

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Introduction: *Listeria monocytogenes* is a ubiquitous pathogen that can survive the technological hurdles usually applied for manufacture of salami. Bacteriocins produced by lactic acid bacteria have a potential technological application in inhibiting the growth of this pathogen. *Lactobacillus curvatus* MBSa2 is a bacteriocin-producing strain isolated from salami, with anti-*Listeria* activity in culture medium.

Purpose: The purpose was to evaluate the activity of the partially purified bacteriocin (PPB) produced by the MBSa2 strain on the control of *L. monocytogenes* in salami, during ripening period.

Methods: PPB was obtained by precipitation with ammonium sulfate followed by SepPak C_{18} chromatography. Minimal inhibitory concentration (MIC) of PPB was determined by serial dilutions in BHI broth, using *L. monocytogenes* Scott A as indicator for antimicrobial activity. Samples of salami mixture, prepared with beef meat (10%), pork meat (75%) and lard (15%) and NaCl, spice mix and commercial starter culture T-SPX, were experimentally contaminated with *L. monocytogenes* Scott A (10^4-10^5 CFU/g). The PPB was added to the mixture at the determined MIC value, stuffed into collagen casings and stored for ripening at recommended temperatures. Controls, without added bacteriocin, were also prepared. *Listeria* counts were performed on days 0, 4, 10, 20, 30.

Results: The MIC value of the PPB against *L. monocytogenes* was 200 AU/ml. Addition of the PPB resulted in reduction of 0.56 log in the counts of *L. monocytogenes* immediately after addition of the bacteriocin. After 10, 20 and 30 days, the counts of the pathogen were 1.98, 1.77 and 1.01 log lower than in the salami without added bacteriocin, respectively.

Significance: The PPB of *L. curvatus* MBSa2 can be used as an additional hurdle for the control of *L. monocytogenes* in salami.

P2-119 Antimicrobial Activity of Lactiguard® against Foodborne Pathogens in Laboratory Media

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Introduction: Foodborne pathogens, including *Salmonella* and *Listeria*, give rise to severe issues in food industry by causing a substantial health risk to consumers. Lactiguard®is particularly good at reducing pathogenic risks because this product reportedly has very strong antimicrobial properties.

Purpose: The objective of this study was to evaluate the effect of dose and temperature on the inhibition activity of the GRAS bacterial anti-microbial preparation against *Salmonella* and *Listeria* in laboratory media.

Methods: The cocktail of NP51+NP3+NP7+NP28 was individually added at $1x10^7$, $1x10^8$ and $1x10^9$ CFU/ml into TSB broth containing *Salmonella* or *Listeria* (Ca. $1x10^4$CFU/ml). Then, these samples were incubated under anaerobic conditions at 37 and 4 °C, respectively. Pathogen enumeration was evaluated at hours 0, 2, 4, 8, 12, 24 and 48 at 37 °C, and on days 0, 1, 2, 3, 5 and 7 at 4°C.

Results: Our results indicated that at 37°C, $1x10^8$ and $1x10^9$ CFU/ml of the preparation have led to a significant ($P < 0.05$) reduction in *Salmonella* since hour 4, while a significant ($P < 0.05$) reduction in *Listeria* with $1x10^9$ of the preparation was observed since hour 2. And, 1×10^8 CFU/ml of the preparation led to a significant ($P < 0.05$) reduction in *Listeria* only from hour 8 to 24; however, at 4°C, a significant ($P < 0.05$) reduction in *Salmonella* was observed only with $1x10^7$ CFU/ml of the cocktail on days 2 and 3. For *Listeria*, $1x10^8$ and $1x10^9$ CFU/ml of the cocktail have led to a significant ($P < 0.05$) reduction since days 2 and 1, respectively, and $1x10^7$ CFU/ml of the cocktail only attributed to a significant ($P < 0.05$) reduction on day 7.

Significance: The use of Lactiguard® is a very effective intervention to control *Salmonella* and *Listeria* in laboratory media and its inhibition activity in food products must continue to be tested.

P2-120 Antimicrobial Activity of Apple Skin Polyphenol and Thyme Essential Oil in Acai Edible Films and Their Effects on Film Properties

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Developing Scientist Competitor

Introduction: Diseases caused by foodborne pathogens are of great concern to the food industry. The consumption of foods with chemical preservatives has led to increased consumer concern and demand for natural and minimally processed foods. In addition, environmental concerns are generated from current domestic and industrial use of synthetic plastics. As a result, there is great interest in developing antimicrobial packaging materials using natural antimicrobial compounds and biopolymers.

Purpose: The purpose of this study was to develop biodegradable edible films with antimicrobial activity by incorporating both apple skin polyphenol (ASP) and thyme essential oil (TEO) into films made from Açaí frozen puree, a tropical fruit product from Brazil.

Methods: The antimicrobial effects of açaí edible films incorporated with ASP and TEO were investigated using a central composite design (CCD). The CCD consisted of 11 treatments. Antimicrobial activity of films against *Listeria monocytogenes*as well as film properties, including mechanical properties, color and thermal resistance, were evaluated. **Results** were analyzed using response surface methodology.

Results: Antimicrobial effect of both compounds on açaí edible films was observed. Combination of 3.1% ASP and 6.07% TEO resulted in the film with highest inhibition zone against *Listeria monocytogenes,* with mean value of 685.4 mm^2. Film stiffness was influenced by the interaction of both antimicrobials. Film lightness was influenced by ASP and interaction of both compounds. Film redness was affected by both compounds. Incorporation of both antimicrobials resulted in slightly increased values of b* (yellowness). Incorporation of ASP resulted in enhanced thermal stability of films.

Significance: Knowledge of antimicrobial and physical properties of packaging materials is essential for industrial food application. This work showed the antimicrobial synergy of ASP and TEO when incorporated in açaí films, and demonstrated their potential application on food preservation due to their antibacterial activity and good contribution to film properties.

P2-121 Evaluation of Microbiological Safety for Collagen Foods in Korea

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Introduction: In Korea, consumption of collagen foods has increased, but microbiological safety has not been evaluated for these foods.

Purpose: The objective of this study was to evaluate microbiological safety of collagen foods in Korea.

Methods: A mixture of *Staphylococcus aureus* strains (NCCP10768, NCCP10778, NCCP11596, NCCP13236, and NCCP10862) and a mixture of *Salmonella* strains (*S.* Enteritidis NCCP14545, *S.* Enteritidis NCCP14546, *S.* Agona NCCP12231, *S.* Typhimurium NCCP10725, *S.* Typhimurium NCCP10747) were inoculated with *Samgyetang* (5 g), *Ugultang* (5 g), *Odolbyeboguem* (25 g), *Jeonyak* (25 g), and *Jokbal* (15 g) at 4 log CFU/g. The samples were then incubated at 4°C (6 days), 10°C (6 days), 20°C (3 days), and 30°C (36 h). Cell counts of *S. aureus* and *Salmonella* were enumerated on mannitol salt agar and xylose lysine deoxycholate agar, respectively. In addition, minimum bactericidal concentrations (MBC; AU/ml) of ingredients (clove, cinnamon, pepper, ginger and jujube) in *Jeonyak* were determined.

Results: Of the five collagen foods, most collagen foods allowed *S. aureus* and *Salmonella* growth, but *S. aureus* and *Salmonella* cell counts decreased ($P < 0.05$) only in *Jeonyak* during storage. For the ingredients in *Jeonyak*, clove, cinnamon, pepper and ginger extracts showed antimicrobial activities (MBC: 3.13-50 AU/ml) to *S. aureus*, and clove and cinnamon extracts showed antimicrobial activities (MBC: 50 AU/ml) to *Salmonella.*

Significance: The result indicates that *Jeonyak* has antimicrobial activity to *S. aureus* and *Salmonella* due to the antimicrobial ingredients in the food.

P2-122 Effects of Grape Seed Extract on the Growth of Top Six Non-O157 Shiga Toxin-producing *Escherichia coli*

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Developing Scientist Competitor

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) is a leading cause of foodborne infections. Centers for Disease Control and Prevention (CDC) "top-six" non-O157 STEC are growing concerns to the food industry due to the rising foodborne illnesses from these strains.

Given their ability to cause fetal hemolytic uremic syndrome and global prevalence in foods, developing intervention methods for these non-O157 strains is necessary, and natural ingredients are among top choices. Grape seed extract (GSE) contains abundant polyphenols which have antimicrobial activity in addition to its health beneficial effects.

Purpose: The objective is to explore the inhibitory effects of GSE on the growth and swimming motility of the "top-six" non-O157 STEC.

Methods: Swimming motility was assayed using 0.3% LB agar plates. The growth curves were conducted in 96 well polystyrene microtiter plates.

Results: At 5×10^5 CFU/ml inoculation level, 0.5-4 mg/ml GSE effectively inhibited the growth of O26:H11, while 4 mg/ml GSE prevented the growth of all other non-O157 STEC strains tested including O45:NM, O103:H2, O111:H2, O121:H19 and O145:NT. However, GSE up to 4 mg/ml had a limited inhibitory effect on top-six non-O157 STECs when the initial inoculation level increased to 1×10^7 CFU/ml. In addition, GSE significantly inhibited swimming motility of O26:H11, O103:H2, O111:H2, O121:H19 and O145:NT at concentrations as low as 0.25 mg/ml, and completely blocked their motility at 4 mg/ml.

Significance: GSE can effectively inhibit swimming motility of non-O157 STEC, however, its inhibitory effects toward the growth are strain and population dependent. Such information is useful for its application as an antimicrobial in food industry.

P2-123 Effect of Commercial Natural Antimicrobials Based on White Mustard and Citrus on Foodborne Pathogens and Spoilage Microorganisms

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Introduction: Natural antimicrobials derived from plant sources are of great interest to the food industry. Before they can be used in commercial products, their efficacy against microorganisms of concern to the food industry, including pathogens and spoilage organisms, must be evaluated.

Purpose: This study aimed to determine the activity of 2 commercial natural antimicrobial products against several organisms including enteric pathogens, spoilage *Bacillus* spp. and *Enterobacter* spp., lactic acid bacteria and yeasts of concern to the food industry. Product A consisted of white mustard essential oil containing 4-hydroxybenzylisothiocyanate (4-HBITC) and Product B was derived from citrus.

Methods: Product A was added to bacterial and yeast cultures in broth to final concentrations of 250, 500 and 1000 ppm based on 4-HBITC and growth (CFU/ml) monitored for 48-72 h at 22°C by spread plating. Absence of live cells was verified by incubation in Dey-Engley broth for 24 h followed by spread plating. Product B (19.53 - 5,000 ppm) was tested for minimum inhibitory concentrations (MICs) against bacteria and yeasts at pH 7.0, 6.5 and 6.0 using a microbroth-dilution assay. Minimum lethal concentrations (MLCs) were determined by direct plating method.

Results: Product A significantly inhibited the growth of all organisms tested at 1000 ppm, and was most effective against *Salmonella* Enteritidis and *Schizosaccharomyces pombe*, which were not detected at 500 ppm after 48 h and at 250 ppm throughout the trial, respectively. Product B inhibited all organisms within the concentrations tested, but was most effective against *Listeria monocytogenes*, *Staphylococcus aureus* and *Bacillus* spp. with MICs of 156.25-312.5 ppm at all pHs tested.

Significance: The results suggest that the antimicrobials tested are effective against a wide range of pathogens and spoilage organisms and have the potential to be evaluated in food products for improving safety and/or increasing product shelf life.

P2-124 Antimicrobial Effect of Mexican Oregano Essential Oil in Combination with Olive Oil against Foodborne Pathogens

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Introduction: The use of natural preservatives is becoming increasingly important for consumers. Oregano essential oil (OEO) has been demonstrated as an excellent antimicrobial against bacteria, yeasts and fungi. One disadvantage is the high concentrations needed for its effect, which affects sensorial properties. On the other hand, food composition can be favorable or unfavorable for the in antimicrobial effect of OEO, including temperature, water activity and pH.

Purpose: The aim of this work was to determine the antimicrobial effect of Mexican oregano EO in combination with olive oil, simulating fat content in food against food contamination bacteria.

Methods: Microorganisms used were *Bacillus cereus, Escherichia coli*, *E. coli 0157: H7*, *Listeria monocytogenes*, *Salmonella* Typhimurium and *Staphylococcus aureus*. A Checkerboard Method in glass test tubes with 2000 µl of TSB were used, with the following concentrations of the factors studied: olive oil (control, 1-7%); Mexican OEO (50, 100, 200, 300, 400,500, 600, 700, 800, 900 mg/l). Tubes were inoculated with the bacterial strains and incubated for 24 hours at 37° C. After incubation, 200 µl of each tube were transferred to the microplate and revealed with 50 µl of 0.1% resazurin. Fractional inhibitory concentrations (FIC= FICA + FICB) were calculated to demonstrate synergism, antagonisms or indifference.

Results: An antagonism is shown for the *Bacillus cereus, Listeria monocytogenes* and *S. aureus* strains since the obtained numerical value were 5.1428, 7.7142 and 6.2857, respectively. While for strains *E. coli*, *E. coli* O157: H7 and *Salmonella* Typhimurium results are 2.1428, 2.6428 and 3.1428, respectively, and showed an indifferent effect.

Significance: Results demonstrate that the antimicrobial effect of oregano can be influenced by the composition of the food where the antimicrobial is added, and should be considered as a problem when natural antimicrobials are considered for hurdle technology.

P2-125 Antibacterial Activity of Red Cactus Pear (*Opuntia humifusa*) and Green Cactus Pear (*Opuntia ficus-indica*) against *Escherichia coli* O157:H7

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Introduction: Plants, herbs, and spices are rich in naturally occurring compounds that have many health benefits and could serve as strong antimicrobial agents in food products. Red cactus pear (*Opuntia humifusa*) and green cactus pear (*Opuntia ficus-indica*) have demonstrated strong anticancer and antioxidant characteristics and could have to antimicrobial activity.

Purpose: This study was conducted to determine the antimicrobial activity of red and green cactus pear against *Escherichia coli* O157:H7 in laboratory culture medium (BHI).

Methods: Extracts from pulp and skin of red and green cactus pear were tested against three strains of *E. coli* O157:H7 individually. Growth over time assay and agar well diffusion assay were used to determine the inhibitory effect of cactus pear.

Results: Our results showed that in control samples, *E. coli* O157:H7 continue to grow and reached an average of 8.86 ± 0.69 logs CFU/ml, whereas the addition of 20% extract of the red or green cactus pears skins caused the bacterial population to remain within 3.42 ± 0.15 logs CFU/ml, respectively, after 10 h of incubation at 37°C. The minimum inhibitory volumes (MIV), the lowest concentration with significant inhibitory effect, were 400 and 475 µl/ml (V/V) for the skin of red and the skin of green cactus pears, respectively.

Significance: These results indicated that skins of red and green cactus pears have potential antimicrobial effects against the growth *E. coli* and could provide a natural means of controlling pathogenic contamination.

P2-126 Use of Plant-derived Essential Oil Compounds, Naturally-occurring Apple Aroma Compounds and Apple Juice Flavoring Mixtures to Control *Escherichia coli* O157:H7

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Introduction: Antimicrobial compounds derived from natural sources are of interest as agents to control foodborne pathogens in juices.

Purpose: The antimicrobial activity of three essential oil (EO) compounds (thymol, eugenol, and trans-cinnamaldehyde) alone and in combination with three naturally-occurring apple aroma (AA) compounds (hexanal, trans-2-hexenal and 1-hexanol) were evaluated to identify the minimum inhibitory concentrations necessary to inhibit *Escherichia coli* O157:H7. Additionally, three commercial apple juice flavoring mixtures (natural apple cinnamon, natural apple spice and natural red apple) were tested alone for antimicrobial activity against *E. coli* O157:H7.

Methods: Minimum Inhibitory Concentrations (MIC) were determined using the standard agar dilution (SAD) method. Antimicrobial compounds at concentrations of 0.2 to 51.2 mg/ml were dissolved into separate containers of molten TSA, containing 0.5% Tween-20. Molten agar solutions were mixed for one minute then poured into individual petri dishes and allowed to solidify. Individual plates were then divided into thirds and spot inoculated with 2.5 µl of *E. coli* culture (diluted to log 106 CFU/ml). Plates were incubated at 35°C and evaluated after 24-48 hours for presence or absence of growth.

Results: Among EO compounds, cinnamaldehyde exhibited the highest degree of antimicrobial activity against *E. coli* (MIC = 0.2 mg/ml). Thymol and eugenol inhibited *E. coli* O157:H7 at an MIC value of 1.6 mg/ml. Among AA compounds, trans-2-hexenal exhibited the highest degree of antimicrobial activity (MIC = 1.6 mg/ml). Hexanal and 1-hexanol were significantly less active, with MICs of 12.8 and 6.4 mg/ml, respectively. Of the apple juice flavoring mixtures tested, apple cinnamon exhibited the highest degree of antimicrobial activity (MIC = 12.8 mg/ml), followed by apple spice with an MIC of 51.2 mg/ml. Combinations of essential oil and apple aroma compounds resulted in an indifferent effects.

Significance: Essential oil, apple aroma and apple flavoring compounds were shown to inhibit *E. coli* O157:H7.

P2-127 Efficacy of Roselle (*Hibiscus sabdariffa*) Calyx Formulations against *Escherichia coli* O157:H7 on Bagged Organic Leafy Greens

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Introduction: The increased trend of fresh produce consumption in the past decade has been accompanied with an increase in associated outbreaks. *Escherichia coli* O157:H7 has been repeatedly implicated in these outbreaks. Since consumers prefer natural over synthetic products, and due to limitations of available sanitizers, we investigated the possibility of an alternative approach to washing organic leafy greens by evaluating the effectiveness of roselle (*Hibiscus sabdariffa*) formulations against *E. coli* O157:H7.

Purpose: The effectiveness of roselle tea and aqueous extract against *E. coli* O157:H7 in organic leafy greens was evaluated.

Methods: Organic leafy greens: baby and adult spinach, and romaine and iceberg lettuce were washed in sterile water, inoculated with a cocktail of *E. coli* O157:H7 (10^6 CFU/ml), and dried to facilitate attachment. Roselle tea was obtained from dried calyces boiled in distilled water. Roselle aqueous extract (10, 20, and 30%; v/v in phosphate buffered saline; PBS), was prepared from fresh calyces. The inoculated leafy greens were individually treated with the roselle extract or tea and stored at 4°C. Hydrogen peroxide, water, and PBS were used as the controls. Bacterial populations were enumerated from treated samples at days 0, 1, and 3 of storage.

Results: The antimicrobial activity of the tea and extract was dependent on antimicrobial concentration, storage time, and the type of produce ($P < 0.01$). By day 3, 30% roselle extract showed a reduction in bacterial population by 3.7 and 3.1 logs for baby spinach and romaine lettuce, respectively, and by 2 logs for the rest of the greens. By day 3, roselle tea showed a reduction in bacterial population of 3.4 logs for romaine lettuce, and of 1.1- to 2.3-logs for the rest of the greens.

Significance: Both, roselle tea and extracts can be used as effective wash treatments to inactivate *Escherichia coli* O157:H7 on organic leafy greens.

P2-128 Antibacterial Effects of Olive, Apple and Grapeseed Extracts against *Escherichia coli* O157:H7 on Organic Leafy Greens Stored at 4°C

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Developing Scientist Competitor

Introduction: There has been an increase in foodborne outbreaks due to contaminated fresh produce in the past two decades. *Escherichia coli* O157:H7 has been implicated in many such outbreaks. Due to growing consumer preference for natural products and to limited availability of approved sanitizers for organic produce, there is a need to test the effectiveness of plant-derived extracts against pathogens on organic leafy greens.

Purpose: Various plant extract formulations were evaluated on four types of organic leafy greens contaminated with *E. coli* O157:H7.

Methods: Organic baby and adult spinach, and romaine and iceberg lettuce were washed in sterile water and inoculated with a cocktail of *E. coli* O157:H7 strains (10^6 CFU/ml). Apple, olive, and grapeseed formulations were prepared in phosphate buffered saline (PBS) at 1, 3, and 5% concentrations. The inoculated greens were washed in each treatment solution, bagged and refrigerated at 4°C. Surviving bacteria were enumerated on days 0, 1, and 3 of storage.

Results: Of all the extracts tested, olive extract was the most effective, resulting in a 4-5 log reduction for each leafy green by day 3. Olive extract (5%) showed complete bacterial inhibition in romaine lettuce by day 3. For iceberg lettuce, no *E. coli* O157:H7 was recovered on day 1 with 3

Poster

and 5% olive extract treatment. No *E. coli* O157:H7 was recovered on day 3 from adult spinach treated with 3 and 5% olive extract. By day 3, apple extract showed reductions of 3.4 and 3.6-logs in romaine and iceberg lettuce respectively, and 1.4- to 2.2-logs for the rest of the greens whereas grapeseed extract resulted in 4.4-log reduction for romaine lettuce, and about 2-log reduction for the rest of the greens.

Significance: This study demonstrates the potential of natural plant extract formulations to inactivate *Escherichia coli* O157:H7 strains on organic leafy greens.

P2-129 Comparison of Nitrite Derived from Natural and Synthetic Sources on the Inhibition of *Clostridium perfringens* during Extended Cooling of Turkey Breast

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Introduction: Natural and organic processed meats cured with vegetable juice powder as an alternative to synthetic $NaNO_2$ typically have lower nitrite levels than traditional products and hence have lower ability to inhibit pathogens such as *Clostridia*. However, the antimicrobial activity of nitrite from the two sources has not been compared when added at equivalent levels.

Purpose: To compare the inhibition of *Clostridium perfringens* during extended cooling of turkey breast cured with cultured celery juice powder vs. $NaNO_2$.

Methods: Seven treatments of deli-style turkey breast (76% moisture, pH 6.1-6.3, 1.4-1.5% NaCl) were prepared with 0, 40, 80, or 120 ppm nitrite derived from cultured celery powder or synthetic $NaNO_2$, and inoculated with *C. perfringens* spores (three-strain mixture) to yield 3-log CFU/g. Individual 100-g portions were vacuum-packaged, cooked to 71.1°C and cooled to 4°C during a 10-hour linear cooling protocol. Triplicate samples were assayed for populations of *C. perfringens* by plating on tryptose-sulfite-cycloserine at 0, 2.5, 5, 7.5 and 10 h; experiments were replicated three times.

Results: Control samples (0 ppm NO_2) without antimicrobial supported an average 1.8, 2.9 and 3.6 log increase of *C. perfringens* at 5, 7.5 and 10 hours, respectively. Addition of 40 ppm NO_2 derived from either source, but without cure accelerator, had no additional inhibition compared to the control. Inhibition was not significantly different between the two 80 ppm NO_2 treatments with an average 0.8, 1.9, and 1.9 log increase at 5, 7.5 and 10 hours, respectively. Both 120 ppm NO_2 inhibited growth to < 1-log increase during the 10 hour cooling.

Significance: This study confirmed that the concentration of NO_2, rather than source, is the primary factor in the inhibition of *C. perfringens* during the extended cooling of meats. Additional studies should be completed to determine the effect of cure accelerators and nitrite from a natural source during extended cooling.

P2-130 Inhibition of Spoilage Yeasts by Spice Essential Oils and Their Components

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Developing Scientist Competitor

Introduction: While the antimicrobial activity of spice essential oils (EOs) and their components against pathogens has been studied extensively, less is known about the compounds against spoilage fungi. Spoilage yeasts are a particular problem in products such as salad dressings, leading to decreased product shelf life that is costly to manufacturers and consumers. EOs are attractive for use in these type of products because of their potential for sensory compatibility.

Purpose: The objective of this study was to determine whether thyme, cinnamon, and clove oils or cinnamic acid, cinnamaldehyde, carvacrol, eugenol, thymol or allyl-isothiocyanate were effective against the spoilage yeasts *Torulaspora delbrueckii* (TD), *Schizosaccharomyces pombe* (SP), *Candida krusei* (CK) or *Zygosaccharomyces bailii* (ZB).

Methods : Yeasts were isolated from spoiled commercial products. An agar dilution assay in yeast extract glucose agar was conducted to determine minimum inhibitory concentrations (MICs) of EOs and EO components. Yeasts were spot inoculated and plates incubated at 32°C for 7 days. MIC was the lowest concentration with no growth.

Results : Susceptibility of yeasts to cinnamaldehyde, cinnamon bark oil, thymol, thyme oil and carvacrol was similar with MICs of 50, 50, 200, 400 and 200 µg/ml, respectively, for all species. Clove bud oil and eugenol had an MIC of 300 µg/ml against TD and CK while the MIC for SP and ZB was 200 µg/ml. Cinnamic acid inhibited CK and TD at 500 µg/ml, SP at 400 µg/ml and ZB at 200 µg/ml. Allyl-isothiocyanate was inhibitory only at > 500 µg/ml for all organisms.

Significance: The first step in evaluating antimicrobials is to determine their effectiveness against the target microorganism. All yeast species tested were susceptible to most EOs and components at low concentrations. While application in foods will require much higher concentrations for complete control, the potential sensory compatibility of EOs for products such as salad dressings, makes them good candidates for application studies.

P2-131 Antimicrobial and Antioxidant Capacity of Peumo (*Cryptocarya alba*) and Arrayan (*Luma apiculata*) Leaves and Fruits

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Introduction: Native Chilean berries, such as Peumo (*Cryptocarva alba*) and Arrayan (*Luma apiculata*), are commonly used in the traditional medicine and local cuisine in the south part of Chile. Hot infusion beverages are made with the leaves, while the fruits are used to season meals due to their characteristic flavor and color.

Purpose: In this study, we characterized the antimicrobial and antioxidant activities of Peumo and Arrayan leaves and fruits.

Methods: The disk diffusion and a modified micro dilution methods were used to determine the antimicrobial activity, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against different strains of *Salmonella, Escherichia coli, Listeria,* and *Staphylococcus aureus* bacteria. The antioxidant activity was characterized by the determination of total polyphenol content (TPC) and the samples antioxidant capacity (AC). Methanol extracts were analyzed at two ripening stages (unripe and ripe), and the antioxidant activity was compared to commercial blueberries.

Results: Overall, fruit extracts were effective in inhibiting both gram positive and gram negative foodborne bacteria. While leave extracts were only efficient in inhibiting one *Staphylococcus aureus* strain. Arrayan extracts showed a higher antimicrobial activity as compared to Peumo extracts ($P < 0.05$). Fruit extracts MIC ranged between 143 and 250 mg/ml for Arrayan and Peumo extracts, respectively. While MIC for leaves extracts were 153 and 150 mg/ml for Arrayan and Peumo, respectively. Antioxidant activity for both native species were greater than commercial blueberries ($P < 0.05$). The TPC and AC were higher for ripe than unripe fruits, with ripe Arrayan fruit having the highest AC (83.6 g AG/100 g and 19.2 µM, TPC and DPPH, respectively).

Significance: Our results suggest a potential application for the use of these traditional berries as natural antimicrobial agents to control foodborne pathogens in foods, while the high antioxidant content adds the value of the use of the fruits as alternative food ingredients.

P2-132 Antibacterial and Antioxidant Activities of Methanolic Extracts of Five Varieties of Nopal Cactus

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Developing Scientist Competitor

Introduction: Nopal cactus has a wide geographic distribution and great diversity of habitats. Cladodes from nopal are currently used as food and feed, value-added products, and have medicinal and therapeutic uses. The scientific information about the antimicrobial and antioxidant activities of nopal is very limited.

Purpose: To evaluate the antimicrobial activity of methanolic extracts of varieties of nopal cactus and correlate this activity with the content of antioxidant compounds.

Methods: Five cultivars of nopal (four of *Opuntia* and one of *Nopalea*) were used in this study. Plant material was washed and dried and macerated in methanol. Preliminary antimicrobial assays were performed using the agar well diffusion technique, using LB agar, PCA agar and MH agar supplemented with 5% sheep blood for *Vibrio cholerae, Campylobacter perfringens* and *C. jejuni/coli,* respectively. Minimum bactericidal concentrations (MBCs) of nopal varieties were evaluated by a microdilution method. Assays for bioactive compounds and antioxidant activity included total phenols, total flavonoids and DPPH radical scavenging activity (%RSA).

Results: All extracts showed antimicrobial and antioxidant activities. The MBC ranged from 0.5-4.0 mg/ml, 0.4-2.5 mg/ml and 0.5-7.0 mg/ml for *C. perfringens, C. jejuni/coli* and *V. cholera,* respectively. High content of total phenols and flavonoids was found in *Opuntia ficus-indica* var. *Villanueva,* (193.02 mg GAE/g, and 139.7 mg CAE/g, respectively), while the extracts of var. *Copena* showed the lowest phenols and flavonoids content (43.53mg GAE/g and 29.31 mg CAE/g, respectively). DPPH radical scavenging activity was related with content of bioactive compounds. The *Villanueva* variety has the highest % RSA at 94.7, while the lowest activity was recorded for *Copena* at 42.7%.

Significance : Our results demonstrate the antimicrobial activity of all varieties of nopal that parallel the antioxidant activity of the extracts. These characteristics can be exploited in this abundant vegetable for its use in the food industry.

P2-133 Antifungal Effect of Starch Edible Films Containing Mexican Oregano (*Lippia berlandieri* Schauer) Essential Oil and Different pH Values

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Introduction: In recent years *Rhizopus* and *Aspergillus* became the main genres that cause postharvest decay, major economic losses, and food spoilage in various horticultural products. Recently fungal resistance is developed to chemical products and the use of higher concentrations, increases the risk of high levels of toxic residues. An alternative to this is the use of plant extracts like essential oils (EOs) is believed to be safer to consumers and the environment. In this sense, edible films can incorporate these extracts to provide microbiological stability and reduce the risk of microbial growth on food surfaces.

Purpose: The aim of this study was to evaluate the antifungal effect on *Rhizopus* and *Aspergillus* spp. of starch edible films containing Mexican oregano (*Lippia berlandieri* Schauer) EO and different pH values.

Methods: Starch edible films were formulated with Mexican oregano EO concentrations of 0.00, 0.25, 0.50, 0.75, 1.00, or 2.00%, each concentration at pH values of 5, 6, or 7. Mold radial growth was evaluated inoculating spores in 2 ways: edible films were placed over inoculated agar, Film/Inoculum mode (F/I), or the edible films were first placed in the agar and then films were inoculated, Inoculum/Film mode (I/F). Radial growth curves were described by the Gompertz modified equation.

Results: There was significant difference ($P < 0.05$) in growth parameters between the 2 modes of inoculation. Starch edible films exhibited better antifungal effectiveness at pH 6 (MIC 0.50%) for *Aspergillus,* pH 5 (MIC value of 0.25%) for *Rhizopus*. The modified Gompertz model adequately described growth curves. A significant ($P < 0.05$) change of Gompertz parameters was observed among essential oil concentrations, increasing the lag phase and decreasing radial growth rates as oil concentration increased.

Significance: Starch edible films, with acid pH values, added with Mexican oregano essential oil are effective con control fungal growth of *Rhizopus* and *Aspergillus* spp.

P2-134 Allyl Isothiocyanate Reduces Foodborne Pathogens on the Surface of Whole Cantaloupe

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Introduction: Cantaloupes (*Cucumis melo* L.) can be washed or dipped in 200 ppm chlorine or 5% hydrogen peroxide to reduce the microbial load on the surface; however, neither treatment is completely effective. Allyl isothiocyanate (AITC), a plant derived antimicrobial compound, has been shown to have antimicrobial activity in its liquid and vapor states, and may be successful in reducing foodborne pathogens on the surface of cantaloupes.

Purpose: The purpose of this study was to determine if AITC (in liquid and vapor phases) reduced populations of *Salmonella enterica* serovar Michigan and *Listeria monocytogenes* on the surface of 'Athena' and 'Hales Best Jumbo' cantaloupes.

Methods: Fifty µl of *S.* Michigan or *L. monocytogenes* were inoculated onto whole cantaloupes in 22 mm diameter circles, allowed to dry for 90 min resulting in 7.05 log CFU/circle, and received either AITC liquid treatment (5 µl; applied directly), vapor treatments or control (no treatment). For vapor treatments, liquid (100 or 300 µl) was applied to Whatman No. 1 filter paper attached to the lid of a glass desiccator. All cantaloupes were stored in sealed glass desiccators and after 24 h, the 22 mm diameter sections of the rind were removed, homogenized and plated onto appropriate agar.

Results: No significant differences existed between cantaloupe cultivars. Concentrations of *S.* Michigan and *L. monocytogenes* on the control melons increased to 7.3 and 7.8 log CFU/10 g, respectively. Log reductions from each treatment were not significantly different ($P < 0.05$) for each pathogen. However, the reduction in *S.* Michigan on the 'Athena' cultivar was significantly different for the vapor treatment levels (3.9 log CFU/10 g for 300 µl and 1.9 log CFU/10 g for 100 µl, respectively).

Significance: Liquid and vapor phases of AITC reduce populations of *S.* Michigan and *L. monocytogenes* on the surface of cantaloupe up to 3.9 log CFU/10 g.

P2-135 Comparison of Grape Seed, Pomegranate and Cranberry Extracts against *Salmonella enterica* serovar Typhimurium and *Salmonella* Enteritidis

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Undergraduate Student Award Competitor

Introduction: Combating foodborne bacterial disease is an ever-evolving battle as bacteria such as *Salmonella enterica* develop resistance to medical/antibiotic treatments and successfully adapt to previously thought safe environments. Natural alternative therapies such as bioactives from cranberries, grapes and pomegranates are becoming increasingly popular associated with their nutritional, antioxidative and pharmacological benefits, including antimicrobial properties.

Purpose: This research compared the effects of extracts from cranberry, grape seed and pomegranate against *Salmonella enterica* serovars Typhimurium and Enteritidis.

Methods: Overnight bacterial cultures in Tryptic Soy Broth (TSB) were washed and resuspended in phosphate buffered saline (PBS, pH 7.2), and individually treated with grape seed extract (GSE, 1 or 2 mg/ml), pomegranate polyphenols, (PP, 2 or 4 mg/ml), cranberry proanthocyanidins (CPAC, 0.3 or 0.6 mg/ml) or PBS (untreated-control) at 37°C for 2, 4, 6, or 24 h. After each time point, bacteria were first serially diluted in TSB containing 10% beef extract, followed by serial dilutions in PBS, and plated on selective Xylose-Lysine Tergitol 4 (XLT4) agar. Each experiment was replicated thrice in duplicate. Data were statistically analyzed using Tukey's test and ANOVA ($P < 0.05$).

Results: Both *S. enterica* serovars from initial titers of ~4.67 log CFU/ml were reduced to non-detectable levels with the two tested concentrations of all extracts after 24 h PP at 2 and 4 mg/ml and GSE at 2 mg/ml also showed complete reduction of both serovars after 4 and 6 h, but no significant reduction was observed ($P > 0.05$) after 2 h. CPAC (0.3 or 0. 6 mg/ml) showed complete reduction of both serovars after 24 h only. Overall, at the tested concentrations, GSE and PP were found to be more effective than CPAC against *Salmonella*.

Significance: These tested extracts show potential as therapeutic options against *Salmonella enterica* infections. However, the bioavailable physiological effects must be tested *in vivo* holistically before any health claims can be made.

P2-136 Reduction of *Cronobacter sakazakii* by Blueberry Juice and Blueberry Proanthocyanidins

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Developing Scientist Competitor

Introduction: *Cronobacter sakazakii* is an opportunistic pathogen of neonates and immune-compromised individuals. As alternate therapeutic options, blueberry extracts have increased consumer appeal due to their high antioxidative, nutritional and health value. Blueberry juice and blueberry polyphenols reportedly have antimicrobial properties against foodborne pathogens, without much known currently on their effects against *Cronobacter sakazakii*.

Purpose: The objective of this research was to determine the effects of blueberry juice and blueberry proanthocyanidins against *C. sakazakii* over 24 h at 37°C.

Methods: Overnight cultures of two *C. sakazakii* strains (ATCC 29004 and ATCC 29544) grown in Tryptic Soy Broth (TSB) at 37°C were washed and resuspended in phosphate buffered saline (PBS, pH 7.2). Each washed culture was individually treated with filter-sterilized commercial blueberry juice (BJ, pH 2.8), neutralized blueberry juice (BJ, pH 7), blueberry proanthocyanidins (B-PAC; 5 mg/ml), malic acid (pH 3.0), or PBS and incubated at 37°C for 30 min, 1 h, 3 h, 6 h or 24 h. After each time-point, bacteria were initially serially diluted in TSB containing 10% beef extract, further serially diluted in PBS, surface-spread plated on Tryptic Soy Agar, and enumerated after incubation at 37°C for 24 h. Treatments were replicated thrice in duplicate and data were statistically analyzed.

Results: *C. sakazakii* strains 29004 and 29544 were reduced to undetectable levels after 1 h from 8.25 ± 0.12 log CFU/ml and 8.48 ± 0.03 log CFU/ml, respectively with BJ (pH 2.8) or B-PAC, while malic acid showed ~1.3 log CFU/ml reduction for both strains. Reductions of ~1 and 1.50 log CFU/ml were obtained for strains 29004 and 29544, respectively, after 30 min with BJ or B-PAC. Neutralized BJ did not reduce both strains even after 24 h.

Significance: Nutritionally beneficial BJ and B-PAC show potential use for preventing and/or treating *C. sakazakii* infections. Further studies on their mechanism of action and *in vivo* studies are warranted.

P2-137 *In Vitro* Control of *Enterococcus faecalis* by *Zataria multiflora Boiss, Origanum vulgare L* and *Mentha pulegium* Essential Oils

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Developing Scientist Competitor

Introduction: Essential oils are aromatic, oily liquids that are obtained from different parts of plants and known for their antimicrobial and anti-oxidative properties. These natural materials are replacing synthetic preservatives and antioxidative agents due to an increasing demand for natural food additives. *Enterococcus faecalis* is a commensal microorganism of the human intestinal tract, and is unusual among prokaryotic organisms because of its ability to produce extracellular superoxide. This anion damages colonic epithelial cell DNA and can lead to colorectal cancer. Therefore, a safe, effective and inexpensive method for controlling this bacterium in foods would be useful.

Purpose: This study evaluates the antioxidant properties of the Essential Oils (EOs) from *Zataria multiflora Boiss (ZMB)*, *Origanum vulgare L (OVL)* and *Mentha pulegium (MP)* and their influence on the growth, membrane permeability and cell surface characteristics of *Enterococcus faecalis*.

Poster

Methods: Two methods (disk diffusion and micro-well dilution) were tested for the antimicrobial activity of EOs. The mechanism of the antimicrobial action of EOs against *E. faecalis* was also studied by measurement of the release of cell constituents and observations of cells by electron microscopy. The antioxidant activities of *ZMB*, *OVL* and *MP* at MIC concentrations were evaluated by inhibition of free radicals using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method.

Results: The minimum inhibitory concentrations (MICs) of *ZMB*, *OVL* and *MP* were 2, 8 and 42 mg/ml, respectively. The antioxidant activities of *ZMB*, *OVL* and *MP* at MIC concentrations, as evaluated by inhibition of free radicals using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, was 26.47%, 3.2% and 34.67%, for *ZMB, OVL* and *MP,* respectively. The electron micrographs obtained from the scanning microscopy showed that slight morphological changes can be seen in cells treated with EOs at MIC concentration. These data revealed an increasing release of the 260 nm absorbing material according to the oil concentration.

Significance: Our results confirmed higher antioxidant activity of *ZMB* than two others, suggesting it can be used to control of O_2^- (produced by *E. faecalis*) may in turn minimize chromosomal instability and ultimately lead to reduced risk of colorectal cancer.

P2-138 Efficacy of Pecan Shell Extracted Organic Antimicrobials Inhibiting the Growth of *Listeria* spp. and *Listeria monocytogenes* Strains Adhered on Chicken Skin

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Introduction: Growers and processors of Certified Organic foods are in need of suitable organic antimicrobials. This study addresses utilization of organic antimicrobials against food pathogens that are increasingly exhibiting resistance to conventional antimicrobials and presents the efficacy of the tested natural antimicrobials in organic food system of poultry meat products.

Purpose: The purpose of our current research was to develop and test effective chemical-free natural antimicrobials derived from an all-natural byproduct, organic pecan shells.

Methods: Unroasted and roasted organic pecan shells were subjected to solvent free extraction of antimicrobials that were tested against *Listeria* spp. and *L. monocytogenes* serotypes to determine the minimum inhibitory concentrations (MIC) of antimicrobials. The effectiveness of pecan shell extracts were further tested using a poultry skin model system and the growth inhibition of the *Listeria* cells adhered onto the skin model were quantified.

Results: In addition to inhibiting the native bacteria on the skin, the solvent free extracts of pecan shells inhibited *Listeria* strains at MICs of 0.38%. The antimicrobial effectiveness tests on poultry skin model exhibited nearly a 2-log reduction of the inoculated cocktail mix of *Listeria* strains when extracts of pecan shell powder were used. The extracts produced higher than 4-log reduction of the native spoilage bacteria on the chicken skin.

Significance: The pecan shell extracts may prove to be very effective alternative antimicrobials against food pathogens and supplement the demand for effective natural antimicrobials for use in organic meat processing.

P2-139 Inhibitory Effect of Mexican Oregano (*Lippia berlandieri* Schauer) Essential Oil on *Pseudomonas* spp. Biofilm Formation

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Introduction: Biofilms are defined as adherent, matrix-enclosed bacterial populations. In nature, microorganisms usually attach to solid surfaces, especially at the liquid–solid interface. In general, biofilms are very resistant to some physical treatments, and are considered a potential hazard and a source of bacterial contamination of foods. An alternative of this is the search for natural compounds, like essential oils (EO's), with antimicrobial properties, which could be used in the biofilm formation.

Purpose: The aim of this study was to evaluate the inhibitory effect of Mexican oregano (*Lippia berlandieri* Schauer) EO on *Pseudomonas* spp. biofilm formation.

Methods: *Pseudomonas* spp. minimal inhibitory concentration (MIC) was determined with EO concentrations of 0 to 2000 ppm. Biofilm formation in stainless steel surfaces was evaluated with growth curves using concentrations below the MIC values. Gompertz equation parameters were used to describe biofilm formation.

Results: Mexican oregano EO MIC value was 1600 ppm whereas biofilm formation in stainless steel surface is affected at lower MIC values (800 ppm of EO) and inhibitory film formation at this concentration is immediately. Gompertz model adequately described the biofilm formation growth curves. Significant differences ($P < 0.05$) between the Gompertz parameters was observed among essential oil concentration, increasing the lag phase, decreasing maximum growth and growth rate as EO concentration increased.

Significance: Mexican oregano EO could inhibit the development of *Pseudomonas* spp. biofilm formation on stainless steel surfaces and could be an alternative for cleaning these kinds of surfaces and equipment.

P2-140 Antimicrobial Efficacy of Cinnamaldahyde, Carvacrol and Citral against *Escherichia coli* O157:H7 on Organic Leafy Greens Held at 4°C

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Developing Scientist Competitor

Introduction: Several foodborne outbreaks of *Escherichia coli* O157:H7 have been linked to the consumption of fresh produce, including organic leafy greens. There is a need for alternative natural interventions for organic leafy greens since chemicals are not permitted in organic production. The essential oils of cinnamon, oregano, and lemongrass are known to effectively reduce *E. coli* O157:H7 in many food products. Further studies are therefore needed to determine if their main chemical constituents are responsible for antimicrobial activity.

Purpose: To determine the efficacy of assorted plant-derived compounds against *E. coli* O157:H7, during washing and storage of organically grown leafy greens.

Methods: Baby and adult spinach, and romaine and iceberg lettuce, were inoculated with a cocktail of *E. coli* O157:H7 at 5-log CFU/ml. Inoculated leafy greens were washed in different concentrations of the constituents (0.1, 0.3 and 0.5%, v/v in phosphate buffered saline, PBS) and refrigerated for three days. Hydrogen peroxide, PBS, and water were included as controls. The survivors were enumerated on days 0, 1 and 3 of storage.

Results: Significant reductions ($P < 0.01$) in *E. coli* O157:H7 populations were observed for all three compounds, at all concentrations and in all the leafy greens. The most effective being carvacrol, with a log reduction of 4.1, 4.9 and 4.5 on day 0 for 0.1, 0.3, and 0.5% concentrations, respectively. On day 0, citral had a log reduction of 2.5, 3.4, and 3.9 and cinnamaldahyde had a log reduction of 3.0, 3.4 and 4.4 for 0.1, 0.3, and 0.5% concentrations, respectively. An increasing log reduction pattern after each day was also seen for all concentrations of compounds and in all types of leafy greens.

Significance: Plant-derived compounds, carvacrol, cinnamaldahyde, and citral, are effective natural antimicrobials and can be used as a wash treatment for bagged leafy greens stored at refrigerated temperatures.

P2-141 Determining the Efficacy of Disinfectants against Human Noroviruses among 20 Commercially Available Disinfectants in Korea and Japan

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Introduction: Korea and Japan have seen increased norovirus outbreaks in the winter of 2012 from which multiple human deaths have been reported. The majority of transmission of norovirus occurs through environmental contamination and human-to-human contacts. Therefore, use of disinfectant can be important means to prevent transmission of norovirus. However, currently available disinfectants have been tested against either Murine norovirus and or Feline Calicivirus, both surrogates of human norovirus and therefore proper efficacy of disinfectants against human norovirus is unclear. We tested 20 commercially available disinfectants for their true efficacy against human norovirus.

Purpose: Our aim is to screen most commonly used top 20 commercial disinfectants for their true efficacy against human norovirus and report our results so that consumers are better informed about the products they are using in protection against spread of norovirus.

Methods: Using peptide sequence antigen, we produced monoclonal antibodies against two strains of norovirus, Norwalk virus (GI.1) and GII.4. The resulting two monoclonal antibodies were conjugated to magnetic beads to create immunomagnetic bead (IMS) reagent to capture human norovirus. This IMS reagent was used to capture intact norovirus after the treatment of disinfectant. The IMS method was shown to not only capture norovirus, but also effective in removing free RNA and PCR inhibitors. We tested the efficacy of varying concentrations of ethanol, sodium hypochlorite, quaternary ammonium compounds along with 20 commercially available disinfectants using IMS method followed by RT-qPCR.

Results: The sensitivity of this procedure was approximately 100 virus particles for GI.1 and GII.4 virus, respectively. The average virus log reduction by various disinfectants was all below log 0- log 1.5 at best. However, only one product and sodium hypochlorite showed more than 4 logs of reduction against both GI.1 and GII.4.

Significance: Our results indicate that majority of disinfectants are not effective against human norovirus. Many previous reports also showed that naked envelope viruses such as norovirus are resistant to vast types of disinfectants. Therefore, it is very important to report and inform consumers on true efficacy of various disinfectants against human norovirus, not the surrogates.

P2-142 Morphological Effects of Pulse Ultraviolet Light and Anolyte Treatment on Bacterial Cell Wall

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Introduction: Pulse ultraviolet light (PUV) is effective to inactivate bacterial contaminated surfaces. Anolyte, the product of passing an electric current through a solution of sodium chloride, is currently being used in the food industry as a sanitizer and is equipment and environment friendly. There is little information about the cellular damage when the PUV treatment is combined with an anolyte wash.

Purpose: In this study PUV for 15 and 30 s, an anolyte wash with a 300 ppm residual chlorine for 3 min and a PUV treatment followed by the anolyte wash was used to determine the morphological effect on *Salmonella*, *Listeria monocytogenes* (LM) and *Staphylococcus aureus* (SA) cells suspended in buffer peptone water. Following the PUV treatment, the cells were harvested and fixed for TEM examination.

Methods: After the anolyte wash and PUV treatment/anolyte wash the anolyte was inactivated before harvesting and fixing for TEM examination.

Results: The TEM images showed disruption of the *Salmonella*'s outer cell membrane with some cytoplasm leakage after both the PUV and anolyte wash. Increased cell wall damage and cytoplasm leakage was observed for the PUV/anolyte treated *Salmonella* cells. The LM and SA outer cell membrane showed less damage with no cytoplasm leakage after the PUV or anolyte treatment, but increased cell wall damage did occur after the combined treatment of PUV followed by the anolyte wash.

Significance: These results indicate that the gram negative bacteria are more sensitive to the PUV and anolyte treatments than the gram positives. However, the hurdle treatment (PUV followed by anolyte wash) is an effective way to inactivate the bacteria pathogens.

P2-143 Efficacy of Commercial Citrus-based Disinfectants to Inhibit Growth, Swarming, and Biofilm Formation of *Salmonella* and Decontaminate Parsley

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Introduction: Biofilms allow bacteria to adhere to biological or nonbiological surfaces and are difficult to remove, whereas swarming enables the rapid colonization of nutrient-rich environments. Synthetic compounds have been widely used by the produce industry to reduce microbial contamination; however, there is a consumer-driven trend toward fewer synthetic food additives and more natural products.

Purpose: The efficacy of six commercial citric-based antimicrobial formulations to control growth, biofilm production, and swarming of *Salmonella* was determined. Furthermore, the efficacy of Citrik Agro to disinfect contaminated parsley was established.

Methods: Minimum bactericidal concentrations (MBCs) of the disinfectants against five *Salmonell*a strains were evaluated by the microplate-dilution method. For the swarm motility test, subinhibitory concentrations of the disinfectants were mixed with Luria–Bertani agar. Biofilm formation was quantified in microplates with broth after staining with safranin. Parsley was artificially contaminated with *Salmonella*, then washed with the product and the presence of *Salmonella* was determined after several days.

Results: The MBCs of the disinfectants ranged from 81 to 922 µg/ml. The product was the most effective inhibitor of *Salmonella* growth (MBC: 81–105 µg/ml). Most disinfectants inhibited biofilm formation at 75% of the MBC, and a reduction was observed at lower concentrations. However, the product inhibited biofilm formation even at 25% of the MBC, and it also produced a higher ($P < 0.05$) swarming reduction (75%)

when 75% of the MBC was used, compared to the other disinfectants. In addition, the product reduced more than 2 logs of *Salmonella* in parsley. This reduction was higher ($P < 0.05$) than that observed by the chlorine treatment.

Significance: Citric extract-based products could be a natural alternative to reduce the risk of *Salmonella* contamination in fresh produce.

P2-144 Antimicrobial Efficacy of Novel Low-residue Peracetic Acid-based Sterilant for Use in Vapor Aseptic Applications

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Introduction: A new peracetic acid (PAA) based sterilant (patent pending) was developed at FMC Peroxygens for use in vapor aseptic applications. The formulation was tested for antimicrobial efficacy against organisms of concern in the beverage industry, using inoculated containers and a test vapor apparatus. Tests were also completed to assess the development of residue in equipment.

Purpose: In order to assess the antimicrobial efficacy of the low residue PAA formulation, tests were conducted using various concentrations in the vapor apparatus against several species of *Bacillus* spores inoculated into beverage containers. Studies were also conducted to determine residue levels.

Methods: *Bacillus* spp. spores were spot-inoculated into 1 l containers, allowed to dry then treated using the FMC sterilant under various parameters of temperature, concentration and contact time. The sterilant was diluted and flash vaporized into a heated airstream applied to the container, and neutralized immediately afterwards using 50 ml neutralizing broth containing 0.5% sodium thiosulfate. Containers were then sonicated 5 minutes and vortexed 30s, and the fluid diluted and plated using APC Petrifilm.

Results: Complete reduction of the inoculum (approximately 6 log *B. atrophaeus* spores) was achieved using 4000 ppm PAA at 5 s contact, and at 8000 ppm PAA in 2s contact at 90°C. Similarly, complete reduction of the inoculum (approximately 6 log *B. subtilis* spores) was achieved using 4000 ppm PAA at 80°C for 5 s. *B. cereus* required additional concentration and temperature, but complete reduction was achieved in 5 s at 150°C and 20000 ppm PAA.

Residue tests showed little to no solid residue in tests comparing the new sterilant with standard grade 15% PAA and 35% hydrogen peroxide.

Significance: This novel sterilant effectively controlled organisms of concern, with little to no formation of residue in lab tests, compared with standard grade PAA and hydrogen peroxide. Pilot scale studies were also conducted and showed encouraging results.

P2-145 Reductions in *Salmonella* and *Campylobacter* on Poultry Parts and Trim Due to Antimicrobial Treatments

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Introduction: Based on data collected from a nationwide microbiological baseline study, the United States Department of Agriculture Food Safety Inspection Service (USDA FSIS) is expected to develop performance standards for *Salmonella* and *Campylobacter* on poultry parts and trim.

Purpose: The purpose of this study was to evaluate the reductions in *Salmonella* and *Campylobacter* populations on raw poultry parts and trim due to USDA approved antimicrobial treatments.

Methods: Raw, boneless, skinless chicken leg meat was portioned (50 g), and inoculated with *Salmonella* (five-strains; 5.0-5.8 log/g) or tested for *Campylobacter* (contamination from the slaughter process). Samples (n = 10) were immersed in acidified sodium chlorite (ASC; acidifiers sodium acid sulfate or citric acid, 900-1000 ppm, pH 2.7-2.8), peroxyoctanoic/peroxyacetic acid mixture (POA; 90-100 ppm and 180-200 ppm), and peroxyacetic acid (PAA; 90-100 ppm) for 15 s, 30 s, or 60 s. After treatment, samples were neutralized in 50 ml of Dey Engley broth (*Salmonella*) or rinsed with phosphate buffered diluent water (*Campylobacter*). Appropriate serial dilutions were plated onto Salmonella-Shigella agar and Campy-CEFEX agar for *Salmonella* and *Campylobacter* enumeration, respectively. Data were analyzed using the general linear model (counts) or a chi-square test (incidence) and means were separated using Tukey's method ($\alpha = 0.05$).

Results: Antimicrobial selection and exposure time are key influences to achieving *Salmonella* and *Campylobacter* reductions on poultry parts and trim. All antimicrobial applications tests reduced ($P < 0.05$) *Salmonella* populations from inoculated levels of 5.8 ± 0.1 log CFU/g. Specifically for ASC, the fewest *Salmonella* (4.1 ± 0.5 log CFU/g) were recovered from samples treated for 15 s when CA was the acidifier; however, a comparable result (4.2 ± 0.2 log CFU/g) was achieved when SAS was the acidifier and samples were treated for 30 and 60 s. The lowest concentration of POA (90-100 ppm) at shortest immersion time (15 s) tested reduced ($P < 0.05$) *Salmonella* inoculated onto parts to 4.5 ± 0.5 log CFU/g. Increasing the POA concentration to 190-200 ppm, however, did not increase its efficacy. Treatment of uninoculated parts with POA (90-100 ppm) reduced ($P < 0.05$) *Campylobacter* incidence from 80.0% to 20.0% and populations from 0.7 ± 0.8 to 0.1 ± 0.1 log CFU/g.

Significance: These data and application parameters can be used to support and validate antimicrobial treatments for poultry parts and trim in secondary processing prior to packaging or grind process to meet current and future USDA regulatory standards.

P2-146 The In-plant Performance of Acidified Sodium Chlorite as Affected by Application Parameters

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Introduction: Validating application parameters of an antimicrobial intervention is essential for HACCP compliance.

Purpose: The purpose of these experiments was to define the optimal application parameters for acidified sodium chlorite (ASC) applied to beef sub-primals and parts and trim within a processing plant.

Methods: Portioned (100 cm^2) beef sub-primals or parts and trim were inoculated with United States Department of Agriculture Food Safety Inspection Service approved pathogenic surrogate organism (5 *Escherichia coli* strains, 5 log CFU/cm^2). Sub-primal samples were stored at 4°C or -20°C and tempered at 4°C (6 h) before conditioning and treatment. The surface of sub-primal samples was conditioned with water or a peroxyoctanoic and peroxyacetic acid mixture (POAA; 180-210 ppm, 1-2 s) directly before treatment with ASC (800 – 1100 ppm, pH 2.35 – 2.50) acidified with citric acid (CA) or sodium acid sulfate (SAS). ASC was applied in-plant to beef sub-primal samples within a top and bottom spray cabinet at 6.8, 7.9, 11.4, or 18.9 l/min for 18 or 30 s. Beef parts and trim were stored at 4°C and treated through an auger application system at 13.2 l/min for 15 s. Surviving *E. coli* (2 plants, 2 replicates per plant, 3 samples per repetition) were enumerated from samples after treatment. Counts (log CFU/cm^2) were analyzed in the general linear model of Minitab and means were separated (α=0.05) using Fisher's least significant difference.

Results: Fewer *E. coli* ($P < 0.05$; 0.4-0.5 log CFU/cm^2) were recovered from sub-primals stored at 4°C than from those that were frozen and tempered (application surface temperatures of 4.3±1.0°C and -1.0±0.3°C, respectively). In general, conditioning the sub-primal surfaces before ASC treatment with water or POAA treatment and increasing the application volume above 6.8 l/min did not enhance ($P \geq 0.05$) its efficacy. CA may be preferred as the acidulant of ASC as fewer ($P < 0.05$) *E. coli* were recovered from sub-primals (0.5-0.7 log CFU/cm^2) and parts and trim (1 log CFU/cm^2) than when SAS was the acidulant.

Significance: These data may be used for the in-plant validation of ASC and indicate the optimal surface temperature, application volume, and acidulant for ASC when applied on beef sub-primals and parts and trim.

P2-147 Delmopinol Hydrochloride Spray Reduces *Salmonella* on Cantaloupe Surfaces

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Introduction: Melons can be contaminated at centralized packaging facilities during post-harvest operations and are vulnerable to microbial cross contamination due to contaminated water tanks, grading/sorting equipment, transport vehicles, and workers. Appropriate post-harvest washing and sanitizing procedures can help control *Salmonella* and other pathogens on cantaloupe or other melons. Since the surfaces of cantaloupes are highly rough or irregular, bacteria can easily attach to these surfaces and become difficult to remove.

Purpose: Delmopinol Hydrochloride (Delmopinol) is a cationic surfactant that is effective for treating and preventing gingivitis and periodontitis. The application of Delmopinol to cantaloupe may be an alternative post-harvest technique to reduce the frequency and level of *Salmonella* contamination.

Methods: Cantaloupe (Athena and Hale's Best Jumbo cultivars) ring plugs (2.5 cm. dia.) were inoculated with a broth culture of *Salmonella* Michigan (approx. 1.0 x 10^{9}CFU/ml). After 15 min, plugs were sprayed with 10 ml of a Delmopinol solution (0% or 1.0%) and held at 37°C for 1 h and 24 h. Melon plugs were diluted with Butterfield's Phosphate Buffer, shaken and sonicated, and solutions were enumerated on tryptic soy agar. The texture quality and color of additional melon samples were evaluated after Delmopinol spray treatments over 14 days storage at 4°C.

Results: A 1.0% (vol/vol) application of Delmopinol reduced *Salmonella* levels 1.6 - 4.4 log CFU/ml in comparison to the field control ($P < 0.01$). No significant differences ($P > 0.05$) were observed in the texture and color of Delmopinol treated melons. Storage of melons treated with 1.0% Delmopinol solution for 1 h had a greater effect on reducing attachment of *Salmonella* compared to 24 h treatment.

Significance: A surface spray application of Delmopinol Hydrochloride can be an alternative antimicrobial post-harvest treatment that will interfere with the mechanism of biofilm adherence, which can make surface bacteria more susceptible to sanitizers or physical removal.

P2-148 Efficacy of Delmopinol against *Campylobacter jejuni* on Chicken, Stainless Steel and High-density Polyethylene

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Introduction: *Campylobacter* spp. are the second leading bacterial cause of confirmed human foodborne illness in the U.S. *Campylobacter* sources include raw poultry, raw milk, drinking water and foods or food contact surfaces that were cross-contaminated. New antimicrobial chemicals that prevent bacterial attachment may be effective for reducing *Campylobacter* contamination of food and food contact surfaces.

Purpose: Delmopinol Hydrochloride (Delmopinol) is a cationic surfactant that is effective for treating and preventing gingivitis and periodontitis. This study evaluated the effectiveness of Delmopinol for reducing attachment of *Campylobacter jejuni* to chicken, stainless steel and High-Density Polyethylene (HDPE).

Methods: Chicken breast meat pieces, stainless steel coupons and HDPE coupons were spot-inoculated with 0.1 ml of a *Campylobacter jejuni* culture. After 10 min, samples were sprayed with 0.5% or 1.0% delmopinol, 0.01% sodium hypochlorite, or distilled water. Spray contact times were 1, 10, or 20 min prior to rinsing with buffered peptone water. Rinses were serially diluted onto Campy Cefex Agar for enumeration. For additional samples, spray solutions were applied first, followed by inoculation with *C. jejuni* after 10 min. Cultures remained undisturbed for 1, 10, or 20 min. Then samples were rinsed and plated as above.

Results: When *C. jejuni* was inoculated before spray treatments, the 1% delmopinol application led to mean log reductions of 1.26, 3.70, and 3.72 log CFU/mL, greater than distilled water, for chicken, steel and HDPE, respectively. When *C. jejuni* was inoculated after spray treatments, 1% delmopinol reduced *C. jejuni* by 2.72, 3.20, and 3.99 mean log CFU/mL more than distilled water for chicken, steel and HDPE, respectively. Application of 1% delmopinol, either before or after bacteria inoculation, resulted in a significantly ($P < 0.05$) greater log reduction than 0.01% sodium hypochlorite or distilled water.

Significance: A surface spray application of Delmopinol Hydrochloride can be an alternative antimicrobial treatment for poultry and pertinent food contact surfaces.

P2-149 Cetylpyridinium Chloride (CPC) Spray Treatments Reduce *Salmonella* on Cantaloupe Surfaces

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Introduction: Melons can be contaminated at centralized packaging facilities during post-harvest operations and are susceptible to microbial cross contamination due to contaminated water tanks, grading/sorting equipment, transport vehicles, and workers. Appropriate post-harvest washing and sanitizing procedures can help control *Salmonella* and other pathogens on cantaloupe or other melons. Since the surfaces of cantaloupes are highly rough or irregular, bacteria can easily attach to these surfaces and become difficult to remove.

Purpose: Cetylpyridinium chloride (CPC) is the active ingredient of some antiseptic oral mouthrinses, and has a broad antimicrobial spectrum with a rapid bactericidal effect on Gram-positive pathogens. The spray application of CPC solutions to cantaloupe may reduce the level of *Salmonella* surface contamination.

Methods: Cantaloupe (Athena and Hale's Best Jumbo cultivars) ring plugs (2.5 cm. dia.) were inoculated with a broth culture of *Salmonella* Michigan (approx. 1.0 x 10^9 CFU/ml). After 15 min, plugs were sprayed with 10 ml of a CPC solution (0, 0.2, 0.5 or 1.0%) and held at 37°C for 1 h and 24 h. Melon plugs were diluted with Butterfield's Phosphate Buffer, shaken and sonicated, and solutions were enumerated on tryptic soy agar. Texture, quality and color of additional melon samples were evaluated after CPC spray treatments over 14 days storage at 4°C.

Results: A 1.0% (vol/vol) application of CPC reduced *Salmonella* levels 4.2 - 6.3 log CFU/ml in comparison to the control ($P < 0.01$). No significant differences ($P > 0.05$) were observed in the texture and color of CPC treated melons. *Salmonella* levels on melons, treated with 1.0% CPC,

were lower after 1 h storage as compared to 24 h. And the Hale's Best Jumbo cultivar was more susceptible to CPC treatments than the Athena cultivar.

Significance: A surface spray application of cetylpyridinium chloride (CPC) can be an alternative antimicrobial post-harvest treatment to reduce pathogen contamination of cantaloupe melons.

P2-150 Effectiveness of Chemical Intervention Treatments against *Escherichia coli* O157:H7, Non-O157 STEC, *Listeria monocytogenes*, and *Salmonella*

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Introduction: A recent increase in consumption of fruits and vegetables has been associated with an increased number of foodborne illness outbreaks linked to these products. As a result, safe and effective sanitizing interventions must be identified for the treatment of produce to destroy foodborne pathogens within the industry.

Purpose: In this study, experiments were conducted to determine the effectiveness of six different categories of chemical intervention treatments against foodborne pathogens in broth. Chemical interventions tested included sodium hypochlorite, hydrogen peroxide, liquid chlorine dioxide, peroxyacetic acid/hydrogen peroxide combinations, organic acid combinations, and quaternary ammonium.

Methods: The chemical products were tested against: *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella*, and a cocktail of non-O157 STEC (O26, O45, O103, O111, O121, and O145) in broth. Treatments were applied to suspensions of each pathogen at various concentrations, and log reductions were determined.

Results: There were significant differences observed between treatments ($P<0.05$). The most effective categories of sanitizers were hydrogen peroxide, liquid chlorine dioxide and peroxyacetic acid/hydrogen peroxide with 8-9 log CFU/ml reductions for *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella*, and non-O157 STEC. The sodium hypochlorite and quaternary ammonium products resulted in 4-6 log CFU/ml reductions depending on the foodborne pathogen. The organic acid combination product was least effective with reductions of 3-5 log CFU/ml depending on the foodborne pathogen.

Significance: Based on study results, we determine that hydrogen peroxide, liquid chlorine dioxide and peroxyacetic acid/hydrogen peroxide products were most effective against the foodborne pathogens of concern within broth. The information from this study will be utilized by researchers to conduct further analysis on the effect of chemical treatments against foodborne pathogens on whole fruit. This study also may be of interest to public health personnel and the fruits and vegetable industry, for the control of these pathogens in the food supply.

P2-151 Efficacy of Sanitizers in Reducing *Salmonella* on Pecan Nutmeats During Cracking and Shelling

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Introduction : Outbreaks of salmonellosis and *Escherichia coli* infections associated with the consumption of contaminated tree nuts continue to be a public health concern. Pecans and other tree nuts are exposed to pre- and post-harvest environments that can result in contamination, yet highly effective sanitizer treatments have not been described.

Purpose : The purpose of this study was to evaluate chlorine and selected organic acids for their efficacy in killing *Salmonella* on pecan nutmeats. The effect of applying intermittent vacuum and atmospheric pressure to inoculated nutmeats during exposure to sanitizers was studied.

Methods : Pecan pieces and halves were immersion- or surface-inoculated with a five-serotype mixture of *Salmonella*, dried, and stored at 4°C for 3 to 6 weeks. Nutmeats were treated with chlorine (200, 400, 1,000 µg/ml), lactic acid (0.5, 1, 2%), levulinic acid (0.5, 1, 2%), sodium dodecyl sulfate (SDS, 0.05%), lactic acid plus SDS, levulinic acid plus SDS, and a mixed peroxyacid sanitizer (Tsunami 200; 40, 80 µg/ml) for up to 20 min. Intermittent vacuum and atmospheric pressure was applied in selected tests. The number of *Salmonella* surviving treatment of nutmeats was determined.

Results : The addition of SDS to lactic and levulinic acid solutions resulted in generally higher reductions of *Salmonella* compared to reductions achieved with other sanitizers. However, regardless of type or concentration of sanitizer, treatment for up to 20 min failed to reduce the pathogen on immersion-inoculated nutmeats by more than 1.1 log CFU/g; maximum reductions on surface-inoculated pieces and halves were 0.7 - 2.6 log CFU/g and 1.2 - 3.0 log CFU/g, respectively. Intermittent vacuum and atmospheric pressure treatments did not enhance lethality.

Significance : Prevention of contamination of pecans during cracking and shelling is critical. Once nutmeats are contaminated, the effectiveness of sanitizers at concentrations tested in this study is minimal.

P2-152 Presence of Disinfectant Resistance Genes in *Escherichia coli* Isolated from NARMS Retail Meats

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Introduction: Retail meats are commonly contaminated with *Escherichia coli* and probably also an important reservoir of antimicrobial-resistant *E. coli*. The frequent use and misuse of quaternary ammonium compounds (QACs) in food processing industries may have imposed a selective pressure and contributed to the emergence of resistant microbes. However, little information is available regarding the QAC resistance genes in *E. coli*isolates from retail meats.

Purpose: The objective of this study was to determine the prevalence of QAC resistance genes, the correlation of their presence and resistance to QAC and other antimicrobials in *E. coli*isolated from retail meats in the U.S.

Methods: Five hundred seventy *E. coli* isolated from National Antimicrobial Resistance Monitoring System (NARMS) retail meat program in 2006 were included in this study. The presence of 10 QAC resistance genes (*qacE, qacEΔ1, qacF, qacG, emrE, sugE(c), sugE(p), mdfA* and *ydgE/F*) were determined by PCR. Minimum inhibitory concentrations of QACs were examined using agar dilution method.

Results: Chromosomally encoded *emrE, sugE(c), mdfA* and *ydgE/F* were widely distributed in *E. coli*, with a prevalence ranging from 77.2% (n = 440) to 100% (n = 570). However, *qacE* was not detected in any of the isolates. The prevalence of the other genes ranged from 22.3% (*qacEΔ1*, n = 127) to 0.4% (*qacG*, n = 2). *sugE(p)* was detected in 6.8% (n = 39) of the isolates. A significant correlation was found between the presence of *sugE(p)*, *qacEΔ1* and antibiotic-resistance isolates ($P < 0.01$). Also *qacEΔ1* was significantly more common ($P < 0.05$) in *E. coli* isolated from poultry meats than from beef and pork. MICs showed that all isolates showed reduced susceptibility to QACs compared with the control strain.

Significance: *E. coli* could serve an important reservoir for the dissemination of QAC resistance. The use of QACs in food processing environments may have played a role in the emergence of QAC-resistant bacteria.

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P2-153 Efficacy of Antimicrobial Solutions on Ground Poultry Skin-on Poultry Products

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Introduction: Recent outbreaks of *Salmonella* illness due to consumption of ground poultry products have resulted in several recalls and stricter regulations concerning pathogens in these products.

Purpose: Determine the efficacy of three antimicrobials on poultry parts when applied to whole parts and ground.

Methods: Fresh skin-on boneless poultry parts were sampled. Control samples were untreated; treated samples were treated with the following antimicrobials: cetylpyridinium chloride (CPC, trade name Cecure®) - 0.6%; hydrochloric and citric acid (ACID, trade name Citrilow™) - pH 1.2; peracetic acid (PAA, trade name Peragonn™) - 200 ppm.

For each treatment set, samples were tested as whole or ground product. Samples were tested for Aerobic Plate Count (APC) and *Enterobacteriaceae* (ENT) bacteria (3M Petrifilm™). Data was converted to log CFU per ml for whole parts and per g for ground product.

Results: Whole control samples had an average APC log count of 4.71. The average APC counts for CPC, ACID, and PAA were 2.62, 2.74, and 3.77, respectively; CPC showed the greatest reduction of 2.09 logs. The average ENT count for the whole control samples was 3.50. The average ENT counts for CPC, ACID and PAA were 1.31, 1.56 and 3.05, respectively; CPC showed the greatest reduction of 2.19 logs.

Ground control samples averaged 4.71 logs of APC. The CPC, ACID and PAA results were 2.77, 2.82, and 3.82, respectively; CPC showed the greatest reduction of 1.94 logs. The control ENT log count was 3.40. CPC, ACID and PAA log results were 2.15, 2.69 and 3.42, respectively; CPC showed the greatest reduction at 1.25 logs. All results (except PAA APC result) showed reductions.

Significance: The use of CPC or ACID antimicrobial on poultry parts for ground products can significantly reduce the APC and *Enterobacteriaceae* counts on the finished products, providing a safer product for the consumer.

P2-154 The Mechanism of Antimicrobial Action of Oleanolic Acid on *Listeria monocytogenes*, *Enterococcus faecalis* and *Enterococcus faecium*

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Introduction: Recently, the use of oleanolic acid has been suggested to control foodborne pathogens, but the mode of oleanolic acid on the pathogens has not been studied.

Purpose: Therefore, the objective of this study was to elucidate the mechanism of antimicrobial action of oleanolic acid on *Listeria monocytogenes*, *Enterococcus faecalis*, and *Enterococcus faecium.*

Methods: The inocula of *L. monocytogenes* ATCC15313, *E. faecalis* ATCC35038, and *E. faecium* ATCC19434 were inoculated in 96-well microtiter plates containing oleanolic acid at 0 to 512 μg/ml. The plates were then incubated at 37°C for 24 h to determine minimal inhibition concentrations (MIC). To determine cell viability of the pathogens, bacterial cell suspension was subjected to 2×MIC of oleanolic acid, and viable cell numbers were enumerated on brain heart infusion agar plates without oleanolic acid. The action of oleanolic acid on cell membrane permeability of the pathogens was evaluatd by propidium iodide uptake assay using FACS analysis and measuring leakage of 280 nm absorbing material.

Results: The MICs were 8-16 μg/ml, 32 μg/ml, and 32 μg/ml for *L. monocytogenes*, *E. faecalis*, and *E. faecium*, respectively. Cell viabilities of the pathogens decreased by 3-4 log CFU/ml after oleanolic acid at 2×MIC was added. In addition, microbial membrane permeabilities of three pathogens increased as oleanolic acid concentration added at 2×MIC.

Significance: This result indicates that oleanolic acid should be useful in destroying foodborne pathogens by disrupting microbial membrane of foodborne pathogens.

P2-155 Antimicrobial Effects of Lauric Arginate against *Campylobacter jejuni* and Spoilage Organisms on Chicken Breast Fillets

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Introduction: *Campylobacter jejuni* is one of the major causes of the foodborne illnesses in the United States. Poultry is considered as the major reservoir of this pathogen. Lauric arginate (LAE) is a USDA approved food antimicrobial and found effective against foodborne pathogens.

Purpose: The objectives of this study were to: a) determine the antimicrobial efficacy of LAE against *Campylobacter jejuni in vitro* and b) study the effect of LAE treatments on growth of spoilage microorganisms on chicken breast fillets.

Methods: *Campylobacter jejuni* (ATCC 33291; ~6 log CFU/ml) was treated with 0 (control), 50, 100 and 200 ppm of LAE solutions for 2h at 4°C in 0.1% peptone water. In second experiment, uninoculated skinless chicken breast fillets were treated with 0, 200 and 400 ppm of LAE packaged and stored at 4°C for analysis after 0, 3, 9, and 14 days for growth of mesophilic and psychrotrophic organisms. All samples were analyzed in duplicate and both experiments were replicated two times.

Results: LAE was highly effective against *C. jejuni in vitro* with no detectable survivors after exposure to 50, 100, and 200 ppm. All LAE treatments caused more than 5 log reduction of *C. jejuni* as compared to control. Treating chicken breast fillets with 400 ppm of LAE caused 2.3-log reduction of psychrotrophs ($P < 0.05$) as compared to control on day 0 of storage. However, no difference existed ($P > 0.05$) in the growth of mesophiles and psychrotrophs on chicken breast fillets after treatment with 200 and 400 ppm LAE as compared to control after 3 days.

Significance: The results of study indicated that LAE is effective in inactivating *C. jejuni* but has no effect against spoilage microflora at the currently approved level of 200 ppm in poultry products. Higher concentrations of LAE may be needed to increase the shelf life of poultry products.

P2-156 Effectiveness of Potassium Sorbate on Controlling the Growth of *Listeria monocytogenes* in Meat Salad

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Introduction: Delicatessen salads are ready-to-eat products that do not require heating prior to consumption. These products may become a health hazard to consumers if they are contaminated with *Listeria monocytogenes* and stored for an extended period of time. Since a thermal kill step is not a suitable remedy for eliminating *L. monocytogenes* in mayonnaise-based salads, it is appropriate to use antimicrobials as a control measure.

Purpose: This study examined the effect of potassium sorbate on the growth of *L. monocytogenes* in meat salad.

Methods: Ground ham was inoculated with a four-strain mixture of *L. monocytogenes* and mixed with mayonnaise containing potassium sorbate. The final pHs of salads were 5.4-5.8 and sorbate concentrations were 0-0.2%. Samples were stored at 4°C for up to 4 weeks, and the net increases of *L. monocytogenes* population in salads were determined.

Results: The initial level of *L. monocytogenes* in salads was 2.1 log CFU/*g*. Without sorbate, the net increases of *L. monocytogenes* in salads of pHs 5.4-5.8 were 2.7-6.4 log CFU/*g*. Addition of sorbate significantly reduced the growth of *L. monocytogenes*. The net increases were reduced to 2.4-5.2, 1.0-3.7, and 0.2-2.0 log CFU/g in salads containing 0.1, 0.15, and 0.2% sorbate, respectively. Generally, salads formulated with lower pHs and higher concentrations of sorbate had lower net increases of *L. monocytogenes*. A model was developed to correlate the population increase to salad pH and sorbate concentration. It suggests that the increases of *L. monocytogenes* population in salads would be < 1.0 log CFU/g if pHs are ≤ 5.6 with 0.2% sorbate and ≤ 2.0 log CFU/g if pHs are ≤ 5.4 with ≥ 0.13% sorbate, pH 5.5 with ≥ 0.15% sorbate, and pH 5.6 with ≥ 0.18% sorbate.

Significance: Findings from this study may be used by salad producers to formulate the salad pHs and sorbate levels that can reduce hazard associated with *L. monocytogenes* in mayonnaise-based salads.

P2-157 Sodium Polyphosphate Enhances the Antimicrobial Activities of Whole and Fractionated Peanut Skin Extract against *Zygosaccharomyces bailii* in a Model Juice System

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Introduction: Spoilage of fruit juices by osmotolerant yeasts such as *Zygosaccharomyces bailii* represents a major detriment to product quality and shelf life. Because *Z. bailii* and related spoilage yeasts may be resistant to traditional food preservatives, alternative approaches are needed. Peanut skin extract (PSE) has been demonstrated to inhibit the growth of *Z. bailii* and other yeasts in apple juice medium (AJM), but at unacceptably high levels.

Purpose: The goal of this work was to evaluate the ability of sodium polyphosphate (SPP), a commonly used food additive, to enhance the antifungal efficacy of whole or fractionated PSE against *Z. bailii* in AJM. SPP is added to juices for various reasons, including metal sequestration and color stabilization. It is also known to possess antimicrobial properties. We hypothesized that SPP and PSE might show cooperative antimicrobial effects, allowing effective use of PSE at lower levels.

Methods: *Z. bailii* ATCC 60483 (10^5 CFU/ml) was incubated for up to 120 h in AJM or in AJM to which SPP (0.013% w/v), PSE (2 mg/ml), or combinations thereof were added. Both whole PSE and 9 individual HPLC-separated PSE fractions were tested. Growth suppression or lethality was determined for each treatment using a Bioscreen C automated turbidimeter and by plating onto YM agar.

Results: When combined with SPP, whole PSE and all 9 PSE fractions showed some antifungal activity against *Z. bailii*. Specifically, Fractions A, B, and D inhibited, but did not prevent, growth. The remaining fractions and whole PSE were more active, some fungicidal. Although whole PSE was ineffective alone, growth was completely inhibited when combined with SPP.

Significance: The cooperative antimicrobial action of SPP and PSE enabled complete suppression of *Z. bailii* growth in AJM, a real juice system. This approach represents a value-added natural antimicrobial solution for inhibition of spoilage yeasts in juices at lower levels of PSE.

P2-158 Antibacterial Effect of Silver Nanoparticles on Intestinal Bacteria

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Introduction: The application of engineered nanoparticles (NPs) for food safety is increasingly being explored. Silver is a non-toxic, inorganic antibacterial agent used in the pharmaceutical and medical industries. Silver (Ag) NPs have a significant potential for a wide range of biological applications, including as an antifungal agent, antibacterial agent for antibiotic resistant bacteria and for preventing infections. However, not much is known about the behavior of Ag NPs upon ingestion and whether they inhibit natural gut microflora.

Purpose: The objective of this study was to investigate the effects of Ag NPs on the intestinal bacteria, *Escherichia coli*, *Lactobacillus acidophilus*, and *Bifidobacterium animalis*.

Methods: Ag NPs at concentrations of 0, 100, 200, and 300 mg/ml were used in tube dilution tests. Tryptic soy broth or Lactobacilli MRS broth without NPs and containing NP-free solution were used as controls. Cells were exposed to the different Ag NP concentrations in broth for 12 h, before plating on respective agar media.

Results: At the end of 12 h, numbers of all three bacterial strains treated with 200 and 300 mg/ml of Ag NPs were significantly different from those of the controls. Ag NPs had no significant effect on *E. coli* up to 6 h of exposure, but after a 9-h exposure, the numbers were significantly lower than the controls ($P \leq 0.0001$). Numbers of *L. acidophilus* and *B. animalis* treated with 200 and 300 mg/ml of Ag NPs were also significantly lower than those of the controls ($P \leq 0.05$) at 9 h of exposure.

Significance: This research provides evidence that Ag NPs have effects on *E. coli*, *L. acidophilus* and *B. animalis*. Further research is needed to examine effects of other NPs on intestinal bacteria, and effects of Ag NPs on human epithelial cells.

P2-159 Viability of *Listeria monocytogenes* on Uncured Turkey Breast Commerically-prepared with and without Buffered Vinegar during Extended Storage at 4°C and 10°C

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Introduction: The antimicrobial effectiveness of lactates for controlling *Listeria monocytogenes (Lm)* in ready-to-eat (RTE) meat products is well documented. However, levels of these antilisterial ingredients commonly used by the meat industry are less effective at controlling *Lm* on uncured RTE meats than on cured RTE products.

Purpose: Determine viability of *Lm* on uncured turkey breast containing buffered vinegar (BV) and surface treated with a stabilized solution of sodium chlorite in vinegar (VSC).

Methods: Commercially-produced, uncured, deli-style turkey breast was formulated with or without BV (2.0, 2.5, or 3.0%), sliced (ca. 1.25 cm thick), and subsequently surface inoculated (ca. 4.3 log CFU/slice) with a five-strain cocktail of *Lm*. Next, 1 ml of a 2 or 10% solution of VSC was added to each package before vacuum sealing and then storing at 4°C or 10°C.

Results: Without antimicrobials, *Lm* increased by ca. 6.2 log CFU/slice after 90 and 48 days of storage at 4°C or 10°C, respectively. At 4°C, *Lm* increased by ca. 0.4 to 1.9 log CFU/slice on turkey breast formulated with 2.0 or 2.5% of BV and treated or not with 2% VSC, whereas when

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treated with 10% VSC, *Lm* levels remained relatively unchanged over 90 days. However, when turkey breast was formulated with 3.0% BV and treated or not with VSC, pathogen numbers decreased by ca. 0.7 to 1.3 log CFU/slice. At 10°C, *Lm* increased by ca. 1.5 to 5.6 log CFU/slice after 48 days when formulated with 2.0 to 3.0% of BV and treated or not with 2% VSC. When formulated with 2.0% BV and treated with 10% VSC Lm increased by ca. 3.3 log CFU/slice, whereas when formulated with 2.5 to 3.0% BV and treated with 10% VSC Lm decreased by ca. 0.3 log CFU/slice.

Significance: Inclusion of BV, alone or in combination with VSC, in uncured turkey breast suppressed outgrowth of *Lm* during shelf life.

P2-160 Adaptation to Extremely High Antibiotic Levels ("Hyper"-resistance) Impacts the Stress Tolerance of *Listeria monocytogenes* and *Salmonella* spp

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Introduction: The overuse of antibiotics in animal agriculture may lead to development of multi-drug resistant pathogens and possibly impart cross-tolerance to environmental stresses encountered during food processing.

Purpose: (i) To assess the innate antibiotic resistance of *Listeria monocytogenes* and *Salmonella* spp. isolates from meat and dairy products, feeds or food-contact surfaces, and (ii) to evaluate the thermo-, acid- and alkaline-tolerance of selected strains, following adaptation to the maximum achievable Minimum Inhibitory Concentration (i.e., 36-800-fold higher than the innate MIC) of selected antibiotics.

Methods: Using the disk diffusion method, thirty strains of each pathogen were exposed to ampicillin, amoxicillin/clavulanic acid, cefotaxime, chloramphenicol, ciprofloxacin, gentamicin, erythromycin, rifampicin, streptomycin, tetracycline. Antibiotics which showed the highest variation in the inhibition zone of *L. monocytogenes* (rifampicin, streptomycin, ampicillin) and *Salmonella* (rifampicin, streptomycin) were selected for further investigation. For each organism, three strains of low (20.5-30.5 mm), intermediate (16.5-26.0 mm) and high (0-15 mm) innate resistance to each of the selected antibiotics were exposed to gradually increasing concentrations of the same agent, selecting for "hyper-resistant" (HR) cells. The HR and the corresponding parental strains were exposed in triplicates to: (i) 60°C in capillary tubes, (ii) TSB of pH 3.5 (lactic acid), and (iii) TSB of pH 10.5 (NaOH).

Results: Contrary to *L. monocytogenes*, *Salmonella* isolates from meat products showed higher ($P < 0.05$) innate antibiotic-dependent resistance than other isolates. Rifampicin-HR strains of both pathogens were more sensitive ($P < 0.05$) to all lethal conditions, compared to the parental strains. Ampicillin- or streptomycin-HR *Salmonella* strains showed similar or lower sensitivity to lethal stresses than parental strains. In contrast, streptomycin-HR strains of *L. monocytogenes* showed 2.0 to 4.1 log CFU/g higher ($P < 0.05$) survival than the parental strains, after 45 minutes exposure to pH 3.5, indicating potential utilization of cells efflux pumps for both antibiotics and protons disposal from the cytoplasm.

Significance: Adaptation of *L. monocytogenes* to high concentrations of antibiotics may induce cross-protection to acid but not to heat or alkaline lethal conditions. Such risk is limited for *Salmonella*.

P2-161 The First Detection of Florfenicol Resistant Gene in Shiga-like Toxin Producing *Escherichia coli* Isolated from Pork in Korea

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Introduction: Florfenicol is fluorinated derivative of chloramphenicol and represent highly protein inhibitors of bacterial protein biosynthesis. It is used in veterinary medicine or feed additives for pigs in Korea. The transfer of antibiotic resistance gene among the bacterial strains has become a problem worldwide, so drug resistant bacterial phenotypes should be identified.

Purpose: In this study, antimicrobial resistance and resistance gene were investigated for *Escherichia coli*, especially Shiga-like toxin producing *E. coli (*STEC) isolated from pork in Korea.

Methods: We monitored 301 pork samples in slaughter houses and retail markets, and isolated 50 strains of *E. coli*. Among these isolates, six isolates resulted in STEC. Minimum inhibitory concentration (MIC) on six strains was performed for 14 antibiotics, ampicillin, amoxicillin/clavulanic acid, cefoxitin, ceftiofur, cephalothin, florfenicol, ciprofloxacin, colistin, gentamicin, nalidixic acid, neomycin, streptomycin, tetracycline and trimethoprim/sulphamethoxazole, and three strains showed high MIC to florfenicol and chloramphenicol (64 μg/ml). PCR was conducted to detect the florfenicol resistant gene (*floR*) and the chloramphenicol resistant gene (*cat*).

Results: All of 3 strains contained the *floR*, while none of them had the *cat*. These PCR products were sequenced and aligned to obtain homology with other available genes in reference GenBank. A BLAST search showed that they contained sequences with homology to the *floR* gene of *E. coli* or *Salmonella enterica* serovar Heidelberg.

Significance: This is the first report to detect *floR* gene in STEC isolated from slaughtered pigs in Korea. These results suggest that some STEC isolates in Korea carry florfenicol resistant gene.

P2-162 Drug-resistant and Virulent *Salmonella* Serovars Can Colonize Pre-harvest Poultry Facilities in the Absence of Selective Antimicrobial Pressure

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Introduction: Reduction in human illnesses associated with raw and processed poultry meat necessitates comprehensive control at the production facilities.

Purpose: There is limited data on the genetic characteristics of *Salmonella* isolated from turkeys, particularly in flocks that have not been exposed to antimicrobials.

Methods: *Salmonella* (n = 95) were isolated from ceca, feed, drinkers, leftover feed, litter and insects from a turkey production facility. Isolates were serotyped and fingerprinted by *Xba*I-PFGE. Antimicrobial susceptibility profiles (15 antimicrobials), 34 virulence genes, plasmid profiles and replicon incompatibility grouping (18 replicons) were evaluated.

Results: Six serotypes (Agona, Anatum, Kentucky, Montevideo, Senftenberg and Worthington) and roughs were identified, mostly from ceca and feed. PFGE dendrogram clustered the strains into 6 distinct groups based on their serotype. Isolates (36%) were resistant to 2 to 4 antimicrobials. Anatum and Worthington were mostly resistant to gentamicin (GEN), streptomycin (STR) and sulfaxisole (SUL), while Kentucky and roughs were resistant to tetracycline (TET), sulfaxisole and trimethoprim/sulfamethoxazole (SXT). Fifty-three percent of virulence genes were detected in all isolates. Isolates harbored genes encoding for *Salmonella* Pathogenicity Islands (SPI 1 to 4), including adhesion (*fimH*), cytoplasmic/effector proteins (*rhuM*, *rmbA*, *sopE* and *avrA*), entry/survival into macrophages (*sipB*, *spaN*, *msgA*, *pagC* and *spiA*), host recognition/invasion (*cdtB*, *hilA*, *invA*, *lpfC*, *orgA*, *pefB*, *prgH*, *tolC* and *sopB*) and iron acquisition (*iroN* and *sitA*). Plasmids ranging from ~1 to 95 kb were detected. Isolates with resistance to GEN, STR and SUL were associated with incompatibility group IncI1 plasmids while those resistant to TET and SXT were associated with groups IncT and HI2.

Significance: Nearly one third of the isolates were resistant to multiple antimicrobials even though the flock was untreated with any antimicrobials. With the exception of the *lpfC* gene, there was no association between serotypes and virulence genes. There is a need to evaluate how on-farm practices affect *Salmonella* colonization dynamics and to develop guidelines that will interrupt the "cycles" of *Salmonella* transmission between humans, animals and the production environment.

P2-163 Temperature Affects Macrolide Resistance in *Campylobacter* spp

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Introduction: *Campylobacter* spp. are frequently associated with foods of animal origin (especially poultry) and are among the most common bacterial causes of human gastroenteritis in many industrialized countries. Significant increases in macrolide resistance reported over the past two decades pose a public health concern.

Purpose: The purpose of this study was to compare the susceptibility of *C. jejuni* and *C. coli* to five macrolides and 235 other potentially inhibitory compounds at 37 and 42°C, two commonly used incubation temperatures representing the body temperatures of humans and chickens, respectively.

Methods: *C. jejuni* 11168 and *C. coli* 49941 were subjected to phenotype microarray (PM) analysis using an Omnilog machine (Biolog). Briefly, cells in a defined medium containing a redox dye were inoculated into 96-well plates to test for chemical and antibiotic resistances including antimetabolites, respiratory inhibitors, membrane active agents, and toxic metals, at different concentrations. Cells were grown, harvested, inoculated into PM plates, incubated, and tested under microaerobic conditions (5% O_2, 10% CO_2, 85% N_2). Duplicate series of PM plates were analyzed for each organism at 37 and 42°C. Programming languages perl and R were used to build a data analysis pipeline to statistically analyze and graphically summarize the PM data in a high-throughput fashion.

Results: Our data analysis pipeline rapidly identified significant differences ($P < 0.005$) in susceptibility at 37 versus 42°C for a number of compounds including: sodium dichromate, lincomycin, m-cresol, and plumbagin. Significant effects of temperature on macrolide susceptibility were observed with increased inhibition at the higher incubation temperature.

Significance: The differential response of *C. jejuni* 11168 and *C. coli* 49941 to inhibitory compounds demonstrates the importance of considering temperature when developing selective media and isolation protocols and in reporting antibiotic susceptibilities. Differences in macrolide susceptibility were especially interesting as growth inhibition was significantly decreased at normal human body temperature.

P2-164 Control of *Listeria monocytogenes* by Antimicrobial Edible Films on Ham Slices and Microwave Reheating of Frankfurters

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Introduction: Antimicrobial packaging may be used to control *Listeria monocytogenes* on post-processed contaminated Ready-To-Eat (RTE) meat products.

Purpose: (i) To evaluate the efficacy of edible films (EFs) containing commercial (traditional) alcoholic distillates (ADs) to inhibit growth of *L. monocytogenes* on ham slices and (ii) To evaluate the impact of EFs with ADs followed by microwave reheating on the reduction of pathogen on frankfurters.

Methods: Ham slices (three/package; 10x10 cm) and frankfurters (two/package; 5 cm length x 2 cm diameter) were inoculated with 2.0 to 3.0 log CFU/cm^2 of a 4-strain composite of *L. monocytogenes*. Alginate-Na (1.5% v/v) EFs were immersed in pure ethanol (40% v/v), or traditional ADs (39-41% alcohol), namely 'raki', 'tsipouro', and 'ouzo' for 3 min. Samples without EFs or with EFs but without ADs or ethanol were used as controls. Frankfurters were placed between two EFs, while one EF was placed between two ham slices. Samples were packaged under vacuum and stored at 4 and 10°C (n=4). Frankfurters were placed in containers with water (200 ml) and reheated in microwave (1000W/60 sec; 56±2°C) on days 0, 20 and 40 or 0, 9 and 15 during storage at 4°C or 10°C, respectively.

Results: On ham slices, all EFs with ADs caused maintained levels of *L. monocytogenes* at least 5.0 log CFU/cm^2 lower ($P < 0.05$) than the controls after 80 and 50 days at 4 and 10°C, respectively. On frankfurters treated with EF-ADs, *L. monocytogenes* population remained 4.0-4.5 log CFU/cm^2 lower ($P < 0.05$) than controls after 40 days of storage at 4°C, while at 10°C, limited growth suppression of 0.8-2.0 log CFU/cm^2 was observed after 15 days. Unlike 10°C, survivors of *L. monocytogenes* on EF with ADs frankfurters stored at 4°C and reheated in microwave, increased by 1.5-2.5 log CFU/cm^2 by day 40 compared to the day of packaging (day 0). Notably, the initial thermotolerance of controls remained unchanged (2.5 log CFU/cm^2; $P \geq 0.05$) throughout storage.

Significance: Food safety regulations enabling antimicrobial interventions for the safety of RTE meat products should ensure both low levels of *L. monocytogenes* during storage and minimum tolerance to recommended consumer practices, such as microwave reheating.

Poster

P2-165 The Effect of Various Food Preservatives on Growth of *Listeria monocytogenes* Mutants with Altered Growth Phenotype in High Salt and Refrigeration Temperature

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Introduction: *Listeria monocytogenes,* the causative agent of human listeriosis, grows in refrigeration and high salt environments. Recently characterized deletion mutants have identified genes implicated in growth in these environments. To better understand the mechanisms underlying stress tolerance, we investigated the effect of certain GRAS chemicals on these mutants.

Purpose: To identify if any of the previously characterized genes are linked to growth alterations specific to some commonly used food preservatives that may aid in the explanation of their role in stress survival.

Methods: The strains were grown in BHI broth with and without 7% NaCl. Their growth (absorbance at 600 nm) was compared to parallel cultures containing an added GRAS chemical at 37°C. In some cases, growth was assessed via colony counts to verify the phenotypes.

Results: Growth profiles (lag time, doubling time, and maximum growth) in the presence of diacetate, sorbate, deoxycholic acid and nitrite was assessed. The *gbuABC* mutant showed the greatest growth reduction of all the mutants in the combined NaCl/preservative conditions. In the presence of NaCl and deoxycholic acid, the peak absorbance (maximum growth) for the *gbuABC* mutant was 0.119 at 43 h vs. the parent strain's 0.159 at 33 h. The effect on *murC* (0.139 at 38 h) and *lstC* (0.141 at 29 h) was less pronounced and *ftsK* was unaffected. When comparing the time to reach peak absorbance in the other GRAS chemicals, the *gbuABC* mutant displayed marked delays compared to the parent strain in diacetate (32 h vs. 21 h), sorbate (47 h vs. 28 h) and nitrite (34 h vs. 21 h). These differences were not seen with the other mutants. The effects of these chemicals in absence of added salt stress was not significantly different from the parent strain.

Significance: Analysis of these phenotypes could enhance our understanding of how GRAS preservatives and NaCl affect *Listeria* and its stress survival genes, improving our understanding of *Listeria*'s tolerance to environmental stresses. This could lead to improved technology for controlling *Listeria* in foods.

P2-166 Evaluation of Coated Nisin Containing Films Formulated to Inhibit *Listeria monocytogenes* on Vacuum Packaged RTE-Foods for Commercial Converting Purposes

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Introduction: Antimicrobial food packaging may extend shelf-life, reduce spoilage, maintain food quality and reduce foodborne pathogens in ready-to-eat (RTE) foods. Nisin is a polypeptide that demonstrates natural antimicrobial activity.

Purpose: Objectives of this study are to compare two different coated antimicrobial films containing nisin for eventual application in a commercial setting. Formulations of the coatings were developed for commercial coating application versus batch lab process produced in the past. Specifically, evaluation of the film's efficacy against *Listeria monocytogenes* and evaluation of the coating properties were performed.

Methods: Pectin (C1) and methyl cellulose (C2) were used as the carriers for the antimicrobial nisin in two separate coatings. Coatings contained 10,000 IU/g of nisin A. Coatings were produced same day and coated onto a PET laminate film by draw down method. Films were dried for 24 hrs before evaluation for the following; color (Δ E), coating weight, thickness (mil), percent solids (%), viscosity (Zahn cup #2 and Brookfield), pH and inhibition against *L. monocytogenes* using the film on lawn method. Control films did not contain nisin. Methods were completed in triplicate.

Results: Coatings achieved inhibition against *L. monocytogenes* compared to the control ($P < 0.05$). The pH of C1 averaged 2.5 and 4.38 for C2. Percent solids average was 9.5 (C2) & 4.73% (C1). Average coating thickness was 0.1 mil. Average Δ E values were less than 1.0. Average coating weight was 1.5 (C2) &1.64 lbs/ ream (C1). Zahn Cup time averaged 40-45sec. Viscosity was determined to be in the pump-able range for commercial coatings (Centipoise and torque were measured).

Significance: The coating properties collected allow for novel commercialization of coated antimicrobial films which industry currently is demanding. Both coatings were effective against *L. monocytogenes* and could be produced utilizing current industry equipment. To the authors' knowledge, an antimicrobial coated film developed for commercial production has not been previously performed.

Poster

P3-01 Developing a Universal Enrichment Broth for the Foodborne Bacterial Pathogen *Salmonella*

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Introduction: For successful prevention of foodborne illness, rapid and reliable methods are needed for detection. Depending on the food matrix, varying pre-enrichment broths are used by FDA field labs as outlined in Bacteriological Analytical Manual for the detection of *Salmonella*. In addition to the preparation of multiple pre-enrichment broths being labor intensive and costly, the use of multiple enrichment broths is a major roadblock when trying to develop pathogen detection methods in multiple food matrices.

Purpose: The purpose of this study is to evaluate and modify current enrichment broths to propagate the foodborne bacterial pathogen, *Salmonella* Typhimurium.

Methods: *Salmonella* Typhimurium (4.65 ± 0.89 log CFU/ml) was inoculated into candidate enrichment broths, incubated at 37°C and samples were taken periodically over 30 h. Candidate broths included the currently used Lactose broth (LB), Tryptone Soy Broth (TSB), Universal Pre-Enrichment Broth (UPB), and Modified Buffered Peptone Water (mBPW) as well as *Yersinia pestis* Enrichment Broth (YpE). Two modified broths, mBPW with 0.25% glucose and YpE broth with a buffer system (buffered YpE), were also examined. Growth was characterized by CFU/ml from TSA plates, optical density from absorbance readings at 600nm (OD_{600}), and Ct values from TaqMan qPCR.

Results: After 24 h in LB, *Salmonella* Typhimurium had the lowest growth (7.93 ± 0.17 log CFU/ml, 30.98 ± 1.54 Ct, $P < 0.05$). *Salmonella* Typhimurium growth was greatest after 24 h in YpE broth (OD 1.27 ± 0.02) which was significantly greater ($P < 0.05$) than buffered YpE broth (OD_{600} 1.10 ± 0.01). *Salmonella* Typhimurium in mBPW with 0.25% glucose had significantly greater growth ($P < 0.05$) than in mBPW without glucose with OD_{600} readings of 0.57 ± 0.04 and 0.19 ± 0.10, respectively.

Significance: Growth characteristics of *Salmonella* Typhimurium varied significantly in the different enrichment broths examined. The identification of the vital constituents for *Salmonella* growth in the enrichment broths will ultimately be used for the identification of a universal enrichment broth for the detection of *Salmonella* Typhimurium in multiple food matrices.

P3-02 Comparison of Enrichment Broths for the Recovery of Healthy and Heat-injured *Salmonella* Typhimurium on Duck Wing

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Developing Scientist Competitor

Introduction: Some salmonellosis have been reported by consumption of duck meat, a widely consumed poultry in Asian countries. In general, these pathogens in food matrix are present in low concentration and sub-lethally injured due to the harsh processing conditions, which may cause false negative results if no appropriate enrichment was applied.

Purpose: This study compared available enrichment broths to find out a better broth to recover the healthy and heat-injured *Salmonella* Typhimurium with low concentrations on duck wing.

Methods: Healthy or heat-injured *S.* Typhimurium was inoculated at the level of 10^2, 10^1 or 10^0 CFU/25g on duck wing. Five non-selective enrichment broths, tryptone soy broth (TSB), lactose broth (LB), buffered peptone water (BPW), nutrient broth (NB) and universal pre-enrichment broth (UPB), and four selective enrichment broths, ONE broth-*Salmonella* (OB), selenite broth (SB), *Salmonella* AD media (AD), and MP media (MP) were evaluated in this study. Four growth parameters, LPD (lag phase duration), MGR (maximum growth rate), DT (doubling time), and MPD (maximum population density), from the growth model using DMfit were calculated. Mean values were compared using ANOVA.

Results: Most of enrichment broths recovered *S.* Typhimurium by more than 6.0 log CFU/ml. Among broths, *Salmonella* in AD, MP and OB had significantly ($P < 0.05$) higher MGR (0.9-1.0/h) and lower DT (0.7-0.8 h). BPW, AD, MP and OB recovered healthy and 50%-injured cells at low inoculum levels by more than 6.0 log CFU/ml and OB had the highest level (7.62 and 7.92 log CFU/ml) of *Salmonella* cells after 24-h incubation. However, the 85%-injured cells were only recovered in OB by more than 7.0 log CFU/ml.

Significance: This study suggests that OB may be a suitable enrichment broth for the *Salmonella* detection to avoid false negative results, thereby ensuring the safety of duck meat.

P3-03 Surveillance of Shiga Toxin-producing *Escherichia coli* O157:H7 Sampled from the Fresh Produce of the Local Market

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Introduction: Increasing trend of importation of fresh produce to the US market increases the risk of expose to different types of pathogens and outbreaks of foodborne illnesses. Multiple foodborne illness outbreaks have been linked to the intake of fresh produce contaminated with various strains of *Escherichia coli* O157.

Purpose: The objectives of this study were to isolate *E. coli O157:H7* and to determine the presence of *stx1* and *stx2* genes of *E. coli* O157:H7 from cilantro, green onion, spinach, alfalfa sprouts, and lettuce collected from the local market.

Methods: Fresh produce consisting of cilantro, green onion, spinach, alfalfa sprouts and lettuce were collected. The total numbers of samples collected were 455, and the samples were analyzed for the presence of *E. coli* O157:H7, *stx1* and *stx2* genes. The strains were isolated and identified according to the procedures of the Bacteriological Analytical Manual with some modification to identify the presence of *E. coli* O157:H7. DNAs were extracted using PrepSEQ Rapid Spin Sample Preparation Kit according to the manufacturer's instructions, and then PCR, Gel electrophoresis, Serotyping, and Pulsed-Field Gel Electrophoresis assays were performed to confirm the presence of *E. coli* O157:H7.

Results: Biochemical and serological analysis showed that 33 (7%) isolates were confirmed to be *E. coli* O157:H7. Out of them 36.4%, 33.3%, 18.18%, 6.06%, and 6.06% were isolated from cilantro, green onion, spinach, sprouts, and lettuce samples, respectively. Results also indicated that twenty-three (70%) of the presumptive positive samples carried the virulence genes of *stx1* or *stx2,* ten (30%) of the presumptive samples showed no virulence genes amplification.

Significance: Outcomes of this project showed that *E. coli* O157:H7 can be found on the surface of the produce. Thus, to provide safe fresh produce to the consumer, a novel approach using bioactive compounds as well as a rapid and close monitoring system needs to be implemented.

P3-04 Evaluation of a Rapid Lateral Flow Method for the Detection of *Salmonella* in Food Samples

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Introduction: The bioNexia *Salmonella* assay is a rapid qualitative Lateral flow device for the detection of *Salmonella* in enriched food samples and for confirmation of *Salmonella* from a selective agar.

Purpose: The objective of this study was to evaluate the new assay for both detection and confirmation of *Salmonella*.

Methods: Samples were pre-enriched in buffered peptone water (BPW) for 18-24 hours at 37°C and then enriched in the selective SX2 broth overnight at 41.5°C. For the confirmation protocol, a *Salmonella* presumptive colony was re-suspended into 1 ml of BPW. Samples were boiled for 5 minutes before testing. Positive results visualized as a blue line were obtained in 20 minutes.

Results: The comparative study between the new method and the ISO 6579 reference method was performed on 276 products. Most of the positive samples were artificially inoculated with stressed *Salmonella* at a very low level. One hundred and fourteen samples were confirmed positive by the reference method and 109 by the new method. No statistically significant difference ($X^2 < 3.84$) was observed between both the methods. In the confirmation protocol, the new assay was compared with an other commercial lateral flow assay and with a commercial latex assay on 59 *Salmonella* strains representing 18 different serovars. Fifty-seven were confirmed as *Salmonella* by the bioNexia assay, 50 with the latex assay and only 35 with the other commercial lateral flow assay. The two missed strains were further confirmed after a short enrichment in BPW.

Significance: This study demonstrates that the bioNexia *Salmonella* method can be a valuable alternative for detection of *Salmonella* in food samples. Without requiring skilled personnel and well-equipped laboratory, it can be a simple and rapid method for detection of *Salmonella*. Thanks to the selected antibody cocktail this assay showed significant higher rate of *Salmonella* identification compared with two commercial assays.

P3-05 Evaluation of Lateral Flow Devices for Detection of *Escherichia coli* O157:H7 in Raw Milk and Produce

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Introduction: Detection of *Escherichia coli* O157:H7 in produce and raw milk by standard cultural methods can be difficult due to the high background microflora. Assays such as lateral flow devices can offer rapid screening of enriched samples and are cost effective.

Purpose: This study evaluated the reliability of using lateral flow devices for *E. coli* O157:H7 detection using FDA BAM methods or modified BAM procedures.

Methods: Romaine lettuce, cilantro, raw milk, spinach and strawberries were inoculated at a low level (for fractional recovery) and a higher inoculum level. Twenty replicates at each inoculum level were tested per food matrix, as well as eight uninoculated controls. Rinsates (125 ml) prepared from inoculated Romaine lettuce, cilantro, and spinach were added to 125 ml 2XmBPWp for enrichment. For strawberry samples, 1 pound samples were enriched in 1 l of broth. Raw milk was enriched (25 g) in 225 ml of mBPWp. Each food was enriched for 5 hours at 37°C then acriflavin, cefsulodin and vancomycin (ACV) were added, and the enrichments were incubated for an additional 15 h at 42°C. The enrichments were all screened with RapidChek *E. coli* O157, Neogen Reveal 1.0 for *E. coli* O157:H7, Neogen Reveal 2.0 for *E. coli* O157:H7 and VIP Gold EHEC lateral flow devices, qPCR and cultural detection on selective media.

Results: Lateral flow devices were as efficient as cultural recovery for identifying EHEC in produce rinses and strawberries. However at the low level (0.1 CFU/g), the Neogen 2.0 test device was significantly less effective than cultural recovery for raw milk.

Significance: *E. coli* O157:H7 lateral flow devices accelerated detection of *E. coli* O157:H7 from leafy greens using a rinsate procedure, strawberries with a soak procedure and raw milk with direct enrichment of 25 g samples. However, the Neogen 2.0 device showed reduced sensitivity detecting *E. coli* O157:H7 at low contamination levels in raw milk.

P3-06 Evaluation of Culture Methods for Shiga Toxin-producing *Escherichia coli*

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Introduction: Non-O157 Shiga toxin-producing *Escherichia coli* (STEC) are enzootic in cattle and constitute an emerging zoonotic threat. A current research goal of the USDA-NIFA is the development of diagnostic tests for STEC O26, O45, O103, O104:H4, O111, O121, O145, and O157:H7.

Purpose: The purpose of this study was to evaluate selected currently available culture media to detect STEC O26, O45, O103, O104, O111, O121, O145, and O157.

Methods: Selective broth enrichment and agar plating media were inoculated with 80 strains, 10 each of the targeted serogroups in pure culture and bovine feces. A total of 22 non-STEC strains of varying genera were used as controls. Possé selective enrichment broth and colorimetric selective differential agar were evaluated for detection of STEC O26, O45, O103, O104, O111, O121, O145, and O157:H7. In addition, we tested the utility of several other plating media for detection of STEC from these same serogroups.

Results: Enrichment conditions described by Possé et al. resulted in a trend of suppressed growth. Possé selective differential agar provided a plating medium from which all 8 serogroups could be selected by means of 3 colony phenotypic colors (red-purple, blue-purple and green). Modified Rainbow Agar® O157 resulted in multiple colony colors in the purple-gray family, which did not allow for differentiation of non-O157 STEC from several other types of flora. As expected, CHROMagar™ O157 did not allow for differentiation of non-O157 STEC serogroups. CHROMagar™STEC tended to inhibit growth of the representative strains.

Significance: The enrichment conditions of Possé require further optimization to allow uninhibited growth across these 8 serogroups. The use of Possé differential agar plating allowed for the screening of all 8 serogroups of interest by 3 distinguishable colony colors and was more economical than other media tested.

P3-07 Comparative Evaluation of a New Selective Chromogenic Agar for the Isolation of Non-O157 STEC Serovars in Conjunction with Immunomagnetic Separation and Acid Enrichment

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Introduction: Non-O157 Shiga toxin-producing *Escherichia coli* (STEC) are one of the most important foodborne pathogens in meat products. The selective isolation of non-O157 STEC colonies on agar media is critical for their confirmatory identification. chromID EHEC® is a new selective chromogenic agar for the selective isolation of non-O157 STEC colonies from foods.

Purpose: Comparative evaluation of chromID EHEC agar and modified Rainbow® agar for the selective isolation of non-O157 STEC from artificially inoculated beef samples.

Methods: A panel of 30 strains of O26, O45, O103, O111, O121 and O145 serovars was evaluated for their selective isolation on this new chromogenic media and compared to modified Rainbow agar. Briefly, 375 g samples of fresh raw beef were spiked with 25-100 CFU of different strains and following enrichment either immunomagnetic separation (IMS) or a combination of IMS and acid treatment was performed according to USDA-MLG guidelines. Isolation of non-O157 STEC colonies was performed on both media followed by their confirmation using PCR targeting *stx* and *eae*genes.

Results: Sensitivity of the new chromogenic agar was 100% following the IMS and acid treatment as all tested strains were successfully isolated from the beef samples. Confirmed colonies for the tested strains ranged between 28-100% for the new media and 0-100% for modified Rainbow agar. Some of the strains such as *E. coli*O103:H11 (BAA-2215) were not recovered on modified Rainbow agar. Acid treatment was helpful in enhancing the specificity of the non-O157 STEC isolation procedure for both media.

Significance: Due to high sensitivity, desired specificity and differential colony coloration, the chromID EHEC agar could be implemented for the selective isolation of non-O157 STEC from foods. The new chromogenic media supported the growth of all non-O157 STEC strains studied ensuring their recovery from contaminated food samples.

P3-08 Simultaneous Immunomagnetic Separation (IMS) of Five *Escherichia coli* STEC O-groups with Subsequent Differentiation Using Modified Rainbow Agar

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Introduction: The USDA FSIS regulation establishes 7 STEC strains to be adulterants in non-intact beef products. The FSIS testing protocols for STECs rely mostly on the use of PCR-based methods. Unfortunately, they are not the most practical approach, especially when isolation and enumeration of the microorganisms is required.

Purpose: To 1) determine whether magnetic beads available for five *Escherichia coli* O groups (O157, O26, O145, O111, and O103) can be combined in a single IMS assay, and 2) evaluate if O group differentiation is possible using a chromogenic agar.

Methods: A cocktail of the five O groups was prepared by incubating each strain in tryptic soy broth for ca. 18 h at 37°C, combining the strains in equal aliquots and performing serial dilutions to achieve final culture concentrations of ca. 3 and 4 log CFU/ml. Each concentration of cocktail was subjected to IMS using two levels (20 and 10ml of magnetic beads with beads of the five O groups combined for a single IMS. Recovered cells were plated on modified Rainbow agar and incubated for ca. 24 h at 37°C. Colonies were confirmed using latex agglutination and counted to evaluate the ability to recover all O groups simultaneously on one plate.

Results: Statistical analysis revealed no significant difference ($P > 0.005$) between the two volumes of beads at any of the bacterial concentrations tested. Difference between the cells recovered by each microorganism was found ($P< 0.05$); however, serogroups O157, O26, O145, and O103 were recovered as expected based on IMS detection limits, and O111 did not grow at expected concentrations.

Significance: This study revealed that a simultaneous IMS could be conducted to recover *E. coli* O157, O26, O145, and O103 at once using a selective media that allows for O group differentiation. Because the amount of beads can be reduced and less time is required this could be considered as a cost and time efficient alternative to isolation of STECs one strain at a time.

P3-09 Prediction of *Escherichia coli* O157 Load Using Immunomagnetic Separation and Regression Analysis

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Developing Scientist Competitor

Introduction: Immunomagnetic separation (IMS) is commonly used for detection and isolation of *Escherichia coli* O157. Positives samples that are to be enumerated undergo additional steps that include enrichment and plating on selective media, which can be expensive and time consuming.

Purpose: The purpose of this study was to investigate the use of a regression formula to predict the *E. coli* O157 concentration of a sample following IMS.

Methods: A five-strain cocktail of *E. coli* O157 was freshly prepared by incubating each individual strain in tryptic soy broth for 18 to 24 h at 37°C, combining equal amounts of the grown cultures, and preparing serial dilutions to achieve final culture concentrations of ca. 1, 2, and 3, log CFU/ml. IMS was performed using an automated bead retriever and recovered cells were plated on tryptic soy agar and incubated for 20 to 24 h at 37°C; obtained colonies where confirmed with latex agglutination. A regression analysis was applied to estimate the relationship between the initial bacterial concentration and the colonies obtained after IMS.

Results: A relationship ($P < 0.01$) was detected between initial culture concentration (log CFU/ml as the response variable) and cells recovered after IMS (log CFU/ml from washed IMS beads). In other words, concentration of *E. coli* O157 recovered from IMS beads may be used to predict concentration of *E. coli* O157 in sample (given the simple matrix included in the study described herein). Estimates of the model intercept and slope values were 1.4049 and 0.7170, respectively.

Significance: This method can serve as an alternative to quantification of *E. coli* O157 as a semi-quantitative measurement to predict high shedding animals and can be considered as a cost and time efficient option. Results also can also be used to estimate the capture efficiency of the magnetic beads binding to *E. coli* O157 cells.

P3-10 Evaluation of Immunomagnetic Separation Techniques for the Recovery and Re-growth Potential of *Escherichia coli* O157:H7 in Finished Composts from Manure, Biosolids, and Yardwaste Feedstocks

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Introduction: Mature, finished compost made from various feedstocks should undergo testing for the presence of *Escherichia coli* O157:H7 to ensure thermal destruction of the pathogen during composting. Immunomagnetic separation (IMS) –based techniques may provide assays which can be conducted within 24-48 h to determine presence or absence of *E. coli* O157:H7 in finished compost.

Purpose: 1) To compare the ability of two IMS methods – automated recirculating (AR) and direct plating (DP) - to recover *E. coli* O157:H7 from compost samples containing biosolids, manure, or yardwastes; 2) to determine the ability of compost samples to support regrowth of *E. coli* O157:H7 in compost.

Methods: Twenty-nine USCC-STA certified compost samples were collected from across the U.S. Three aliquots (400 g each) were inoculated with *stx*-2 positive *E. coli* O157:H7 at 10^{1}-10^{2} CFU/g. AR homogenized samples were incubated 5 h at 37°C. DP samples were serially diluted and incubated 2 h at room temperature, then 42°C for 6 h. All samples were incubated overnight at 4°C before addition of immunomagnetic beads. AR sample recirculation (30 min) and DNA purification from immunomagnetic beads, was followed by real-time PCR targeting the *stx*2 gene. For DP, immunomagnetic beads were added to diluted samples, mixed, removed, and then plated onto chromagarO157.

Results: The AR and DP methods both recovered *E. coli* O157:H7 from 30/ 30 (100%) samples. PCR detection of *E. coli* O157:H7 was greatly enhanced by the removal of polyphenols and humic acids from DNA samples. Regrowth of *E. coli* O157:H7 in compost varied based on feedstock.

Significance: IMS recovery, coupled with either direct plating or real-time PCR detection, is an efficient method to recover *E. coli* O157:H7 from finished compost within 24 – 48 h. Regrowth potential of *E. coli* O157:H7 in finished composts is affected by physicochemical parameters of compost.

P3-11 A Complete AOAC-Approved Workflow for the Detection of *Salmonella* spp. in Pooled Food Samples

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Introduction: The Pathatrix Auto™ pathogen isolation platform provides a workflow that is able to process volumes up to 50mL containing as many as ten individual food enrichments in the same sample pool. This new workflow provides food producers with a PCR-based pathogen detection technology without the high costs associated with the traditional one-sample-per-assay-well relationship.

Purpose: AOAC approval was recently obtained for this workflow which allows Food Safety professionals to utilize a sample pooling strategy prior to screening by Real-Time PCR. We demonstrate that this workflow can robustly process a diverse array of food sample types, has high fidelity in correctly detecting the presence of *Salmonella*, and is applicable to other bacterial pathogens of interest found in food.

Methods: The core technology is the automated isolation of pathogenic *Salmonella* serovars from food matrices by antibody-conjugated magnetic beads. The captured bead-bound bacteria are then lysed and the supernatant is added to a lyophilized Life Technologies MicroSEQ® *Salmonella* spp. Real-Time PCR assay previously validated by AOAC and AFNOR. Confirmation of sample calls was determined by selective plating and previously validated Life Technologies Real-Time PCR workflows.

Results: By combining the specificity of antibody-based capture and the sensitivity of Real-Time PCR, our workflow is able to reliably detect 1 CFU of *Salmonella* in 25-375g food product samples. A wide variety of sample types were tested in the course of this validation study. In all sample types tested, this workflow correctly identified all positive and negative samples (100% specificity and sensitivity; N=60 sample types, N≥20 enrichments per sample type to obtain fractional positives).

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. This creates an economic benefit to food producers by providing a workflow that is able to rapidly and inexpensively screen for rare contamination events. This work demonstrates that by using this workflow, one can attain equivalent-or-better results than traditional culture methods, in far less time, for a significantly less cost burden than other PCR-based platforms.

P3-12 Evaluation of Commercial Test Kits for Detection of *Salmonella* in Alfalfa Sprout Spent Irrigation Water

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Introduction: *Salmonella* contamination accounts for most of the reported sprout outbreaks and alfalfa sprouts are the most frequently linked vehicle. Microbiological testing of spent irrigation water has been recommended as part of an overall strategy to reduce sprout-associated outbreaks. An increasing number of pathogen screening kits based on different assay formats are commercially available and many of these kits have been certified by the AOAC. However, very few of them have been validated for detection in sprouts or spent irrigation water.

Purpose: Evaluate the performance of a number of AOAC certified rapid methods in comparison with the FDA BAM method for detection of *Salmonella* in spiked alfalfa sprout spent irrigation water. The methods selected include three lateral flow tests (Reveal, RapidChek, Singlepath), three enzyme immunoassays (Assurance Gold EIA, TECRA VIA, VIDAS UP), and five DNA-based assays (BAX, iQ-Check, BAX, MicroSEQ, GeneDisc, Atlas).

Methods: Six strains of *Salmonella* (Cubana, Mbandanka, Muenchen, Newport, Tennessee and St. Paul) previously associated with sprout outbreaks were used for preparation of the inoculum. Alfalfa sprout spent irrigation water samples were collected from a commercial sprouting facility at approx. 48 ± 12 h of sprouting. Twenty-five ml of spent irrigation water was inoculated with 0, 1, or 10 CFU of the *Salmonell*a cocktail and was subjected to the enrichment and assay protocols recommended by each method. Regardless of the assay results, the presence of *Salmonella* in the enrichments was determined following procedures outlined in the FDA BAM.

Results: All test kits except VIDAS UP were able to detect the presence of *Salmonella* at a level of 10 CFU/25 ml. For samples spiked at 1 CFU/25 ml level, the Assurance Gold and Atlas tests performed the best, probably due to the use of better enrichment media. RapidChek was the most sensitive test among the three lateral flow tests. Real-time PCR based tests required a shorter period of culture enrichment and were easy to perform with the automated systems. False positive results were observed for the BAX test due to sample interference. The performance of VIDAS UP could be improved by adjusting the pre-enrichment temperature from 42°C to 37°C, but false negative results were still observed in samples with the low inoculation level.

Significance: Most of the methods evaluated were able to detect low levels of *Salmonella* in spiked alfalfa sprout spent irrigation water. The availability of additional validated methods will improve the microbial testing programs for sprouts.

P3-13 Comparison of Sampling and Processing Methods for Microbiological Analysis of Fecal Indicators in Large Volumes of Field-Sampled Produce

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Introduction: The most common methods for quantifying indicator organisms on produce are stomaching excised skin or directly swabbing the produce surface. However, these methods have logistical limitations when sampling large sample sizes.

Purpose: To develop and validate a novel method for large-scale sampling of produce and quantification of indicator microorganisms in cantaloupe, melon, and jalapeño pepper.

Methods : Cantaloupe melons and jalapeño peppers were decontaminated using chlorine and ethanol. A cocktail containing 10^6 CFU of *Escherichia coli, Salmonella* (surrogate of fecal coliforms) and *Enterococcus faecalis* was inoculated on the produce surface by spots (melon) or submersion (jalapeño). After allowing the inoculum to dry, microorganisms were quantified using the stomacher, swab or a novel rinse/filtration method. The latter method consisted of rinsing one melon or one jalapeño with peptone water, filtering the rinsate by membrane filtration to concentrate microbes, and incubating the filter over KF and RapidEcoli2 agars for *Enterococcus* and *E .coli*/coliforms, respectively. Six replicates, by three individuals over multiple days were analyzed by the Kruskal-Wallis and Tukey's tests.

Results: The rinse/filtration method was similar or more sensitive in microbial recovery efficiency when compared with the stomacher or swab methods. On inoculated melons, recovery efficiency of *Salmonella* was significantly better ($P < 0.05$) by the rinse/filtration method (3 - 4.5 log CFU versus 2 - 3.5 log CFU for the other methods). For recovery of *E. coli* and *Enterococcus*, the three methods were similar (2.5 - 3.5 log CFU for

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E. coli and 3 – 4.7 log CFU for *Enterococcus*). For jalapeños, the rinse/filtration and swab methods provided better recoveries than did the stomacher method.

Significance: The rinse/filtration method is similar or better in recovery sensitivity than swab or stomacher methods and is logistically easy to use in large-scale sampling of produce.

P3-14 Comparative Evaluation of a New TEMPO® Assay for the Next Day Enumeration of the Total Aerobic Mesophilic Flora in a Variety of Food and Environmental Samples

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Introduction: The enumeration of total aerobic mesophilic flora is used to determine the sanitary quality of food products. Traditional enumeration methods consist of time-consuming and labor-intensive steps; including serial dilution preparation, plating and colony counting. TEMPO® AC assay is a new AOAC-PTM℠ approved automated assay to enumerate total aerobic mesophilic flora within 22-28 h that eliminates these concerns.

Purpose: Comparative evaluation of a new automated assay for the enumeration of total aerobic mesophilic flora in the select food and environmental samples.

Methods: Five replicates of 3 different lots of 15 naturally contaminated foods including meat, poultry, sea foods, fruits and vegetables, dairy, nuts, pet food and environmental samples were processed to enumerate total aerobic mesophilic flora using a new automated assay and aerobic plate count (PCA) reference methods (SMEDP for dairy and AOAC 996.23 for others samples). The logarithmic transformations of final counts were statistically analyzed using t-test ($P < 0.05$) for the lot-wise comparisons and to establish repeatability of the test and reference methods.

Results: Out of total 46 lot-wise comparative evaluations, the overall calculated bias was 0.02 indicating that there was no significant bias between the test and reference methods. The mean log count differences between the alternative method and reference method for all lot-wise comparisons were within the acceptable range of <0.5 log (0-0.43).

Significance: This new AOAC–PTM approved method provides a faster, accurate and simpler approach for the enumeration of total aerobic flora within 22-28 hours without the need of media preparation, serial dilutions, manual counting, and visual interpretation of results. This new assay provides rapid results with the additional advantages of significant economic labor savings, increasing efficiency and better traceability.

P3-15 Rapid Quantitative Enumeration of Yeasts and Molds

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Introduction: Yeast and Molds are ubiquitous food spoilage agents that can grow at a wide range of temperatures, pH values and more importantly at reduced water activities (A_w) thus having the potential for substantial economic losses to the food industry. These organisms have become important indicators for monitoring food quality but the 5-7 day incubation required by traditional enumeration methods poses a burden on food producers. A novel dehydrated film medium was developed to address the need for rapid (48-60 hours) detection of yeasts and molds and the detection technology was optimized to overcome the inherent limitations of yeast and mold appearance and interpretation on dehydrated media.

Purpose: This study was performed to demonstrate comparative enumeration of yeast and molds using a new dehydrated film medium and reference methodology as described in FDA/BAM and ISO21527:2008 (parts 1 and 2).

Methods: The method comparison was conducted using a variety of naturally contaminated food matrices (n = 75) with the new dehydrated film test method and a reference method (FDA/BAM and ISO21527:2008, parts A and B). Each food sample was serially diluted in 0.1% peptone water. The new dehydrated film medium was hydrated with 1ml of the sample and 100 micro-liters were spread plated on DRBC or DG-18 media in replicate.

Results: Results were statistically comparable between the new dehydrated film method at 48-60 h to DRBC and DG-18 at 5 days with no significant differences as indicated by the *P*-values (> 0.05) and regression analysis.

Significance: Rapid quantitative detection of yeasts and molds using the new dehydrated film method was evaluated using a large number (n = 75) of naturally contaminated foods. The new method was found to be reliable and have improved interpretation thus providing end users with actionable results in a shorter time frame.

P3-16 Evaluation of Inoculation and Carcass Rinse Methods on the Recovery of *Salmonella* Enteritidis and *Campylobacter jejuni* from Broiler Carcasses

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Developing Scientist Competitor

Introduction: *Salmonella* and *Campylobacter* continue to be major foodborne pathogens associated with poultry. Current USDA-FSIS protocol uses a 400 ml carcass rinse for microbiological analysis of these organisms on poultry carcasses; however, enhanced sensitivity may be achieved using a lower rinse volume such as 200 ml. In addition, mixed culture inoculations of broiler carcasses with *Salmonella* and *Campylobacter* may also have an impact on recovery of each of these pathogens.

Purpose: This study was designed to evaluate independent and combined bacterial inoculation (*Salmonella* Enteritidis and *Campylobacter jejuni*, either alone or combined), attachment period (0 and 30 min), and carcass rinse volumes (200 and 400 ml) on the recovery of *S.* Enteritidis and *C. jejuni* from inoculated broiler carcasses.

Methods: Commercially processed broiler carcasses were purchased and held at 4°C. *S.* Enteritidis was grown aerobically at 37°C for 18 h in BHI broth and *C. jejuni* was grown microaerobically in *Campylobacter* Enrichment Broth at 42°C for 18 h. Carcasses were inoculated with 10 ml of *S.* Enteritidis or *C. jejuni*, either alone or mixed, and allowed either 0 or 30 min bacterial attachment period. Carcasses were transferred to a sterile rinse bag and rinsed using the USDA-FSIS carcass rinse method with either a 200 or 400 ml Buffered Peptone Water rinse.

Results: Recovery of *S.* Enteritidis and *C. jejuni* were enhanced in mixture in both the 200 and 400 ml carcass rinses, irrespective of bacterial attachment period ($P < 0.05$). Furthermore, there was an increased recovery of both pathogens with the 200 ml rinse compared to the 400 ml rinse ($P < 0.05$).

Significance: A mixture of *S.* Enteritidis and *C. jejuni* can be used for broiler carcass inoculation instead of separate inoculation simulating in-plant contamination scenarios and a 200 ml rinse provides high recovery of both pathogens.

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P3-17 Comparison of Detection Methods for Non-O157 Shiga Toxin-Producing *Escherichia coli* in 375 g Beef Trim

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) strains of serotypes other than O157:H7 have emerged as an increasing concern to public health and the food industry. While O157:H7 remains the primary concern, six other serogroups (O26, O45, O103, O111, O121, and O145, referred to as the Big 6) account for approximately 70% of non-O157 STEC infections in the US from 1983-2002 (CDC). Current testing methods target two virulence factors (*stx* and *eae*) found in the Big 6. Available commercial methods were developed allowing for industry to meet the requirements specified by USDA even though these virulence factors can be present in other *E. coli* serogroups.

Purpose: The objective was to compare commercial detection methods to the reference method (USDA MLG 5B.01) for detection of non-O157 STEC in 375 g beef trim using limit of detection (LOD). In addition, the goal was to evaluate isolation and confirmation protocols used in Europe (EU) compared to strategies provided by USDA MLG.

Methods: Each O group was analyzed separately in a series of inoculated studies. Beef trim intended for 80% lean ground beef production was sourced from a local supplier. Twenty-five (25) g samples were cut, weighed out, inoculated and stored at 4°C to allow adaptation to the product and to cold stress the cells. Three replicate samples at each of 7 dilution levels were inoculated. Each 25 g inoculated sample was combined with 350 g un-inoculated trim and 1.5 l of appropriate pre-warmed enrichment broth and homogenized. Samples were incubated and tested according to the manufacturer's protocol. A Most Probable Number (MPN, 3-tube) was calculated along with 95% confidence intervals.

Results: Overall, the commercial platforms performed well, showing similar levels of sensitivity for detection of presumptive positives for O45, O26, O103 and O121 (screen results only). For O111, one method that utilizes an integrated immunomagnetic separation (IMS) and PCR approach was more sensitive than a PCR-only screen approach. Additionally, one commercial method showed more confirmed positives. There was difficulty detecting O111 with the USDA MLG 5B.01 method, likely due to the high novobiocin concentration in the enrichment media; this is consistent with industry feedback that O111 does not grow to the same levels as the other Top 7 STEC in media containing antibiotic in the same amount of time. The USDA method also was less sensitive for O103 compared to the three commercial methods.

Significance: Use of an IMS tool, such as antibody-coated beads, aided considerably with the confirmation protocol and is an important step when confirming suspect samples. Generally, results from the modified Rainbow plates were better than the EU confirmation plates (MacConkey with or without rhamnose). In summary, detection of non-O157 STEC in 375 g beef trim can be performed by any of the three commercial methods evaluated in this study.

P3-18 Withdrawn

P3-19 Validation of the VIDAS® UP *Listeria* (LPT) for the Detection of *Listeria* spp. in 125-g Samples in Various Food Matrices

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Introduction: The USDA-FSIS *Listeria monocytogenes* sampling plan has been modified to include a sample size of 125 g. Currently, rapid *Listeria* spp. detection methods are validated only for 25 g sample size. The VIDAS® LPT is a phase ligand fluorescent immunoassay to be used on VIDAS® family of instruments to detect *Listeria* in food and environmental samples.

Purpose: To validate the *Listeria* method for the detection of *Listeria* spp. in 125 g test portions of a variety of food matrices.

Methods: Food matrices (125 g), deli-ham, queso fresco, deli turkey and ground beef, were inoculated with either *L. monocytogenes* ATCC 7644, 19114, 51776 or 13932 at low (20 samples; 0.2-2 CFU/test portion), high levels (5 samples, 2-5 CFU/test portion) and 5 uninoculated controls. All test portions were enriched in LPT broth (1:4 dilution) and incubated 24 h at 30 ± 1°C followed by a secondary for 22 h at 30 ± 1°C. Enrichments were analyzed by the *Listeria* assay and confirmed using the USDA-MLG or AOAC reference method, regardless of initial screen result. The Probability of Detection model (AOAC) and chi-squared analysis were used to analyze data.

Results: The method detected 16, 11, 10 and 7 *Listeria* positive samples as compared to 15, 11, 12 and 7 out of the 20 low inoculated samples (0.2-2 CFU/test portion) in deli-ham, queso fresco, deli turkey and ground beef, respectively. POD and chi-squared analysis demonstrated that the method and USDA-MLG and AOAC reference methods were equivalent in detecting *Listeria* spp.

Significance: The VIDAS® UP *Listeria* method was determined to be equivalent to the reference methods for the detection of *Listeria* spp. in 125 g test portions of the food matrices tested.

P3-20 Comparative Evaluation of the VIDAS® UP*Listeria* (LPT) for the Detection of *Listeria* spp. in Various Food Matrices

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Introduction: VIDAS® UP *Listeria* is a phage ligand fluorescent immunoassay for use on the VIDAS® family of instruments for detecting *Listeria* in food and environmental samples, using a single enrichment step in proprietary LPT broth.

Purpose: To validate the method for detecting *Listeria* spp. in a variety of food matrices at 25 gram sample size.

Methods: Smoked salmon, peanut butter, ice cream, mixed salad, cooked shrimp, queso fresco, cantaloupe and ground pepper were inoculated with either *L. monocytogenes*, *L. ivanovii, L. welshmeri, L. innocua, L. seeligeri* or *L. grayi* at low levels (20 samples; 0.2-2 CFU/test portion), high levels (5 samples, 2-5 CFU/test portion) and 5 uninoculated controls. All test portions were enriched in LPT broth (225 ml) with the exception of cantaloupe, which was enriched by adding enough LPT broth to completely submerge the fruit. Enrichments were incubated 26 h at 30±1°C. Enrichments (0.5 ml) were analyzed by the assay and confirmed using the FDA-BAM or AOAC reference method, regardless of initial screen result. The Probability of Detection model (AOAC) and chi-squared analysis were applied to analyze the results.

Results: The method detected 14, 15, 18 , 20, 16, 9, 12, and 8 *Listeria* positive samples compared to 15, 16, 15, 15, 13, 11, 10 and 10 positives out of the 20 low inoculated samples (0.2-2 CFU/test portion) in smoked salmon, peanut butter, ice cream, mixed salad, cooked shrimp, queso fresco, cantaloupe and ground pepper, respectively. POD and chi-squared analysis demonstrated that the method and FDA-BAM and AOAC reference methods were equivalent in detecting *Listeria* spp.

Significance: The VIDAS® UP *Listeria* method with single-step enrichment and next day automated detection was equivalent to the reference method for detecting *Listeria* spp. in 25 g test portions of the food matrices tested.

P3-21 ISO 16140 Extension Study of the a Method for Detection of *Salmonella* in 375 g Raw Beef and Raw Veal Samples

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Introduction: The VIDAS® UP *Salmonella* (SPT) assay is a specific phage protein ligand assay performed in the automated VIDAS instrument in conjunction with a 1-step enrichment procedure. The method has previously been ISO 16140 certified by AFNOR Certification for detection of *Salmonella* in all 25 g food samples.

Purpose: The objective of this study was to extend the application to 375 g sample size of raw beef and raw veal samples .

Methods: Samples (375 g), ¼ diluted in pre-warmed supplemented Buffered Peptone water, were enriched for 22-26 hours at 41.5±1°C. Two protocols were tested, one with the addition of vancomycin and the other with the addition of the proprietary salmonella supplement. After incubation, samples were boiled for 5 ±1 minutes before performing the assay. All presumptive positive samples were further confirmed after streaking on a chromogenic agar plate. This new method was compared with the ISO 6579 reference method, according to the ISO 16140 standard.

Results: In the comparative study, 63 confirmed positive samples were detected by the new method and 66 with the reference method. No statistically significant difference ($X^2 < 3.84$) was observed between both the methods. Using spiked samples with a strain of *Salmonella* Ohio, the 50% level of detection was found between 0.3 and 0.9 CFU/375g for the reference method and between 0.2 and 0.8 CFU/375g for the immunoassay method.

Significance: The results of this study demonstrated equivalence of the method with the traditional reference methods for the detection of *Salmonella* in 375g raw beef and raw veal samples. The ¼ dilution of the matrix into the enrichment broth is an advantage in terms of cost, handling weight for the technician and requirement of space in the incubator.

P3-22 Validation of the LMX Method for the Detection of *Listeria monocytogenes* in 125-g Food Samples

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Introduction: The USDA-FSIS *Listeria monocytogenes* modified sampling plan (2012) tests 125 g samples compared to the previously recommended 25 g. The majority of commercially available rapid pathogen detection methods have been validated using 25 g samples, including the VIDAS® LMX method for the detection of *L. monocytogenes* which was previously validated as a *Performance Tested Method*℠.

Purpose: To validate the LMX method for the detection of *L. monocytogenes* in 125 g test portions of a variety of food matrices.

Methods: Food matrices (125 g), ground beef, deli ham and turkey and Mexican soft cheese – queso fresco), were inoculated with *L. monocytogenes* at low (20 samples; 0.2-2 CFU/test portion), high levels (5 samples, 2-5 CFU/test portion) and 5 uninoculated controls. All test portions were enriched in LPT broth (1:4 dilution) for 24 h at 30 ± 1°C followed by secondary enrichment in LPT broth for 22 h at 30 ± 1°C. Enrichments were analyzed by the LMX assay. All enrichments were culturally confirmed using the USDA (MLG Ch. 8.07) or AOAC (section F, b) reference method, regardless of initial screen result. The Probability of Detection model (AOAC) and chi-squared analysis were applied to analyze the results.

Results: The LMX method detected 7, 11, 16 and 11 *L. monocytogenes* positive samples compared to 7, 11, 15 and 8 by reference method out of the 20 low inoculated samples (0.2-2 CFU/test portion) in ground beef, queso fresco, deli ham and deli turkey, respectively. POD and chi-squared analysis demonstrated that the LMX method and USDA-FSIS and AOAC reference methods were not significantly different in detecting *L. monocytogenes*.

Significance: The LMX method was determined to be equivalent to the reference methods for the detection of *L. monocytogenes* in 125 g test portions of ground beef, deli ham and turkey and Mexican style soft-cheese (queso fresco).

P3-23 Chemiluminescence Competitive Assay for the Detection of Aflatoxin B1 in Corn Using an Aptamer Linked with Hemin/G-quadruplex Horseradish Peroxidase-mimicking DNAzyme

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Introduction: Aptamers are single-stranded oligonucleotides that can strongly and selectively bind to a target molecule and have been regarded as a useful element in the development of biosensor and aptasensor. As far as we know, aptamer assay for AFB1 detection has not been reported.

Purpose: In this study, we developed a chemiluminescence competitive aptamer assay of AFB1 using an aptamer linked with hemin/G-quadruplex horseradish peroxidase-mimicking DNAzyme (HRP-DNAzyme) and validated the aptamer assay with corn samples artificially spiked with known concentration of AFB1.

Methods: Three lengths of oligonucleotides as aptamers linked with HRP-DNAzyme were designed and used for the development of chemiluminescence competitive aptamer assay of AFB1. The optimization of the aptamer assay was carried out by testing key parameters such as selection of coating antigen (AFB1-BSA and AFB1-OVA), kind of blocking reagents (1% BSA and 1% skim milk), and selection of optimum length and concentration of aptamer linked with different numbers of HRP-DNAzyme. The specificity and sensitivity of the aptamer assay was tested. Sample preparation to minimize matrix effect was investigated, and corn samples spiked with AFB1 at 0, 0.5, 1, 5, and 10 ng/g were extracted and analyzed by the aptamer assay.

Results: The AFB1 aptamer linked with the double HRP-DNAzyme that produced sufficient chemiluminescence (CL) values when binding to AFB1-OVA used as a coating antigen was selected. Under conditions optimized by testing key parameters, the aptamer assay exhibited wide dynamic range from 0.1 to 1000 ng/ml and showed the limit of detection of 0.11 ng/ml. Cross reaction to aflatoxin G1 and zearalenone was observed but no cross-reaction to other mycotoxins and herbicide (atrazine) was shown. Aqueous methanol (20%) gave good extraction efficiencies and matrix influence from corn extracts was successfully reduced by 4-fold dilution with water. The recovery, as a practical application, from spiked corn samples averaged from 60.4 to 105.5%.

Significance: The aptamer assay developed in this study is the first application of aptamer for the detection of AFB1. This study provides great opportunities to apply the aptamer toward AFB1 to the development of biosensors and aptamer assays.

P3-24 Recovery of Foodborne Pathogens from Stainless Steel Coupons When Co-inoculated with a Flourescent Compound

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Introduction: GloGerm™and similar fluorescent compounds have been used to visualize potential spread of microorganisms in a variety of settings (hospitals, labs, and food service environments). However, the quantification of a fluorescent compound alone may not correlate to concentrations of pathogens. Inoculation of surfaces with a fluorescent compound pathogen cocktail may be beneficial; the fluorescence would help guide sampling locations for subsequent pathogen quantification.

Purpose: The purpose of this study was to determine if fluorescent compound and foodborne pathogens could be co-inoculated on to stainless steel and result in recoverable populations similar to when a pathogen is inoculated alone.

Methods: *Escherichia coli* O157:H7, *Salmonella* Enteritidis, *Listeria monocytogenes,* and *Listeria innocua* were inoculated (approximately 8 log CFU/ml) on 2" by 2" stainless steel coupons alone and in combination with the compound (2:1, inoculum:compound). Inoculated coupons were allowed to dry for 20 minutes and then sampled using polyester tipped swabs. The swabs were then placed in phosphate buffered saline, serially diluted, and plated on selective media. The experiment was replicated 3 times, sampling 3 coupons each trial, for a total of 9 samples per treatment/bacteria. The recovery rates of the compound/bacteria cocktail were compared to recovery rates of bacteria alone.

Results: 6.86, 7.01, 6.88, and 6.42 log CFU/ml of *L. innocua*, *L. monocytogenes*, *S.* Enteritidis, and *E. coli* O157:H7, respectively, were recovered from the stainless steel coupons when bacteria was inoculated alone. When co-inoculated with the compound 6.75, 7.05, 6.73 and 6.42 log CFU/ml were recovered. There was no significant difference ($P > 0.05$) between bacteria recovered from the control coupons and from the compound/bacteria cocktail coupons for all 4 bacteria.

Significance: Co-inoculating surfaces with fluorescent compound/bacteria cocktails does not affect the recoverability of microorganism which may allow researchers to visually track bacteria through an environment using the fluorescence as a guide where to sample.

P3-25 Cationic Nanocapture for the Concentration of Foodborne Hepatitis A Virus

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Introduction: The low amount of viral particles in contaminated foods makes it challenging to develop methods for their detection. Rapid and easy-to-use methods for separating and concentrating viruses from contaminated foods are needed to help enhance the efficiency of virus detection, improve food safety, and reduce foodborne viral diseases.

Purpose: The purpose of this study was to investigate the recovery efficiency of hepatitis A virus (HAV), as a model of foodborne virus capture, using iron oxide magnetic nanoparticles (20-30 nm; MNP) coated with a cationic food protein, protamine.

Methods: Protamine was covalently coated onto the surface of the MNPs using a three-step chemical reaction. Fourier transform infrared spectroscopy, zeta potential, and transmission electron microscopy were employed to confirm the conjugation of protamine to the MNPs. The feasibility of protamine-coated MNPs for the recovery of HAV from 50 ml of viral suspensions (10^0 10^1, 10^3, or 10^5 PFU, 0.05 M glycine, 0.2% Tween 20) was tested under different pH (4.5, 6, 7.5 and 9), NaCl concentration (0.14, 0.28, 0.56, and 1M), and MNP concentrations (50, 100, 200, and 300 µL). The recovery efficiency was determined by real-time RT-PCR.

Results: The optimal conditions for HAV recovery from 50 ml of viral suspension using cationic MNPs were pH 9, 0.14 M NaCl, and 50 µl of the MNPs. The recovery rate obtained under these conditions was between 24% and 49% in 70 min, depending on the viral inoculation level.

Significance: Protamine-coated cationic MNPs were able to recover HAV in a short time. The recovery efficiency of HAV in food matrices is under investigation.

P3-26 Performance Evaluation of a New Molecular Technology for the Detection of *Escherichia coli* O157 in Food

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Introduction: *Escherichia coli* is a member of the Enterobacteriaceae family, which are some of the most important enteric pathogens. Although most *E. coli* do not cause gastrointestinal illnesses, certain groups can cause life-threatening diarrhea and severe disability.

Many innovative technologies are being used for the microbiological analysis of foods. Unlike traditional methods that rely on specific media to select and grow pathogens, developments in molecular biology have led to innovative methods that are easier and more rapid to perform, requiring only minutes to complete. The 3M™ Molecular Detection System (MDS) combines two technologies – isothermal DNA amplification and real-time bioluminescence detection – in a robust platform that is expected to be less prone to matrix interference.

Purpose: This study was conducted to evaluate the performance of the new system following recommendations from the AOAC guidelines for validation of microbiological methods.

Methods: Samples of foods were spiked with *Escherichia coli* O157 (at 1-10 CFU/25 g) and with *Escherichia coli* O103 and *Enterobacter aerogenes* (10-100 CFU/25 g). Several food categories, including meat, fish and seafood, fruits and vegetable, dairy products, and miscellaneous were evaluated. Each food product was divided into four 25 g samples: (i) negative control, (ii) spiked with *E. coli* O157, (iii) spiked with *E. coli* O103 and *E. aerogenes*, and (iv) spiked with all three organisms. All 120 samples were enriched and incubated according to the manufacturer's recommendation. After incubation, the samples were analyzed in duplicate using the MDS.

Results: No matrix interference was observed for any of the food products tested. From 240 tests conducted, the new technology demonstrated 99% repeatability and 99% accuracy.

Significance: The MDS was found to be not only a suitable, but also a practical, rapid, and sensitive method for the detection of *E. coli* O157 even in the presence of related organisms.

P3-27 Detection of *Cronobacter sakazakii* in Milk Powder Using Loop-mediated Isothermal Amplification

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Introduction: Loop-mediated isothermal amplification (LAMP) is an emerging detection technology for the amplifying DNA under isothermal condition.

Purpose: The aim of this study was to develop a rapid and reliable LAMP technique for the detection of *Cronobacter sakazakii* in milk powder or powdered food.

Methods: Thirty-five *C. sakazakii* strains and 14 pathogenic microorganisms were used to examine the specificity of LAMP on *C. sakazakii*. LAMP products were submitted to restriction enzyme fragment polymorphism (RFLP) to prove the specificity of *C. sakazakii*. The sensitivity of polymerase chain reaction (PCR) and Real-time PCR were compared with that of LAMP in this study.

Results: LAMP for *C. sakazakii ompA* was successfully developed. RFLP pattern of LAMP product with *Hha*I and *Nru*I confirmed the specificity. The LAMP for *C. sakazakii* did not show the cross reactivity with other foodborne pathogens. Based on the sensitivity of *C. sakazakii*, LAMP had 1,000-fold and 100-fold higher sensitivity than PCR and real-time PCR, respectively.

Significance: According to this study, diagnosis time is shortened and early diagnosis is performed by LAMP when food poisoning caused by pathogenic microorganisms like *C. sakazakii* in the future.

P3-28 Evaluation of a Suite of Loop-mediated Isothermal Amplification Assays for the Rapid, Reliable, and Robust Detection of Shiga Toxin-producing *Escherichia coli* in Produce

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) is a leading cause of produce-associated outbreaks in the United States. It is critical to have rapid, reliable, and robust STEC detection methods to better ensure produce safety. A suite of loop-mediated isothermal amplification (LAMP) assays were developed recently for STEC detection. However, they have not been evaluated using an extensive collection of strains or complex produce matrices.

Purpose: The purpose of this study was to further evaluate LAMP performance in comparison with real-time quantitative PCR (qPCR) using a large panel of strains, and to validate the method for the rapid, reliable, and robust detection of STEC in various produce items.

Methods: The specificity of LAMP assay was evaluated with 160 bacterial strains, and the sensitivity was tested with seven STEC strains of adulterant O serogroups in pure culture and spiked produce samples (spinach, lettuce, and sprouts). To simulate real-world contamination events, produce samples were surface-inoculated with low levels (1-10 CFU/25 g) of individual STEC strain, and held at 4°C for 48 h before testing with LAMP and qPCR. Six commonly used DNA extraction procedures were also compared for their effect on assay performance.

Results: All STEC targets and their subtypes were accurately detected. The detection limits of various targets were approximately 2 to 20 cells/reaction in pure culture and 10^5 to 10^6 CFU/25 g in spiked produce samples, except for *stx2c*, *eae*β, *eae*ε*1*, and *eae*γ*2/*θ. In produce samples spiked with low levels of respective STEC strains, LAMP consistently achieved accurate detection after 6 to 8 h of enrichment, with the exception of sprouts. Different DNA extraction methods also yield varied results.

Significance: The research provided a rapid, reliable, and robust method for detecting STEC in produce samples during routine sampling and analysis. The challenge with sprouts detection by both LAMP and qPCR calls for specific attention and further analysis.

P3-29 Rapid Detection of *Escherichia coli* O157:H7, Non-O157 STEC and *Salmonella*, in Contaminated Meat Using LAMP MP Molecular Test and Lateral Flow Assay

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Introduction: Rapid detection of foodborne pathogens is an essential feature of any detection method used in food industry. In this study we present data for the detection of *Escherichia coli* O157:H7, *Salmonella* spp., (AOAC approved method) and non-O157 STEC (Shiga Toxigenic *E. coli*) using LAMP molecular detection kit and a simple lateral flow assay.

Purpose: The objective of this study is to emphasize the importance of simple, easy-to-use, cost effective and rapid immuno-assays that can help in speedy time-to-result testing of food samples.

Methods: Pure culture dilutions were tested with the kit and lateral flow assays to determine the limit-of-detection (LOD). For enrichments, ground beef/turkey, beef trim and chicken carcass samples were obtained from local stores and inoculated with 0 CFU, 2 CFU and 10 CFU/sample. Samples were enriched in SAS™ Enrichment media and tested using SAS™ Molecular detection kit and lateral flow assay.

Results: The LOD for the kit and lateral flow assays were determined by testing pure culture dilutions. For *E. coli* O157:H7 and *Salmonella* spp., the LOD for LAMP and lateral flow assay is 2×10^3 CFU/ml and 1×10^6 CFU/ml, respectively. For non-O157 STEC, the LOD for the kit and lateral flow assay is 1×10^1-10^3 CFU/ml and 1×10^3-10^5 CFU/ml, respectively (depending on the strain).

In 6 hours enriched samples, the kit assay can detect *E. coli* O157:H7 and *Salmonella* spp., to as low as 1 CFU/sample inoculation and non-O157 STEC to as low as 2 CFU/sample inoculation. The lateral flow assays for *E. coli* O157:H7 detect as low as 2 CFU/sample inoculation, non-O157 STEC can detect as low as 2 CFU/sample inoculation. Lateral flow assay for *Salmonella* spp., was able to detect as low as 10 CFU/ml in 16-20 hour enrichment samples.

Significance: The data presented here shows that a simple and rapid lateral flow assay can be used for rapid and easy detection of bacteria in contaminated meat without the need for any costly equipment thus aiding in faster time-to-results.

P3-30 Validation of Test for Detection of *Salmonella* spp. in a Variety of Foods

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Introduction: The ANSR *Salmonella* isothermal nucleic acid amplification test was previously granted AOAC Performance Tested Method℠ status (PTM # 061203) with claims for a variety of raw meat products, oat cereal, and several types of environmental samples. A matrix extension study was conducted to expand the validated claims to include an additional 10 food types.

Purpose: The purpose of the study was to evaluate performance of the ANSR *Salmonella* method for detection of *Salmonella* spp. in ice cream, almonds, soy flour, cocoa powder, dried pasteurized egg, peanut butter, black pepper, spinach, raw frozen shrimp, and dry pet food in comparison to that of the reference culture methods of FDA-BAM or USDA-MLG.

Methods: Food samples were inoculated with *Salmonella* spp. and held under conditions intended to simulate natural contamination with sublethally injured cells. For each food tested, 40 or 50 test portions were inoculated at a level predicted to produce a fractional positive data set,

and 10 test portions served as uninoculated controls. Half of the test portions were analyzed by the ANSR method after both 16 and 24 hours of enrichment. The remaining test portions were analyzed by the reference procedure.

Results: A total of 728 test portions were analyzed in the study. There were a total of 152 positive results by the method (24 hours) and 164 by the reference procedures. Results were analyzed using both chi-square and probability of detection statistical methods. For all 10 foods tested, there were no statistically significant differences in the number of positive results obtained by the ANSR method at 24 hours and the reference procedures. Results with the test method at 16 hours were statistically equivalent to those of the reference methods for 8 foods, with only ice cream and dried egg producing significantly more positive results by the reference method.

Significance: Successful completion of this study has resulted in expansion of the AOAC validated claims for the test method to a total of 15 foods and 5 environmental sample types.

P3-31 Evaluation of Loop-mediated Isothermal Amplification for the Rapid, Reliable, and Robust Detection of *Salmonella* in Produce

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Developing Scientist Competitor

Introduction: *Salmonella* is a leading bacterial pathogen involved in produce-associated outbreaks. Rapid, reliable, and robust detection methods are needed to better ensure produce safety. Loop-mediated isothermal amplification (LAMP) was recently adopted to detect *Salmonella* in produce. However, the assay has not been evaluated using an extensive collection of strains or complex produce matrices.

Purpose: The purpose of this study was to further evaluate LAMP performance in comparison with real-time quantitative PCR (qPCR) using a large panel of strains, and to validate the method for the rapid, reliable, and robust detection of *Salmonella* in various produce items.

Methods: The specificity of the assay was evaluated with 180 bacterial strains. The sensitivity of the assay was tested with ten *Salmonella* strains of different serovars in pure culture and spiked produce samples (cantaloupe, lettuce, pepper, sprout, and tomato). The same produce items were surface-inoculated with low levels (1-20 cells per 25 g of produce) of *Salmonella* and detected after aging at 4°C for 48 h. All samples were tested by both LAMP and qPCR.

Results: No false-positive or false-negative results were observed among 180 strains used to evaluate assay specificity. The limits of detection of various *Salmonella* strains belonging to various serovars were 1 to 10 cells/reaction in pure culture and 10^4 to 10^6 CFU/25 g in spiked produce samples, which were superior to qPCR. In produce samples spiked with low levels of respective *Salmonella* strains, LAMP consistently achieved accurate detection after 6 to 8 h of enrichment, with the exception of sprouts.

Significance: The LAMP assay was demonstrated to be a rapid, reliable, and robust method for the detection of *Salmonella* in produce. The difficult with sprouts detection by both LAMP and qPCR warrant further studies.

P3-32 Development of an Assay to Detect *Salmonella enterica* Serovar Senftenberg by qPCR

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Introduction: *Salmonella* contamination of food is a public health concern because of the potential for widespread outbreaks and the gastroenteritis it can cause. *Salmonella* Senftenberg is a serotype of *Salmonella* that is often detected in humans, and, although it is not listed among the top 20 serotypes implicated in human illnesses, it is ranked in the top ten among cases of non-human diseases. The zoonotic nature of *Salmonella* suggests a potential for this serotype to become significant in human disease. Development of assays to detect *S.* Senftenberg and other exotic serotypes would help define treatment and prevention methods.

Purpose: The aim of this study was to sequence, design, test, and manufacture an assay that detects *S.* Senftenberg while avoiding the detection of other similar serotypes or species.

Methods: Twenty samples presumptive of *S.* Senftenberg were sequenced using the Ion PGM™ Sequencer. After genome assembly, a bioinformatics analysis was performed to identify conserved regions that are unique to *S.* Senftenberg and exhibit low similarity to other *Salmonella*. Candidate qPCR assays were designed using a proprietary bioinformatics tool and evaluated against all available GenBank sequences. The specificity of the assays was tested using an exclusion panel comprised of various serotypes of *Salmonella* and related pathogens, and was determined to be specific for *S.* Senftenberg. The LOD is estimated to be 10 CFU/reaction.

Results: Sequences were obtained in three days, and genome assembly and assay design was completed in five days. The assay was tested against an exclusion panel and found to be specific for Senftenberg. Sensitivity was estimated at 10 CFU/reaction.

Significance: Our *S.* Senftenberg real-time PCR assay is highly specific and is able to distinguish Senftenberg from other *Salmonella* serotypes. Identification and subsequent control of *S.* Senftenberg during food production and processing may help reduce the frequency of food-related illnesses.

P3-33 Validation of a New Real-time PCR Assay for Detection of *Listeria monocytogenes* from Foods and Environmental Surfaces

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Introduction: The Thermo Scientific™ SureTect™ *Listeria monocytogenes* Assay is a new Real-Time PCR test for the detection of *L. monocytogenes* from food and environmental surfaces, which combines pre-dispensed lysis reagent and lyophilized tableted PCR reagents to simplify and improve assay handling, along with dedicated software to interpret and display PCR results.

Purpose: The study was conducted according to the AOAC-RI Performance Test Method℠ validation process to evaluate the Assay for use with a representative range of produce, meat, dairy and sea-food matrices as well as stainless steel surfaces.

Methods: Validation of the SureTect Listeria monocytogenes Assay was conducted by enriching 25g samples of food matrices or surface sponges in supplemented Oxoid™ 24 LEB Broth for 22 hours, followed by PCR analysis according to SureTect method instructions. Cooked deli-ham, smoked salmon, salami, prawns, raw cod, ice-cream, American style cheese, Brie, fresh spinach & lettuce, cantaloupe melon, Frankfurters & stainless steel surfaces were evaluated in comparison to the ISO 11290-1:1998, Amd 1:2004 reference method. Foods were spiked at low (0.2-2

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CFU/25g) and high (2-5 CFU/25g) levels, with low level spiking required to achieve fractional positive rates across 20 replicate samples. All PCR positive results were confirmed using the SureTect confirmation protocol (plating onto *Brilliance*™ Listeria Agar) and by a shortened ISO confirmation procedure.

Results: Internal and external independent validation demonstrated that the Assay gave equivalent or better performance than ISO 11290-1 for all matrices studied. Results from the Assay were in agreement by probability of detection statistical analysis with ISO 11290-1. When compared with the reference method, the mean RLOD for all matrices was 0.705 CFU/25g (0.037-1.427). Inclusivity testing detected all of 53 isolates of *L. monocytogenes* tested. None of the 38 exclusivity isolates were detected by the assay.

Significance: The PCR assay was shown to be an accurate and user-friendly method, due to the use of pre-dispensed lysis reagent, tableted PCR reagents and automatic interpretation of results. Results for a wide range of foods, including challenging matrices, demonstrated the assay was able to reliably detect the presence of *L. monocytogenes*.

P3-34 Real-time PCR Confirmed MPN Enumeration of *Listeria monocytogenes* from Inoculated Food Matrices Containing *Listeria innocua*

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Introduction: The pathogen *Listeria monocytogenes* is associated with infrequent but severe foodborne illness. Because the levels of contamination with *L. monocytogenes* are frequently less than 1 CFU/g, the most-probable-number (MPN) enumeration technique is the most appropriate method for surveying levels in foods. A fundamental problem with the MPN method is that each presumptive-positive tube must be confirmed, which can be complicated further by the presence of additional *Listeria* species.

Purpose: The purpose of this study was to evaluate the use of real-time PCR for confirmation of presumptive positive MPN tubes as a means for quantitatively surveying foods for the presence of *L. monocytogenes* when additional species of *Listeria* are present.

Methods: Produce, seafood, dairy products and grain products were simultaneously challenged with *L. monocytogenes* (mean = 2.3 ± 0.2 log CFU/g) and *L. innocua* (mean = 3.3 ± 0.2 log CFU/g). A 5-tube MPN series (0.1, 0.01, and 0.001 g inoculum levels) was performed following the U.S. FDA BAM method. MPN tubes were confirmed by real-time PCR.

Results: In all but one spiked food matrix, the plate count estimates fell within the 95% confidence interval of the corresponding PCR-MPN and serves as an indication of the accuracy of the method. The exception was inoculated Mexican-style cheese, in which the PCR-MPN routinely underestimated the levels of *L. monocytogenes* present. Triplicate analytical portions were used to determine the precision of the PCR-MPN method. For each microbial challenged food product the resulting three MPN values were in excellent agreement. Replication differences were typically at the 0.001 g inoculum level.

Significance: The PCR-MPN method provides a reliable estimate of levels of *L. monocytogenes* in food samples, including those that also contain *L. innocua*. This method reduces the amount of time required for quantitation by eliminating the need for conventional confirmation of large numbers of MPN tubes.

P3-35 Comparison of Four DNA Extraction Methods in the Real-time PCR Assay for Detection of *Listeria monocytogenes* from Milk Products

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Introduction: *Listeria monocytogenes* is an important foodborne pathogen, commonly isolated from foods of animal origin like raw milk, meat and poultry. Real time PCR assay for rapid detection on pathogen in food has become choice due to its high sensitivity and specificity, so the efficiency of DNA extraction from various food matrices is important.

Purpose: We compared the efficiency by evaluating conventional DNA extraction (boiling) method and three commercially available kits (Qiagen DNeasy blood&tissue kit, ABI rapid spin sample purification kit and GeneAll cell SV mini kit) for DNA extraction of *L. monocytogenes* cultured solution in the real time PCR assay.

Methods: *L. monocytogenes* cultured for 24 h was serially diluted with range from 10^{o} to 10^{5} CFU/ml and spiked in milk and milk products (cheese and milk formula) with cultivation for 4 h at 37°C. DNAs from spiked and cultured samples were extracted with each kit and evaluated by detection limit and standard curve using a TaqMan *Listeria monocytogenes* detection kit (ABI).

Results: The lowest detection limit was 10^{o} CFU/ml in each food matrices, which was obtained with ABI and GeneAll kit, whereas Qiagen kit and boiling method had limits of 10^{1} - 10^{3} CFU/ml. When standard curves compared, GeneAll kit was more efficient in three types of food matrices of milk, cheese and infant milk formula with R^2 of > 0.98 and efficiency of 105-111%, while ABI kit showed R^2 of 0.85 - 0.98 and efficiency of 119 - 200%.

Significance: This result suggested that ABI and GeneAll kit could provide the most sensitive methods in the real time quantification PCR assay for detecting of *L. monocytogenes* from milk and milk products.

P3-36 Simultaneous Detection of *Salmonella* spp., *Escherichia coli* O157 and *Listeria monocytogenes* in a Variety of Cheeses and Spinach Using a Multiplex Real-time PCR Method

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Introduction: Foodborne diseases affect about 48 million people per year in U.S. A major portion of these foodborne illnesses are caused by *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, thereby reducing the incidence of foodborne diseases. At present there is no method available for the simultaneous detection of all three organisms in contaminated foods regulated by FDA.

Purpose: This project aims to evaluate, optimize and adapt a real-time PCR method, originally developed at USDA, to simultaneously detect the presence of *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes* in soft-cheese and spinach.

Methods: Five different cheeses and spinach in a previously described selective medium were spiked with 5-25 CFU/25 g of *Salmonella*, *E.coli* O157:H7 and *L. monocytogenes*, stomached and incubated for 2 hours at 37°C followed by the addition of nalidixic acid, fosfomycin, cycloheximide and acriflavine and then grown overnight. The samples were then used for the extraction of genomic DNA using a DNA extraction kit (Qiagen).

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The DNAs were used to carry out the real-time PCR with primers and TaqMan probes targeting *invA* (*Salmonella*), *rfbE* (*E. coli* O157) and *hlyA* (*L. monocytogenes*) as well as an internal amplification control (IAC).

Results: All three gene targets of the tested pathogens were detected in the spiked cheeses and spinach samples after enrichment, with a sensitivity level of 5-25 CFU/25g. As expected, the gene targets were not detectable except that the IAC was positive in control samples.

Significance: The availability and optimization of this method for the simultaneous detection of *Salmonella* spp., *E. coli* O157 *and Listeria monocytogenes* in different cheeses and spinach will allow the FDA and other organizations to take action and prevent spread of an outbreak.

P3-37 An Independent Laboratory Evaluation of a *Salmonella* spp Detection Kit

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Introduction: The globalized food industry demands rapid, accurate, and easy-to-use pathogen detection systems. Increasingly, real-time PCR is relied upon to meet these demands. The *mericon™ Salmonella* spp. method combines one of two straightforward sample preparation methods with real-time PCR detection using two kits.

Purpose: This internal evaluation was to conduct a comparison of the new method to the ISO 6579 reference method for the detection of *Salmonella* as part of the AOAC-RI™ validation process.

Methods: The method comparison analyzed a total of eight foods including raw ground beef (30% fat), chicken carcass rinses, creamy non-organic peanut butter, fresh spinach, pasteurized whole milk, instant nonfat dry milk, milk chocolate and shell eggs and pet food and environmental samples. Each matrix was inoculated with a different serotype of *Salmonella* at two levels (0.2-2 CFU/25 g and 2-5 CFU/25 g). For each sample, DNA was extracted by both the manual DNA and automated DNA extraction kits, analyzed by the RotorGene real-time PCR system and compared to the ISO 6579 reference method. Test kits were also evaluated for ruggedness, inclusivity and exclusivity.

Results: The method comparison demonstrated no significant differences in the number of positive samples detected between the *mericon* method and the ISO method for all matrices studied (POD statistical model). All 103 inclusivity strains tested were positively detected. All 31 non-target exclusivity strains tested were not detected. The ruggedness evaluation indicated that minor modifications did not affect the outcome of the method.

Significance: This new method is an efficient and reliable alternative to the traditional reference methods of detecting *Salmonella* in a variety of foods, feed and environmental samples.

P3-38 Validation of a Commercial Real-time PCR Test Kit for Screening *Salmonella* in Produce, Meats, Seafood, Dairy, Spices, Infant Formula, Pet Food and Environmental Surfaces

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Introduction: *Salmonella* is found in many food and environmental sources and causes serious illness. Since its isolation can be long and difficult non-culture, rapid methods for its detection are needed.

Purpose: This study evaluated the DuPont™ BAX® System Real-Time PCR Assay for *Salmonella* for detecting *Salmonella* spp. across a diverse range of food and environmental matrices.

Methods: A total of 24 sample types were evaluated from categories including produce, meats, seafood, eggs, dairy, spices, infant formula, pet food and environmental surfaces. Most matrices were spiked at a level sufficient to give fractional results, while two matrices (poultry rinsates and chicken wings) were naturally contaminated with *Salmonella* at fractional levels. For samples for which the enrichment protocols differ between the test method and the reference FDA-BAM, USDA-MLG and/or Health Canada Compendium methods, the reference culture method was also performed on comparable samples enriched using the appropriate reference method enrichment protocol. Most test samples were evaluated with the PCR method before and after a 3-hour secondary enrichment (re-growth) in BHI broth to help ensure that variants of each food type could be tested using this method, even if the grow-back step is required.

Results: In this study 788 presumptive positive results were obtained from 1815 total tests with the PCR test method from either the primary enrichment or the BHI re-growth. Sensitivity and specificity were calculated to be >99.9% for the alternative PCR method when compared against the appropriate culture method results. Analysis using the AOAC International POD model demonstrated no statistically significant difference between alternative and reference culture methods.

Significance: This study demonstrates that the Assay for *Salmonella* is a rapid and sensitive alternative method for detecting *Salmonella* in these matrices using both standard and alternative enrichment methods. Test method results demonstrated no significant difference when compared with the corresponding reference culture methods.

P3-39 Pulsed-field Gel Electrophoresis Subtyping of *Salmonella* Isolates from Carcasses, Lymph Nodes, and Fecal Samples from Cattle at Slaughter Facilities in Mexico

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Introduction: *Salmonella* is one of the leading causes of foodborne illnesses worldwide; in Mexico and other developing countries limited information on the prevalence of this pathogen at pre-harvest exists. Pulsed-Field Gel Electrophoresis (PFGE) is considered the "gold standard" *Salmonella* subtyping method due to its discriminatory power to differentiate among isolates, and its ability to determine relationships between PFGE patterns and particular serotypes.

Purpose: This study aimed to 1) analyze the relationship between *Salmonella* serotypes and PFGE patterns from beef carcasses and feedlot isolates and 2) to molecularly characterize *Salmonella* isolates from lymph nodes and fecal samples of cattle from slaughter facilities in Mexico

Methods: A set of 93 *Salmonella* isolates from beef carcasses and feedlot, previously serotyped, were used to perform PFGE. In addition, 33 *Salmonella* isolates from lymph nodes (mandibular, mediastinal, and mesenteric) and feces from 12 animals were analyzed to assess genetic relatedness. Subtyping was performed according to the PulseNet PFGE protocol for *Salmonella* serotypes.

Results: The serotypes previously identified were *S.* Muenster (26.9%), *S.* Kentucky (19.4%), *S.* Reading (15.1%), *S.* Anatum (12.9%), *S.* Give (5.4%), *S.* Mbandaka (5.4%), *S.* Montevideo (3.2%), and *S.* Tennessee (2.0%). Nine samples (9.7%) were not identified by traditional serotyping, 77.7% of them were associated to a PFGE subtype when analyzed by PFGE. *Salmonella* Kentucky was the most clonal in this study, 100% of the isolates had indistinguishable PFGE pattern; *S.* Muenster was the most diverse, 11 different subtypes were identified. Cluster analysis of PFGE patterns showed great relation (90.3%) between serotypes and PFGE subtypes.

Significance: Results suggest there is a high concordance between serotypes and PFGE subtypes among isolates from beef carcasses and feedlot from Mexico. The diversity observed inter and intra-serotypes is important to discriminate among clones to enhance epidemiological studies. More research is needed to improve interventions at different points in the food chain.

P3-40 Independent Evaluation of a Commercial STEC Method for the Detection of Shiga Toxin-producing *Escherichia coli* in Ground Beef and Beef Trim

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) have been implicated in numerous foodborne outbreaks. With the implementation of routine testing by the USDA of additional serotypes of STEC in beef, a validated commercially available tested method is needed for the rapid detection of all regulated STEC organisms.

Purpose: The objective of this study was to evaluate the Bio-Rad iQ-Check® Real-Time PCR STEC method for the detection of inoculated Shiga toxin-producing *Escherichia coli* in ground and raw beef trim in comparison to the USDA FSIS MLG 5B.02, 5.06 and 5A.02 methods.

Methods: Portions of ground and beef trim were inoculated individually at low (0.2-2.0 cells/25g) or high (2.0-10 cells/25 g) inoculation levels with 5 different serotypes of *E. coli.* The 25 g samples were combined with 350 g or 300 g of uninoculated beef for analysis with the iQ-Check and USDA FSIS MLG methods, respectively.

After 10 and 12 h of incubation in a 1:4 dilution of STEC Enrichment Broth, the samples tested with the iQ-Check method were analyzed with the kit for presence of *stx* and *eae* genes followed screening for the USDA "Top 7" O groups using the kit if the samples contained *stx* and *eae*. The samples tested with the USDA FSIS MLG 5B.02/03 or 5A.02 methods were analyzed with the primer and probe sets as prescribed by the method or with BAX®E. coli O157 MP test. All samples were confirmed for the presence of target STEC organisms regardless of the screening result using the FSIS MLG 5B-02/03 and 5.06 methods.

Results: For inoculated beef trim and ground beef, the commercial method performed statistically as well as the USDA FSIS MLB 5B.02/03, 5.06, and 5A.02 methods for the detection of Shiga toxin-producing *E. coli*.

Significance: The commercial method provides a commercially available option to detect STEC in beef in as little as 10 h.

P3-41 Using a Repetitive Sequence-based PCR System for Molecular Characterization of Shiga Toxin-producing *Escherichia coli*

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Introduction: Shiga toxin-producing *Escherichia coli*(STEC) are of major public health concern due to their frequent contamination of food products and ability to trigger foodborne illnesses such as diarrhea, hemorrhagic colitis, and life-threatening hemolytic uremic syndrome.

Purpose: In this study, we used the DiversiLab rep-PCR system to generate a digital DNA fingerprint profile for 152 STEC isolates (63-O157 and 89-Non-O157) from human, food, and animal sources.

Methods: STEC isolates were collected from Michigan State University (n=104), as well as the Center for Veterinary Medicine (n = 48) within the FDA. Fourteen serogroups were analyzed, including 5 of the "Big 6" *E. coli*serogroups, using the Kullback-Leibler method for similarity calculation.

Results: Excellent reproducibility of rep-PCR profiles was observed with % values ranging from 98.4%-99.7% and 97%-99.8% for intra-run and inter-run studies, respectively. A comparison of Non-O157 isolates revealed that 79/89 (88.9%) isolates clustered according to serogroup status, and peak differences among the Non-O157 isolates ranged from 2 peak differences (94.3% similarity) up to 12 peak differences (59.6% similarity). Furthermore, the dendrogram of STEC serogroups created from rep-PCR profiles mirrors the distinct clonal groups elucidated for STEC strains by other investigators. For our panel of O157 isolates, 58/63 (92.1%) yielded a DNA banding pattern typical of the O157:H7 rep-PCR pattern type. Interestingly, 3 of the 5 O157 STEC that did not exhibit an O157:H7 rep-PCR pattern type were isolated from asymptomatic individuals. Rep-PCR profiles for STEC isolates were also distinct from 12 other bacterial species, which included *S. flexneri*, *L. monocytogenes*, *V. cholera*, *K. pneumoniae*, and *Salmonella enterica*. Together, these results indicate the ability of the DiversiLab rep-PCR system to distinguish between and within O157 and Non-O157 STEC serogroups.

Significance: Rapid PCR-based typing methods, like repetitive sequence-based PCR (rep-PCR), may facilitate the identification of STEC by complimenting the discriminatory power of PFGE.

P3-42 Optimization of Multiplex Real-time PCR Assay for Detection and Quantification of *Vibrio* spp. and Total Bacteria

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Introduction: *Vibrio* spp. are opportunistic human pathogens that cause gastroenteritis with a high lethality rate. The infection is associated with consumption of seafood or with exposure of contaminated water.

Purpose: This study describes the development of a Multiplex Real-time PCR assay for simultaneous detection and quantification of total bacteria (16S rDNA) and three kinds of *Vibrio* spp. such as *Vibrio vulnificus* (*vvh*A), *V. parahaemolyticus* (*tlh*), and *V. anguillarum* (*Tox*R).

Methods: For the multiplex assay, 4 sets of primer pairs and probes were newly designed as well as the Real-time PCR cycling protocol, fluorescent detection parameters, and reaction mixture components were optimized.

Results: The optimal conditions of PCR used the following final concentrations for each 800 nM of the *Tox*R, 200 nM of the 16S rDNA, 50 nM of the *vvh*A, and *tlh* forward/reverse primers and probe. The optimal cycling parameters consisted of the initial denaturation at 95°C hold for

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1 min followed by 40 cycles of DNA amplification: denaturation step at 95°C for 15 s and combined annealing/extension step at 56°C for 50 s. The species-specific targets showed negative results and 16S rDNA was positive against 28 bacterial species including *Vibrio* spp. The assay was optimized for the detection of low numbers up to Log 0 CFU/reaction and the R^2 values were 0.99 for the standard curve of *vvh*A, *tlh*, and *Tox*R and 0.98 for the 16S rDNA.

Significance: The Multiplex Real-time PCR assay could be useful for rapid monitoring of seafood and marine water quality.

P3-43 FERN Multi-laboratory Validation of the BAX qPCR *Vibrio* Assay for Identification of *Vibrio* Isolates

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Introduction: Seafood related *vibrio* illnesses are on the rise in the U.S. and abroad. FDA is in the process of validating various real-time PCR assays for detection and quantification of vibrios in seafood. The first phase in this process is to demonstrate specificity of this assay for the intended target organisms using pure cultures. The BAX Vibrio Assay is a multiplex real-time PCR that includes proprietary targets and reagents for simultaneous identification of total *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*.

Purpose: To evaluate the BAX *Vibrio* assay for use in identification and confirmation of *V. cholerae, V. parahaemolyticus* and *V. vulnificus* isolates by comparing the sensitivity and specificity of the testing method with the BAM method.

Methods: The BAX *Vibrio* Assay was compared to the reference method (API 20E, BAM Chapter 9) using a paired-study design for identification of *V. cholerae, V. parahaemolyticus* and *V. vulnificus* isolates. Each of the six laboratories analyzed 70 randomly coded isolates including 20 *V. vulnificus*, 20 *V. parahaemolyticus*, 20 *V. cholerae* and 10 isolates of other *Vibrio spp*. or other bacterial genera.

Results: There was no significant difference ($P \geq 0.05$) between the BAX *Vibrio* Assay and the selected reference method (API 20E) for identification of isolates. The sensitivity of the BAX *Vibrio* Assay was 98%, 98% and 97% for *V. cholerae, V. parahaemolyticus* and *V. vulnificus*, respectively. The specificity of the BAX *Vibrio* Assay was 96%, 98%, and 97% for *V. cholerae, V. parahaemolyticus* and *V. vulnificus*, respectively.

Significance: The BAX *Vibrio* Assay is a reliable and rapid alternative to API 20E for identification of *V. cholerae*, *V. vulnificus*, and *V. parahaemolyticus* isolates. This validation will advantage laboratories by offering an additional reference method for identification of these pathogenic vibrios, including during the next validation phase for detection of these organisms in seafood.

P3-44 The Step-by-Step RT-quality Assurance Procedure for Reverse Transcription Quantitative PCR: Illustration with the *Bacillus weihenstephanensis* Acid-resistance Biomarkers

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Introduction: Gene expression levels are recognized as relevant biomarkers to describe bacterial behavior and fitness. The use of reverse transcription quantitative PCR (RT-qPCR) enables accurate, sensitive, quantitative and fast measurements of RNAs. However, numerous critical points may arise throughout the entire RT-qPCR workflow, strongly influencing the robustness of the method, and thus the reliability of the results.

Purpose: This study describes appropriate step-by-step RT-qPCR quality controls implied for the selection and quantification of *B. weihenstephanensis* resistance biomarkers.

Methods: The robustness of the developed RT-qPCR method was assessed from the experimental design to the sample extraction, reverse transcription and quality controls.

Results: Primers and probes of 36 genes showed qPCR efficiency from 92% to 104%, r^2 at least of 0.980 and closed intercept of 43.72 ± 0.98. The linearity and the repeatability of the RNA extraction was assessed from 3 log of CFU.ml^{-1} to 8 log CFU.ml^{-1}. The linear correlation coefficient was equal to 0.99, with a slope of 1.01 and the intercept close to 0, with estimated bacterial concentrations ranging from 2.8 ± 0.2 to 7.8 ± 0.03 log CFU.ml^{-1}. RNA extracted from bacterial cells upon sub-lethal conditions gave RQI value greater than 5. For cells submitted to lethal conditions, a repeatable RQI close to 3 was estimated. Efficient removal of contaminating genomic DNAs was estimated over 100 bacterial samples and. No significant variation in the estimated copy numbers ($P < 0.05$) of RNA samples was found underlying the absence of both reverse transcription and qPCR inhibitors. Using 51 bacterial samples, the calculated *M* value of 3 reference genes (*tuf*,16S, 23S) were lower than 1.5 underlining satisfying expression stability to perform RT-qPCR data normalization.

Significance: Provided appropriate controls, RT-qPCR enables relevant gene expression quantification to select acid resistance biomarkers that will further be used to increment bacterial physiology in behavior prediction.

P3-45 Rapid Detection of *Clostridium difficile* by Using Whole Genome Amplification and Real-time PCR

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Introduction: Foodborne pathogens—responsible for causing illnesses and death in humans—are a widely growing concern. In order to effectively prevent foodborne illnesses, implementing a rapid detection method during distribution and manufacturing of food is necessary to eliminate foodborne pathogens. Many detection methods have been developed, of which PCR-based methods are generally preferred. However, PCR-based methods also need a microbial enrichment step to increase the microbial population over the detection limit. Therefore, we think that more rapid methods can be developed by shortening the enrichment time.

Purpose: This study was conducted to ascertain the possibility of replacing microbial enrichment with molecular enrichment. We used whole genome amplification (WGA) for molecular enrichment and compared it with microbial enrichment by using predictive modeling.

Methods: Genomic DNA was isolated using AccuPrep® Genomic DNA Extraction Kit (Solgent co, Korea). WGA was performed using SEQ-TempliGen™ WGA Kit (Solgent co, Korea) and a C1000™ Thermal Cycler (Bio-rad, USA). A primary model (modified Gompertz model) was used to determine the R^2 values of lag time and growth (or increasing) rate of microbial enrichment in BHI broth and of molecular enrichment by WGA. The toxin B (*tcdB*) gene was used as target sequence for real-time PCR amplification.

Results: The lag time of *C. difficile* growth in brain heart infusion (BHI) broth was 3.775 h, but was negligible for WGA amplification of the *C. difficile* genome. Assuming that a single bacterial cell of *C. difficile* is present in 25 g of sample, the minimal time calculated using real-time PCR was 6 h and 30 min for microbial enrichment in BHI broth. However, the same quantities of DNA can be amplified using WGA in just 30 min.

Significance: By replacing the microbial enrichment process with WGA, the detection time can be greatly reduced and more rapid detection methods can be developed for *C. difficile*.

Poster

P3-46 Evaluation of the Atlas® *Salmonella* G2 Detection System for Foods and Environmental Surfaces

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Introduction: *Salmonella* has been implicated as a major cause of human food borne illness worldwide. There is an increased demand to apply effective detection methods that are rapid, accurate and easy to use.

Purpose: To evaluate the Atlas® *Salmonella* G2 Detection Assay for the detection of *Salmonella enterica spp*. in food and environmental surfaces.

Methods: A single 10 or 12 h enrichment for short shelf life products and a 16 h enrichment for long shelf life products and environmental surfaces was used, and after enrichment, the sample was processed for bacterial lysis, template specific sample extraction, amplification, and probe detection per assay protocol. A total of 6 foods and three environmental surfaces were compared to the USDA MLG 4.05 or FDA BAM-5 reference method in an internal study and one food and one environmental surface in an external study. Selectivity was evaluated by testing 100 target microorganisms in three different media and 30 non-target microorganisms.

Results: The assay detected 100% of 100 target *Salmonella enterica spp.* and did not detect 30 exclusive microorganisms. It was equivalent or better than the reference method for high fat ground beef (25g and 375g), lettuce (375g), deli turkey (325g) and ground turkey (375g), plastic surface, sealed concrete and stainless steel. Evaluation of one food, ground turkey (375g), is still in progress. The test method provided the final result in 16-30 hours utilizing compared to at least 3 days for cultural methods.

Significance: The Atlas® *Salmonella* G2 Detection Assay detected *Salmonella enterica spp*. In a variety of food and environmental samples.

P3-47 Evaluation of the New Atlas® *Escherichia coli* O157:H7 Detection Assay for High Fat Ground Beef and Beef Trim Samples

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Introduction: Sensitive and specific methods are needed for the detection of pathogenic *Escherichia coli* O157:H7 in beef samples.

Purpose: The purpose of the study was to evaluate the Atlas *E. coli* O157:H7 Detection Assay sensitivity and specificity as well as detection of *E. coli* O157:H7 in artificially inoculated high fat ground beef (HFGB) and beef trim (BT) samples.

Methods: HFGB and BT samples in 375g test portions were inoculated with low levels of *E. coli* O157:H7 and diluted in mTSB or in the media suggested by the reference method. All samples were incubated at 42°C, lysed to release bacterial mRNA and loaded onto the instrument. The instrument combines target capture, transcription-mediated amplification, and hybridization protection assay. The results were compared to the FSIS reference method MLG 5.06. Sensitivity and specificity was evaluated by testing 50 *E. coli* O157:H7 strains at 10XLOD of 1e4 CFU/mL and 30 non-O157:H7 *E. coli* strains at 1e8 CFU/mL.

Results: The assay performed equally compared to the FSIS reference method for 375g HFGB and BT samples. No statistically significant difference was observed between the test method and reference method as determined by POD analysis. The test method showed sensitivity of 1e4 CFU/mL by detecting all 50 inclusive organisms and 100% specificity by not detecting 30 exclusive organisms.

Significance: The Atlas® *E. coli* O157:H7 Detection Assay provides fast and highly accurate detection of pathogenic *E. coli* O157:H7 in 375g HFGB and BT samples and results were comparable to the FSIS reference method.

P3-48 Evaluation of the Atlas® STEC Detection Assay for Fresh Raw Ground Beef and Fresh Raw Beef Trim

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Introduction: Sensitive and specific detection methods are needed for the identification of pathogenic shiga-toxin producing *Escherichia coli* (STEC), including O157, O26, O45, O103, O111, O121, and O145 serotypes.

Purpose: To evaluate the Atlas STEC Detection Assay for the detection of *E. coli* O157:H7 and STECs in artificially inoculated ground beef and beef trim after a single enrichment of 375 g test portions.

Methods: The method included a single enrichment of 375 g test portions in modified Tryptic Soy Broth (mTSB) at 42°C. After sample transfer for bacterial lysis, template specific sample extraction, amplification and probe detection were all performed on the automated instrument. Fresh raw ground beef, 30% fat, and fresh raw beef trim were compared to the FSIS MLG 5.06 and 5B.02 confirmation procedures. Selectivity was evaluated by testing 50 target microorganisms and 30 non-target microorganisms as determined by POD analysis.

Results: The test method provided a positive result for 100% of 50 target microorganisms, and a negative result for 30 non-target microorganisms. No significant differences were observed between the test method and the FSIS MLG 5.06 and 5B.02 confirmation methods.

Significance: *E. coli* O157:H7 and STEC, including the FSIS regulated big six serogroups, can be detected in raw ground beef and beef trim on the Atlas System.

P3-49 Evaluation of Environmental Samples from a Food Plant with the Atlas® *Listeria* Detection Assay Utilizing a Single 90 ml Enrichment Step

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Introduction: *Listeria monocytogenes* poses a significant food safety challenge due to the organism's ubiquitous nature and substantial public health risk. In order to prevent end-product contamination with the human pathogen *Listeria monocytogenes*, food processors employ environmental monitoring programs for *Listeria spp.* to verify the effectiveness of an establishment's food safety program. Taking into consideration the costs associated with environmental monitoring, a reduction in the media volume added to a sponge would be beneficial for reducing the costs of analysis.

Purpose: Utilizing environmental samples collected from a food processing plant, a study was performed to determine the acceptability of a single enrichment medium step with volume reduced from 190mL to 90mL, for testing by the Atlas® *Listeria* Detection Assay.

Methods: Each environmental sponge (n = 200) was massaged in 90mL Half-Fraser, and incubated overnight at 35°C. Enrichment samples (12µL) were added to a sample transfer tube containing a proprietary lysis solution, and then tested on the Atlas System. Sample processing on the instrument included purification via Target Capture, amplification by Transcription Mediated Amplification, and detection by Hybridization

Protection Assay. For culture confirmation, 100µL of the enrichment was transferred to 10mL Fraser and incubated 24h at 35°C, followed by streaking onto Modified Oxford agar. Discrepant assay results were confirmed by retesting another sample from the enrichment bag. Assay performance was analyzed by calculating the probability of detection (POD) at a 95% confidence interval.

Results: For environmental samples enriched in 90mL Half-Fraser, no statistically significant difference compared to culture was observed at a 95% confidence interval based on the POD analysis.

Significance: This study demonstrated that a reduced enrichment media volume using 90 ml Half-Fraser with testing on the Atlas System was equivalent to culture for detection of *Listeria* in environmental samples.

P3-50 Enrichment Media Comparison for Testing Whey Protein Powder Using the Atlas® *Salmonella* Detection Assay

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Introduction: The detection of *Salmonella* in whey protein powder is of interest to a wide variety of food production companies and microbiological reference laboratories. Identifying the correct enrichment protocol is critical in achieving an accurate and reliable result from this traditionally difficult matrix.

Purpose: To compare results using Universal Pre-enrichment Broth (UPB) and Lactose Broth (LB) enrichment media for testing whey protein powder with the Atlas *Salmonella* Detection assay and confirmation via by culture methods.

Methods: Whey protein powder samples were inoculated at 100 CFU/25g and 200 CFU/25g of *Salmonella* Typhimurium. The samples were stored for 2 weeks at room temperature followed by a 24 h enrichment in Universal Pre-enrichment broth (UPB) at 42°C or Lactose Broth (LB) at 35°C. After enrichment, sample processing included purification via Target Capture, amplification by Transcription Mediated Amplification, and detection by Hybridization Protection Assay. Culture confirmation was performed by transferring 1000 µl of enrichment to 10 ml of Tetrathionate Broth for 24h at 35°C and streaking onto ChromAgar *Salmonella* and XLD.

Results: In a one to one comparison of UPB and LB broths used for sample enrichment, the UPB broth yielded better growth of *Salmonella* from whey protein powder samples. At the 200 CFU/25g inoculation level, the testing showed 100% (10/10) correlation between the UPB enrichment protocol and culture results and an 80% (8/10) correlation between the LB enrichment protocol and culture results. At the 100 CFU/25g inoculation level, the testing showed 100% (10/10) correlation between the UPB enrichment protocol and culture results and a 30% (3/10) correlation between the LB enrichment protocol and culture results.

Significance: This study demonstrates that at two inoculation levels in whey protein powder, enrichment with UPB at 42°C shows better growth of *Salmonella* compared to LB enrichment at 35°C after 24 hours of incubation.

P3-51 Performance of the Atlas® *Listeria monocytogenes* Detection Assay (LmG2) after a Single Enrichment

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Introduction: *Listeria monocytogenes* (*Lm*) is a foodborne pathogen resulting in approximately 1,600 infections annually in the US. Listeriosis primarily affects the immunosuppressed, elderly, children, and pregnant women, causing severe health problems such as encephalitis, meningitis, septicemia, and miscarriage. *Lm*control continues to be a food safety challenge given the organism's ability to survive and grow under low pH, low temperature, and high salt.

Purpose: To develop a molecular method for detecting *Lm*in 25g and 125g food portions and environmental samples with testing being performed after a single enrichment.

Methods: Fractionally inoculated food samples and non-inoculated environmental samples are analyzed using the LmG2 assay, a messenger RNA (mRNA) based detection method with target specific to *Lm.* 25g food (ice cream, lettuce, chicken salad, hot dog, cured ham, frozen cream pie, frozen pizza, brie) and environmental samples are enriched at 35°C in PALCAM base with 0.02 g/L of Nalidixic acid for 24h, and a 26h enrichment is used for 125g food (cooked chicken and deli turkey) samples. Enriched samples are transferred to a proprietary lysis buffer, automatically purified via Target Capture, amplified by Transcription Mediated Amplification, and detected by Hybridization Protection Assay. Culture confirmation is performed by transferring 100 µl of enrichment to 10 ml of Fraser for 24h at 35°C and streaking onto MOX plates.

Results: The LmG2 assay provides positive results for 100% of *Lm* strains, and negative results for tested non-target microorganisms commonly found in food and grown to a titer ≥ 1E + 08 CFU/ml. The assay is equivalent to culture for all tested matrices. Similar equivalency is observed on environmental samples where positive samples are *Lm* confirmed.

Significance: The LmG2 assay specifically detects *Lm* in a variety of inoculated food matrices (25g and 125g) and non-inoculated environmental samples after 24h-26h enrichment on the Atlas system with equal sensitivity to culture.

P3-52 Rapid Detection of Pseudomonads in Dairy Products and Process Water

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Introduction: Pseudomonad organisms are a major cause of bacterial spoilage of pasteurized milk and dairy products due to post process contamination. Early detection of pseudomonads can be a predictor of product shelf-life as they are the predominant psychotropic bacteria present. The BioLumix system is an optical system that detects growth of pseudomonads using a CO_2 sensor in selective growth media.

Purpose: To evaluate the ability of the optical system to detect pseudomonads in dairy products and process water, and to,determine the speed to results, sensitivity, selectivity and ability to predict shelf-life.

Methods: The optical system was directly compared to the plate count methodology for milk samples stored at refrigerated temperatures and held overnight at room temperatures. Testing of process water was also accomplished.

Results: Commercial milk products were used to measure the presence of pseudomonads during refrigeration and storage at elevated temperatures. Pseudomonads were present at varying levels in dairy samples and were detected within 16-24 hours using BioLumix vials. All process water samples tested were free of Pseudomonads by both methods. Process water samples inoculated with different Pseudomonads strains were detectable by the optical system. The vials were selective by not allowing for growth of unrelated gram positive and gram negative bacteria,

mold or yeast. The combination of the Pseudomonas vial with overnight pre-incubation of the sample could serve as an indicator of shelf-life of products.

Significance: The data in this study suggested that the BioLumix Pseudomonas vials are capable of early detection of pseudomonads in dairy products and in process water. The system offered a reduction in time to results as compared to the plate methodology.

P3-53 Comparison of Foodproof Real-time PCR Kits to the ISO Method for STEC Screening and O-group Identification

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Introduction: Foodborne illness caused by Enterohemorrhagic *Escherichia coli* (EHEC) claimed ~ 50 lives during one of the largest outbreaks in 2011. EHEC have been encountered in leafy vegetables, sprouted seeds, raw milk and cheeses, as well as fresh, minced, and mixed meat preparations. Most infections have been with *E. coli* O157; however, other strains of non-O157 *E. coli* capable of causing sickness and death also should be examined in food.

Following the current ISO method (ISO/TS 13136), not all variants of the *stx2* pathogenicity gene are detected, particularly *stx2f*. Differences in important serotypes exist between Europe and the United States. ISO/TS 13136 requires five serotypes (O26, O103, O111, O145 and O157). Moreover, there will be an extension of regulation (EC) 2073/2005 specifically for sprouts, which requires these five as well as O104.

Purpose: To evaluate the specificity (inclusivity and exclusivity), sensitivity, and robustness of the ISO/TS 13136 method (a PCR-based method) for STEC screening and serotype identification in comparison to the foodproof STEC Screening and O-group Identification Kits.

Methods: ISO primary enrichment for STEC testing was performed. Enrichments were then tested using ISO/TS13136 described PCR-based method or PCR-based STEC screening kit, which involved DNA extraction from the primary enrichment followed by real-time PCR. The serotypes of STEC-positive samples were then determined using the O-group identification kit.

Results: The sensitivity and robustness of the screening and O-group identification kits fulfill requirements set forth by ISO/TS13136. However, the specificity of the screening kit was superior due to its detection of more Shiga-toxin variants, such as *stx2*, and the O-group Identification Kit determined 3 more O-groups than the ISO/TS 13136 method currently requires.

Significance: The combination of the foodproof STEC Screening and O-group Identification Kits provided the ability to detect and identify all eight serotypes simultaneously and reduce the time-to-result.

P3-54 Capture and Detection of a Representative Human Norovirus Strain Using Target-specific Nucleic Acid Aptamers: Proof of Concept

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Introduction: Human noroviruses (NoV) are the most common cause of acute viral gastroenteritis and a leading cause of foodborne disease. Detection is an important tool in our attempt to reduce disease burden. Aptamers [small, single-stranded (ss) DNA or RNA molecules with target binding affinity] are emerging ligands to facilitate pathogen capture and detection. They demonstrate advantages over traditional capture ligands like antibodies, including reduced cost, ease of production, and high stability.

Purpose: To develop capture and detection methods using pre-selected ssDNA aptamers with binding specificity to Snow Mountain virus (SMV), a prototype human NoV strain.

Methods: Two assay designs were produced. In the aptamer magnetic capture (AMC) design, aptamers (NVII-13, 24) were used as capture ligands, with subsequent detection using virus-specific RT-qPCR. In the second assay (sandwich-based), SMV was captured using antibody-bound beads, which were then exposed to a SMV-specific aptamer (NVII-22). Detection was achieved by amplification of the aptamer by qPCR.

Results: The mean capture efficiency (%) of aptamers NVII-13 and NVII-24 using the AMC-RT-qPCR method as applied to serially diluted SMV stock ranged from 2.5 - 42%, and increased (improved) as virus titer decreased (from 4 log – 1 log GEC/ml). The lower limit of detection (LoD) of the assay was 1 log genome equivalent copies (GEC) SMV/ml. For the sandwich assay format, sample positivity was established as Ct values > 2-3 times the standard deviation associated with the negative control. This criterion was met for samples containing between 1 and 4 log GEC SMV/ml, equating to a lower limit of detection of 1 log GEC/ml. Both of the aptamer-based assays had a 3 log lower (better) LoD when compared to similar immunomagnetic separation- RT-qPCR detection assays.

Significance: This study shows proof-of-concept that nucleic acid aptamers can be used for capture and detection of human NoV, with excellent detection limits. Further studies are underway to evaluate their usefulness in NoV detection in foods.

P3-55 Using the ApoH Protein to Capture and Concentrate Human Norovirus Particles and Virus-like Particles from Various Matrices

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Introduction: Human noroviruses (HuNoV) are a leading cause of foodborne illness. The inability to cultivate these viruses *in vitro* highlights the need for simple and effective methods to concentrate them from relevant sample matrices prior to detection. This is particularly complicated because HuNoV tend to be present in low concentrations in foods, and commonly used capture ligands (antibodies) lack broad reactivity to all HuNoV strains.

Purpose: To evaluate apolipoprotein H (ApoH) as a potential broadly reactive reagent to concentrate HuNoV for detection using quantitative Reverse Transcription PCR (RT-qPCR).

Methods: Experiments were designed to evaluate (i) if ApoH effectively captured HuNoVs or Virus Like Particles (VLPs); and (ii) if so, recovery efficiency. To address the first question, (VLPs) were bound to ApoH coated microplates and evaluated for detection using an ELISA method. To address the second, a representative epidemic GII.4 HuNoV strain obtained as a fecal specimen from an infected individual was used as inoculum. This was serially diluted and mixed with ApoH-conjugated magnetic beads (ApoH Technologies). Subsequent virus detection was done using RT-qPCR targeting the ORF1/ORF2 junction. Capture experiments were done on both 1 and 10 ml sample volumes.

Results: ELISA results indicated significant capture of GII.2 and GII.4 VLPs, as well as the HuNoV surrogate, Tulane virus. Using the ApoH conjugated magnetic beads followed by RT-qPCR, the GII.4 HuNoV strain was effectively concentrated from 1 ml samples at various inoculum levels ranging from 10^6 to 10^1 genome equivalent copies (GEC) with virtually 100% efficiency, i.e., no statistically significant difference ($P > 0.05$) between

input and output virus concentrations as per comparison to a standard curve. In 10 ml scale-up experiments, concentration efficiency was about 10%, as evidenced by a 1 log drop in virus titer after concentration relative to the standard curve.

Significance: ApoH represents a novel and efficient method for capture and concentration of HuNoV. Further studies are underway applying this protocol to artificially contaminated food extracts.

P3-56 Development of a Human Norovirus Indicator Using *Enterococcus* sp. 16S rRNA and *Bacteroides fragilis gyr*B Gene as a Rapid Detection (Monitoring) Tool in Various Environments

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Developing Scientist Competitor

Introduction: Human norovirus (HuNoV) is the cause of more than 21 million cases of acute gastroenteritis in the US and it can persist at any point from farm to fork chain. However, there has been a lack of a rapid indicating system for HuNoV contamination. It has been suggested that the ratio between the phyla Firmicutes and Bacteroidetes is significantly shifted when enteric viruses are present, including HuNoV.

Purpose: The ratio of *Enterococcus* sp. (16S rRNA) and human-specific *Bacteroides fragilis (gyr*B gene) in HuNoV-infected and non-infected human fecal samples as a means to indicate the presence of HuNoV.

Methods: Total DNA and RNA were extracted from 31 HuNoV-infected samples and 17 non-infected human fecal samples using commercial extraction kits. Reverse transcription was performed on RNA template to synthesize HuNoV-specific complimentary DNA (cDNA). Extracted DNA and synthesized cDNA were quantified using real-time Polymerase Chain Reaction (PCR) system. Primers were designed to target the VP1, 16S rRNA, and *gyr*B genes for HuNoV, *Enterococcus* sp. and *B. fragilis*, respectively. Threshold cycles (C_T) values were plotted against the log-RNA copies for HuNoV and log-transformed cell counts [Colony Forming Unit (CFU)/g-feces]. *Enterococcus* sp. and *B. fragilis* counts were compared using Analysis of Variance.

Results: There were statistically significant differences ($P < 0.05$) in the CFU of *Enterococcus* sp. and *B. fragilis* between HuNoV infected and non-infected fecal samples. In infected fecal samples, *Enterococcus* sp. was 1.37 log CFU/g-feces higher while *B. fragilis* was 1.35 log CFU/g-feces lower, averagely. When normalized with *B. fragilis*, a linear relationship was observed between *Enterococcus* sp. and HuNoV (R^2=0.47).

Significance: These results suggest that there is a significant change in the ratio of *Enterococcus* sp. and *B. fragilis* in HuNoV-infected fecal samples. With this newly developed, rapid PCR-based detection system, the presence of HuNoV and other enteric human viruses can be effectively monitored in broad spectrum of environments in a timely manner, such as food, water, and environmental samples.

P3-57 Withdrawn

P3-58 Multiplex PCR for Detection of *Vibrio* spp. and 5 Pathogenic *Vibrio* Species Using Primers Designed by Comparative Genomics

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Introduction: *Vibrio* spp. is the major foodborne pathogen and 11 *Vibrio* species are known as pathogenic species causing *Vibrio* infections represented by *V. cholera*, *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus* and *V. mimicus*. While genetic markers were limited as virulence factor genes in previous PCR for *Vibrio* diagnostics, comparative genomics could suggest primer sets developing a multiplex PCR with increased range of *Vibrio* species and enhanced specificity.

Purpose: The purpose of our study is to develop a *Vibrio* multiplex PCR and to evaluate its specificity with various *Vibrio* strains suggesting novel diagnostics of *Vibrio* spp. and five major *Vibrio* species.

Methods: Total 30 genome sequences of *Vibrio* were subjected through comparative genomics to screen specific genes for *Vibrio* spp. and five major pathogenic *Vibrio* species. Primer sets were designed and each primer sets were reacted with various DNAs of *Vibrio* type strains and other non-*Vibrio* pathogenic bacteria. A multiplex PCR was developed and evaluated with various *Vibrio* strains isolated from Korean local areas.

Results: Primers designed from *Vibrio* genome sequences showed the specificity on their target *Vibrio* spp. and species by PCR with type strains of *Vibrio* and non-*Vibrio*. A multiplex PCR was developed by 6 primer sets enabling detection of *Vibrio* spp. and five pathogenic *Vibrio* species and its specificity was confirmed with various *Vibrio*/non-*Vibrio* type strains. Total 117 *Vibrio* isolates were identified as *Vibrio* spp., *V. parahaemolyticus*, *V. vulnificus* and *V. alginolyticus*. And the specificity of the multiplex PCR was confirmed by sequencing the PCR products.

Significance: The developed multiplex PCR was proved its discriminating ability with various *Vibrio* type strains and isolates. We expect that this multiplex PCR could be applied to the accurate monitoring *Vibrio* species with advantages to protect human health against *Vibrio* infection.

P3-59 Multiplex PCR Assays for Simultaneous Detection of Genetically Modified Organisms (GMO) from Crops and Processed Food

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Introduction: Identification and quantification of genetically modified (GM) crops is challenging due to the increasing varieties available, the expanding number of commercialized events, turnaround time needed to process samples, and the myriad of government regulations and guidelines. Thus, rapid and effective qualitative screening as a first step has becomes more daunting. Multiplex PCR assays (m-PCRs) that simultaneously amplify many distinct GMO-related genetic elements can detect wide range of GMOs in a single reaction and, are therefore cost-effective for qualitative GMO screening.

Purpose: To develop multiplex PCR assays that can specifically screen all currently approved GM crops from agricultural raw materials as well as processed food.

Methods: m-PCRs for screening 8 major GM crops including corn, soybean, cotton, potato, alfalfa, sugar beet, wheat, and rice were developed with primers targeting the most common genetic elements covering currently approved GM crops. Specificity and sensitivity of each assay were determined by testing against commercially available standard GM materials: - 12 GM corns, 8 GM soybeans, 9 GM cottons, 1 GM potato, 1 GM sugar beet, 1 alfalfa, 1 GM wheat and 1 GM rice - in different ratios of spiked GMO contamination. Also evaluated were differently processed foods ranging from bulk crops, cake mixes, and popcorns to highly processed foods such as cereal, nutrition bar, and canned vegetables.

Results: Eight different m-PCRs were developed for screening GM corn, GM soybean, GM cotton, GM sugar beets, GM alfalfa, GM potato, GM wheat, and GM rice. Each PCR assay specifically amplified GMO-specific elements from DNA purified from all 34 standard GM materials tested. All multiplex PCR assays detected 10-100 copies of target gene. The limit of detection was less than 0.01% (wt/wt) target DNA and less than 0.1% (wt/wt) target crop contaminations. These assays also detected GM crops from 55 distinctly processed food samples including powders and liquids as well as highly processed canned.

Significance: The m-PCRs approach developed in this study allows for simultaneous detection of GM crops in one reaction with adequate sensitivity. It is rapid and cost effective, and therefore, highly applicable to the screening of GMO.

P3-60 Universal Multiplex PCR for Detection of *Escherichia coli* O157:H7 and *Salmonella* spp. in Food Samples

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* spp. are major foodborne pathogens. Traditional microbial detection methods such as enrichment culture-based identification is time consuming and labor intensive.

Purpose: Multiplex PCR could be a reasonable solution to detect microbial contamination in food samples. However, multiplex PCR assays have several disadvantages, such as increased complexity from multiple primer sets, low amplification yield, variable efficiency on different templates and poor universal applicability caused by different annealing temperature requirements of multiple primer sets.

Methods: A universal multiplex PCR (UM-PCR) could overcome these kinds of problems and increase the specificity and sensitivity of a detection method. A UM-PCR was developed and used to simultaneously detect *E. coli* O157:H7 and *Salmonella* spp. in food samples.

Results: This UM-PCR method detected the presence of *E. coli* O157:H7 and non-O157 Shiga toxin-producing *E. coli* by targeting *eae*, stx_1 and stx_2. At the same time, *Salmonella* spp. could be detected by targeting *inv*A and *sgx* in the same assay with a sensitivity of 10^4 CFU/ml. We could detect 7 major enterohemorrhagic strains including *E. coli* O157:H7 and 34 serotypes of *Salmonella* in an enriched sample of ground beef and of leafy green vegetables using a 1:1 and 1:3 inoculation, respectively.

Significance: UM-PCR greatly improves the range of applicability of a multiplex PCR assay. In addition, this method could be applied in other areas, such as the analysis of polymorphisms, quantitative assays for pathogens and the identification of bacterial species.

P3-61 Development of an Automated Multiplexed Immunomagnetic Separation System for Isolating Shiga Toxin-producing *Escherichia coli*

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Introduction: In recent years, non-O157 Shiga toxin-producing *Escherichia coli* (STEC) have become an emerging problem.

Purpose: Efforts have been devoted to facilitating and speeding their detection; however, their isolation from high background microbiota foods remains problematic. To solve this problem, immunomagnetic separation (IMS) is commonly used.

Methods: Here we describe the development of an automated multiplexed IMS assay capable of isolating *E. coli* O26, O45, O103, O111, O121, O145 and O157 and *E. coli* expressing intimin using the KingFisher Flex.

Results: This platform presents many advantages: 1) high-throughput setup (up to 96 samples simultaneously); 2) programmable (number of washes, temperature, and mixing speed); and 3) automation. The multiplexed format will save time, labor, reagents, and test sample. The specificity of the serogroup-specific antibodies used was tested against 240 STEC isolates belonging to 29 serogroups. Except for 4 strains belonging to the O111 (3) and O157 (1) serogroups, nearly all strains (98.3%) were correctly identified. Of the 240 strains tested, 161 were genotyped for the attaching-and-effacing gene (*eae*) and expression of the corresponding intimin was confirmed using HeLa cells. A total of 78 strains carried *eae* with 62 expressing intimin and 2 expressing type 1 fimbria, while 83 did not carry the *eae* with 8 expressing type 1 fimbria and 54 expressed some other adhesin(s). A total of 7 intimin antibodies were also developed and tested against 12 of those strains. Of these, 4 intimin antibodies showed promising results (> 90% specificity) for inclusion in our assay.

Significance: Future directions include testing those intimin antibodies against more STEC strains, including other serogroup-specific antibodies, and testing this assay in foods. Our assay will provide meaningful data for releasing safer foods, thus minimizing annual cost and numbers of recalls, enhancing public health, and allowing utilization of the same standard protocol throughout regulatory agencies in a more user-friendly manner.

P3-62 Rapid and Sensitive Detection of *L. monocytogenes* with Loop-mediated Isothermal Amplification (LAMP) Method Targeting *prfA* Gene

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Introduction: *Listeria monocytogenes* is one of the major foodborne pathogens that causes human listeriosis. Developing a rapid and sensitive screening method is important for the detection of the target pathogen present in various food matrices from a public health perspective.

Purpose: The aim of this study was to develop a rapid and sensitive detection tool for screening *L. monocytogenes* by using Loop-mediated isothermal amplification (LAMP) assay targeting *prfA* gene.

Methods: The LAMP assay in pure culture suspension (in 0.85 % NaCl solution) was performed at 62.5°C for 30 min to determine the inclusivity, exclusivity and the LoD of the assay. The LoDs of the LAMP assay in Listeria enrichment broth for 24 h incubation (LEB24) and LEB artificially contaminated with milk for 24 h incubation (LEB24-M) were determined and compared with those of PCR and real-time PCR assays.

Results: The inclusivity and exclusivity were 100 % and the limit of detection (LoD) was 2.22×10^2 CFU/ml at 19.5 min in pure culture suspension. The LoD of the LAMP assay in LEB24 was 2.22×10^0 CFU/ml which is identical with the real-time PCR result but 10^2 times sensitive than the PCR result. The LoD of the LAMP assay in LEB24-M was 2.22×10^0 CFU/ml which is 10 and 10^2 times sensitive than real-time PCR and PCR results, respectively.

Significance: These data indicate that the LAMP assay is a rapid, simple and sensitive assay which could be used as a potential screening tool of *L. monocytogenes*.

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P3-63 Development of Loop-mediated Isothermal Amplification for the Rapid Detection of *Arcobacter* Species in Chicken

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Introduction: *Arcobacter* is one of foodborne pathogenic bacteria which may cause gastroenteritis in human and abortion in animal.

Purpose: The aim of this study was to develop a more sensitive, specific and rapid technique of loop-mediated isothermal amplification (LAMP) for detecting of *Arcobacter* species.

Methods: The LAMP reaction was optimized under different conditions. Specificity and sensitivity of LAMP were compared with multiplex polymerase chain reaction. Bacterial isolation, multiplex PCR, and LAMP were performed in chicken samples collected from retailed markets.

Results: The optimization reaction condition for LAMP was achieved at 61°C for 50 min and ladder-like DNA products were produced with reference strains and field isolates of *Arcobacter* species. Because multiplex PCR showed the cross-reactivity with *Campylobacter* species, the LAMP assay was specific rather than multiplex PCR which was used in previous studies. The detection limit of LAMP was 2-20 CFU/reaction in vitro and 200 CFU/reaction in chicken samples. The sensitivity of LAMP assay showed a tenfold to thousand-fold times more than that of multiplex PCR assay.

Significance: The LAMP assay developed in this research is rapid and reliable detection technique for *Arcobacter* species which can cause food poisoning in human.

P3-64 Novel Agglutination Assays Using Phage Ligand Proteins to Identify the Top 7 Shiga Toxin-producing *Escherichia coli* (STEC) Serogroups

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are an important cause of foodborne illness. *E. coli* O157:H7 is implicated in the majority of the outbreaks and Hemolytic Uremic Syndrome cases. However, other serotypes such as O26:H11, O103:H2, O145:H28, O111:H8, O121 have also been implicated. As a result, the food industry needs fast, sensitive and complete methods for STEC detection to ensure a safe food supply.

Purpose: This study was designed to evaluate latex agglutination assays using phage ligand proteins for the top 7 STEC serogroups.

Methods: The latex test was performed by mixing a colony taken directly from a selective agar with a drop of the blue latex suspension. A positive agglutination is obtained within 1 minute of mixing. For each serogroups, the inclusivity study was performed on 20 specific strains and the exclusivity on 33 non-target strains from other *E. coli* serogroups or other gram negative or gram positive bacteria.

Results: The inclusivity study showed a high specificity: all target organisms produced positive results in less than one minute. The exclusivity study showed that none of the latex assays cross reacted with the other *E. coli* serogroups or bacterial species tested.

Significance: These rapid, sensitive and reliable agglutination assays can be used for the identification of presumptive isolated STEC colonies from selective media. They can be an economic, rapid and sensitive alternative to PCR for identification of the positive colonies to be further studied for the presence of *eae* and *stx* virulence genes, as described in the USDA MLG 5B.03 protocol.

P3-65 Withdrawn

P3-66 Quantitative Validation of Two Novel Selective Media for the Enumeration of *Bacillus cereus* in Fermentation Sauce Samples

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Introduction: In the previous studies, we developed two novel selective media, Blood-yolk-polymyxin B-trimethoprim agar (BYPTA) and modified mannitol-yolk-polymyxin B agar (mMYPA), for the enumeration of *Bacillus cereus* (*B. cereus*) in foods. BYPTA was developed by the addition of egg yolk, laked horse blood, sodium pyruvate, polymyxin B, and trimethoprim, and mMYPA was developed by supplementing mannitol-yolk-polymyxin B agar (MYPA) with trimethoprim.

Purpose: In this study, two novel selective medium were compared with MYPA for the isolation and enumeration of *B. cereus* in naturally contaminated fermentation sauce samples.

Methods: A total of 46 fermentation sauce samples were purchased from a local retail market. Each sauce sample (25 g) was suspended in 225 ml of Butterfield's phosphate-buffered water and homogenized for 2 min. Subsequently, 1 ml of homogenate was serially diluted (10-fold) in PBS followed by the inoculation of 0.1 ml of each dilution onto three plating media in duplicate. MYPA and mMYPA were incubated at 30°C and BYPTA plates at 37°C for 24 h.

Results: No statistical difference was observed in the quantitative enumeration ($P > 0.05$) between three selective media, whereas BYPTA and mMYPA exhibited higher selectivity than MYPA excluding the background flora in fermentation food samples.

Significance: Two novel selective medium could be a useful enumeration tool to assess the level of *B. cereus* in various fermentation sauce samples.

P3-67 Molecular Serotyping and Sub-typing of *Salmonella* Strains by Genome Sequence Scanning

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Introduction: *Salmonella* serovars are the leading cause of foodborne illnesses in the U.S. leading to hospitalization and death. Genome Sequence Scanning (GSS), a single molecule technology, was developed by Pathogenetix for bacterial identification.

Purpose: This study evaluated GSS performance as a molecular serotyping and sub-typing tool for *Salmonella*.

Methods: Samples of bacterial strains were prepared by an automated sample preparation module that carried out extraction, restriction digestion and sparse labeling of genomic DNA with sequence-specific, fluorescent tags. The individual DNA fragments were microfluidically linearized and their fluorescence measured in an automated detection module to generate sequence-specific patterns, "traces". Traces of all strains were then compared using proprietary algorithms that compute a similarity parameter, which was used to cluster strains on a GSS-generated phylogenetic tree. Sequence data from whole-genome sequenced strains were processed *in-silico* and added to the tree.

Results: A collection of more than 230 *Salmonella* strains kindly provided by FDA, representing the most frequently encountered serovars associated with human illness as well as isolated from food products, was used to test the strain typing capability of GSS. In general, strains clustered into serovar-specific branches on the GSS-phylogenetic tree, indicating a clonal lineage origin for most serovars. Polyphyletic lineage serovars like Newport formed more than one distinctly separated branch on the tree.

Significance: The results show that GSS could be used to reliably differentiate and type *Salmonella* serovars significant for public health and food safety and aid in epidemiological investigations with a time-to result of less than 5 h. Serovar-specific cladal organization of *Salmonella* strains on the GSS tree would help to allocate serovar designation to unknown strains based on their position and identity of close neighbors on the GSS tree.

P3-68 The Smart DNA-based Chemiluminescence Resonance Energy Trasfer (CRET) for the Detection of Ochratoxin A in Coffee

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Introduction: The CRET has been one of the ways to reveal bio-molecules and its theoretical background is very similar to Fluorescence resonance energy transfer. The CRET has been investigated as a diagnostic tool in medical fields and expanded to environment and food fields.

Purpose: In this study, we developed a CRET-based biosensor using single strand DNA (aptamer) to detect an OTA in coffee without dilution.

Methods: The CRET was optimized with appropriate concentrations of aptamer toward OTA and hemin, volume of coffee extracts undiluted. To validate the CRET system, OTA-positive coffee samples were prepared by spiking known concentration of OTA at 0.5 to 50000 µg/kg. The coffee samples were extracted with absolute methanol, and the extracts were directly tested by the CRET. Chemiluminescence was immediately measured by Chemi-Doc image (Bio-rad) and analyzed by chemi-doc analyzing program.

Results: As a result, the optimized CRET system could detect 0.005 ng/ml, and cross reaction to other mycotoxins was not observed. Total assay time of the CRET system to detect OTA was in 10 min, more rapid than that of analytical methods previously reported. The optimum concentration of aptamer was 100nM for detecting the chemiluminescence signal in coffee by Chemi-Doc image. Optimal volume of the coffee extracts and aptamer solution was used 4:6 ratio for OTA analysis. Which may be used methanol concentration is important to detect the OTA. Because of extraction method of OTA from real sample had contained methanol and it may used dilution step. In our system removed the dilution step for detection of OTA. As a result, more than 50 ppb of OTA had quenching efficiency of over 20% and less than 0.5 ppb of OTA had had quenching efficiency of over 10%. The over the limit of OTA is 5 ppb and our system can be detect 0.5 ppb in coffee without dilution.

Significance: As a result, we could detected the OTA using quenching or not of chemiluminescence without specific equipment. To detect the chemiluminescence signal just need to the simple image equipment such as CCD camera, CMOS chip and camera of smartphone.

P3-69 Rapid'Sakazakii to Detect *Cronobacter* spp. in Infant Formula: Certification According to the ISO16140 Standard

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Introduction: The RAPID'Sakazakii method is based on a chromogenic principle for *Cronobacter* spp. detection in infant formula. An ISO 16140 method comparison study was conducted, by analyzing 77 infant formula samples in the relative accuracy, sensitivity and specificity part and showing equivalent performances between the test method and the ISO/TS 22964 methods. The relative detection limits of the RAPID'Sakazakii and the ISO/TS 22964 methods were clearly similar and vary from 0.8 and 1.2 CFU/25g. The selectivity and specificity of the alternative method was assessed by testing 52 target strains and 31 non target strains.

Purpose: An independent inter-laboratory study was conducted at ADRIA, to compare the test methods precision to the ISO/TS 22964 one, as part of the NF Validation approval process and according to the ISO 16140 standard.

Methods: The test method protocol includes an overnight enrichment in BPW. The characteristic colonies are confirmed by running oxydase tests and biochemical galleries. Flexibility is offered by storing the enrichment broth at 5±3°C for 3 days, before streaking onto the selective test method agar.

Results: The alternative method was evaluated in a ring trial involving 16 laboratories. Probiotic infant formula containing *Lb. reuteri* was contaminated with the wild *C. sakazakii* strain. Eight blank samples, 8 samples contaminated at a fractional recovery level (0.8 cells/g) and 8 highly contaminated samples (20.6 cells/g) were sent to each collaborator. The calculated relative accuracy, sensitivity and specificity values were, respectively, 97.1, 75.5, and 94.2 %. The results of accordance, concordance, and odds ratio confirm that the test method precision is equivalent to the ISO/TS 22964 standard one.

Significance: Both the ISO 16140 method comparison and inter-laboratory studies clearly show that the test method is a reliable alternative method for *Cronobacter* spp detection in infant formula, offering important economic savings by reducing time to result and handling time.

P3-70 Development of a New Device for the Rapid Detection of *Alicyclobacillus*

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Introduction: A new Soleris® vial was developed to rapidly detect *Alicyclobacillus* contamination in beverages and raw materials. The vial, based on detection of carbon dioxide produced from the metabolism of the organisms, consists of a detection chamber containing carbon dioxide indicators separated by a barrier layer from a chamber containing a growth medium and test sample. The majority of spoilage *Alicyclobacillus* microorganisms can be detected within 48 hours. The vial is used in conjunction with the test instrument system.

Purpose: The purpose of this study was to develop and assess the performance of a device for the rapid detection of spoilage *Alicyclobacillus*-microorganisms in the juice and beverage industries.

Methods: Experiments were performed to select the optimal growth medium and indicator chemistries for the most rapid detection of *Alicyclobacillus* microorganisms. Inclusivity studies used a panel of the target organisms at levels of <100 CFU/ml. The detection time in the test instrument and growth of low inoculum levels of organisms in food matrices were examined. A confirmatory test for guaiacol producing *Alicyclobacillus* was performed using the contents from a vial with positive growth.

Results: The inclusivity test panel consisted of eight *Alicyclobacillus* organisms. The instrument detection times were as low as 12.2 hours for 12 CFU/ml of *A. acidoterrestris* ATCC 49025. In juice, the detection time was 10.9 hours for an inoculum level of 8 CFU/ml of *Alicyclobacillus* spore

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suspension in lemonade pre-incubated for 24 hours. The majority of the other target organisms containing < 100 CFU/ml detected in < 48 hours. The food matrices tested did not interfere with the vial test.

Significance: The new test *Alicyclobacillus* vial provides a system for the rapid detection of spoilage *Alicyclobacillus* microorganisms in many beverage products in < 48 hours compared to the standard incubation time of 5 days for agar plates.

P3-71 Soleris® Direct Yeast and Mold as an Alternative to Dilution Plating for Determination of Yeast and Mold Levels in Foods

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Introduction: Soleris Direct Yeast and Mold (DYM) is an automated, growth-based method for determination of yeast and mold levels in foods. Using selective growth media and an indicator dye system, carbon dioxide is detected as a function of microbial metabolism. The result is available within 48 hours.

Purpose: The study was designed to statistically compare semi-quantitative yeast and mold results produced by the test method with FDA-BAM method dilution plating results. Method performance was assessed for 12 diverse food types: Ranch salad dressing, yogurt, ice cream mix, nonfat dry milk, orange juice concentrate, tomato juice, dried fruit, corn flour, cocoa powder, black pepper, saw palmetto powder, and dry pet food.

Methods: Both naturally contaminated and inoculated foods were used in the study. For each food, a 1:10 sample homogenate was prepared using standard methods. Further decimal dilutions were prepared for both the reference plating method and test methods. Twenty vials were inoculated at each of 3 or more dilutions. The results were analyzed statistically using a probability of detection (POD) model which compared the observed POD for the test method with the POD predicted by the reference method plate count for a particular dilution.

Results: In total, results were analyzed for 1,200 determinations performed from 60 dilutions. Based on predicted POD values, input levels ranged from 0 to 126 CFU/vial. Of 60 statistical tests, there were only 7 cases where the predicted POD did not fall within the 95% confidence intervals of the observed POD. In 6 of these cases, the test method provided a higher estimate of the yeast and mold count compared to the reference method.

Significance: A high level of agreement was observed between yeast and mold levels determined by the test method and dilution plating methods. Soleris DYM provides the user with a rapid, automated alternative to traditional yeast and mold methods.

P3-72 Development and Evaluation of New PNA Probes for Whole Cell Detection of *Candida albicans*

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Introduction: *Candida albicans*, which may be a contaminant in foods ranging from cheeses to dry-cured meats, is an important cause of systemic fungal infection. Techniques able to differentiate *C. albicans* from other *Candida* spp. may enable rapid detection of this pathogen in foods and aid timely application of appropriate antimicrobial therapy. Peptide nucleic acid (PNA) may have significant practical advantages over DNA-based technology in such methods.

Purpose: To develop new PNA-FISH probes specific for *C. albicans* and compare their performance against DNA probes targeting the same regions on the 28S rRNA; to use flow cytometry (FCM) for quantitative analysis of probe hybridization.

Methods: Two sets of DNA probes and their corresponding PNA counterparts were selected from alignments of the 28S rDNA of representative *Candida* species. One target region was recently shown by our group to be addressable with the DNA-FISH probe Calb2208. The other probe region is unique and has not been previously described. Fluorescently-labeled DNA and PNA probes having similar melting temperatures were evaluated against a panel of yeasts, including *C. albicans* and other *Candida* spp. Fluorescence microscopy and benchtop FCM (BD Accuri C6) were used for qualitative and quantitative analyses, respectively. Probes were evaluated for hybridization intensity, staining uniformity and specificity for *C. albicans*.

Results: Overall, the PNA probes were brighter and provided more uniform staining than the DNA probes. PNA CalB2208 was brighter than PNA 28S rRNA-3, but also yielded higher non-specific staining. Ratiometric analysis of FCM data indicated that PNA 28S rRNA-3 was 2.5 – 5-fold more discriminatory than PNA CalB2208.

Significance: PNA-FISH was shown to be diagnostically superior to DNA-FISH in this application. A novel probe, 28S rRNA-3, was developed and shown to be highly discriminatory for *C. albicans*. This probe may enable rapid, selective identification of *C. albicans* in both foods and in clinical samples.

P3-73 Rapid Detection of *Listeria* spp. from Environmental Samples Using a New Immunomagnetic Assay

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Introduction: Ubiquitous *Listeria* species are the microbial targets of most Food Quality Management Programs, particularly those due to *L. monocytogenes*. This species is among the most important causes of death from foodborne infections in all industrialized countries. The preventive activity requires the rapid detection of this pathogen or its indicator to guarantee the production of safe food.

Purpose: The aim of this study was to validate FoodChek™ Listeria spp. assay and to compare its efficacy to the reference method MLG 8.08.

Methods: The performance of the new immunomagnetic lateral flow assay was evaluated using ACTERO™ Listeria Enrichment Media allowing a 24-hour single enrichment step, and following the Guidelines of the AOAC for the Performance Tested Methods℠. The evaluation included a matrix study of *Listeria* spp. detection from environmental samples. There were 121 environmental samples from stainless steel and plastic food contact surfaces tested by the new assay and these were compared with 52 samples analyzed using the USDA-FSIS reference cultural method. The Chi square analysis was performed to evaluate the difference between both methods.

Results: The *Listeria* spp. assay detected the five species most frequently isolated from food (*L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. seeligeri*, *L. welchimeri*). None of the 30 non-*Listeria* strains tested reported a positive signal.

Listeria spp. was more frequently detected from plastic samples by the new assay ($\chi^2 = 4.4$; $P < 0.05$) as compared to MLG 8.08. However, no differences ($\chi^2 = 2.0$) were observed when stainless steel samples had been analyzed. The original assay allows for the detection of *Listeria* spp. from environmental surfaces in less than 25 h, including enrichment and detection steps.

Significance: The new immunomagnetic *Listeria* spp. assay provides food processors with a rapid and reliable tool to monitor and to control *Listeria* spp.

P3-74 Application of VITEK® MS System for the Identification of Pathogens from the Isolated Colonies on Chromogenic Media from a Variety of Food Samples

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Introduction: The VITEK®MS system uses Matrix Assisted Laser Desorption Time-of-Flight Mass Spectrometry (MALDI-TOF MS) to analyze spectral patterns of ionized molecules derived from intact microorganisms for their identification. This novel system provides microbial identification with similar specificity as 16S rRNA gene sequencing and could prove valuable for identification of pathogens from foods.

Purpose: Evaluate a MALDI-TOF MS based system for the identification of *Salmonella*, *Listeria* and *Campylobacter* spp. from isolated colonies on chromogenic media from a variety of food products.

Methods: Thirty strains each of *Salmonella* spp., and *Listeria* spp. and 15 strains of *Campylobacter* spp. were artificially inoculated into a variety of food matrices independently, enriched, and detected using target specific systems and isolated on selective chromogenic media and differential media including ALOA® and PALCAM for *Listeria* spp.; CampyFood Agar for *Campylobacter* spp.; and ASAP™ and XLD for *Salmonella* spp. Typical colonies (2-5) on each media were identified using the MALDI-TOF MS based system.

Results: All *Salmonella*, *Listeria* and *Campylobacter* strains were recovered from enriched food samples on the chromogenic media and differential media studied and correctly identified (100%) using the MALDI-TOF MS based system. Additionally, colonies of all strains on the chromogenic media and sheep blood agar from pure cultures were also correctly (100%) identified.

Significance: Identification of the pathogen after their detection from food samples could be directly performed by VITEK MS using the colonies isolated on selective chromogenic media. This has potential to eliminate the requirement of isolating colonies on non-selective media before their further identification.

P3-75 *In silico* Adaptation of EDNA (E-probe Diagnostic Nucleic Acid Analysis) for Detection of Foodborne Pathogens

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Introduction: Increasing consumption of fresh produce over the past decade has been accompanied by increasing numbers of foodborne illness outbreaks linked to contaminated product. A variety of pathogenic microbes can contaminate fresh produce at different points within the production process. The development of a pathogen detection method capable of providing a comprehensive microbial profile of a complex food sample would significantly enhance our capability to respond to outbreaks.

Purpose: Adapt a bioinformatics pipeline strategy, EDNA, to generate inclusive pathogen profiles using metagenomic data from complex food samples.

Methods: Fecal coliforms, strains of *Escherichia coli* O157:H7, pathogenic *E. coli* strains, and Shiga toxin sequence-specific electronic queries (e-probes) were developed and tested against sixteen mock sample databases (MSDs) containing 10,000 genome segments of both pathogen and a model plant host, *Vitus vinifera* (grapevine), using BLASTn parsed with an e-value of 1×10^{-3}. Decoy e-probe sets, designed to determine background positive levels, were developed and queried against the MSDs for statistical analysis. Precision (true positives/(false + true positives)) was calculated for all e-probe sets, and statistical confidence in positive calls was assessed via t-test.

Results: Optimum E-probe length was established by calculating precision. When microbe abundance in samples exceeded 0.5%, precision of *E. coli* e-probes of 20 and 40 nucleotides averaged 99% and 99.2%, respectively. The EDNA e-probes (20-40 nt) successfully detected *E. coli* at higher concentrations (> 0.5% abundance), and the Shiga toxin e-probes (80 nt) allowed detection when the toxin was > 1.0% abundance (precision = 100%). Longer *E. coli*e-probes also identified the pathogen at concentrations above 5% abundance, but t-test statistical confidences were limited by the total number of e-probes available, despite the fact that precision was 100%.

Significance: This bioinformatics approach to microbial detection has the potential for simultaneous detection of all foodborne pathogens present in a food sample.

P3-76 Evaluation of 3M™ Tecra™ *Staphylococcus aureus* Visual Immunoassay for the Detection of *Staphylococcus aureus* in Selected Foods

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Introduction: The 3M Tecra *Staphylococcus aureus* Visual Immunoassay (STAVIA) is an Enzyme-Linked Immunosorbent Assay (ELISA) performed in a 'sandwich' configuration using proprietary antibodies, intended as a rapid and specific screening test for the presumptive detection of *S. aureus* in food samples.

Purpose: The aim of this study was to compare the method for the detection of *S. aureus* in a range of foods with a standard cultural method, AOAC Official Method 975.55.

Methods: In total, 1200 food samples were analyzed, representing 20 food types at 3 levels of contamination. Both naturally and artificially contaminated samples were included. In addition, an inclusivity and exclusivity study was performed.

Results: Of the 1200 samples tested, 699 were confirmed positive by the method while the Reference Method confirmed 332 positive. Fractional recovery was achieved for all food types at the low level inoculums (< 5 cells/g). Overall, the results of this study show that the test gave an increased proportion of positive results when compared to the Reference Method in its recovery of *S. aureus* from foods inoculated at the low level. Chi-square analysis at the 5% level shows that there was significant improvement in recovery of *S. aureus* contamination from these samples compared to the Reference Method. For uninoculated and high-level samples (10-50 cells/g), the two methods were statistically equivalent.

Significance: The test method provides simple and sensitive presumptive detection of *S. aureus* within 27 hours.

P3-77 Performance of the 3M™ Molecular Detection Assay *Listeria* as Compared to the Canadian Reference Method MFHPB-30

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Introduction: In recent years, about 132 cases of listeriosis on average were reported annually in Canada. *Listeria* is frequently associated with environmental contamination within food production facilities and is a significant food safety concern and cost. The 3M™ Molecular Detection Assay *Listeria* is used with the 3M™ Molecular Detection System for the rapid and specific detection of *Listeria spp.* in enriched food and environmental samples. The 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 Molecular Detection Assay *Listeria spp.* against the Compendium of Analytical Method MFHPB-30 in a variety of environmental surfaces for the inclusion in the Compendium of Analytical Method as a Laboratory Procedure (MFLP).

Methods: The method and comparative reference method (MFHPB-30) were analyzed by testing 9 typical food environmental surfaces (Stainless Steel, Plastic, Ceramic, Rubber, Food-grade painted surfaces, Wood, Sealed concrete, Cast iron, Air filter material). 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 (POD) statistical model and showed perfect performance, exceeding the criteria outlined in the Health Canada MMC.

Significance: The Molecular Detection Assay *Listeria spp.* showed excellent performance and exceeded the Canadian requirements of the MMC. Additionally, modifications to the method (shorter handling times, lower enrichment volumes, shorter stomaching times) offers the capability of detecting *Listeria spp.* in environmental samples rapidly and effectively over other methods.

P3-78 Evaluation of the Aerobic Procedure for the Recovery of Lactic Acid Bacteria with 3M™ Petrifilm Plates™ in Ready-to-Eat Meat Products

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Introduction: The detection and enumeration of lactic acid bacteria (LAB) has relevance among ready-to-eat meat (RTE) products because LAB are responsible of food spoilage, causing the development of flavors, odors and gas which shortens the shelf life of this type of products. The use of alternative methods for this analysis could reduce the cost and time-to-results.

Purpose: The objective of this study was to evaluate the performance of the Aerobic Procedure for Lactic Acid Bacteria with 3M™ Petrifilm™ Aerobic Count Plates compared to the Agar method using acidified MRS (deMan, Rogosa and Sharpe) referred in the Compendium of Method for the Microbiological Examination of Foods.

Methods: A total of 53 samples from a mix of turkey and pork ham were artificially inoculated with LAB strains commonly found in these products (*Leuconostoc mesenteroides* and a mixture of *Lactococcus lactis spp lactis* and *Lactobacillus brevis*) at a medium level of 50-100 CFU/plate. Samples were analyzed in duplicate by standard plate count method using acidified MRS agar to pH 5.5 ± 1 and the Aerobic Procedure for LAB that utilizes the Petrifilm Aerobic Count Plate supplemented with MRS medium and a pH indicator to help identify and enumerate lactic acid bacteria.

Results: The counts from both methods were converted to log values and averaged. A paired *t*-test for two sample means was calculated to analyze statistical differences. Results do not show any significant differences ($P = 0.56$) between the methods, the Pearson correlation coefficient (r) was also determined showing a positive correlation (0.96).

Significance: Results shown that the Aerobic Procedure for the detection and enumeration of LAB is a reliable and easy method that reduces time for confirmation and cost of sample preparation by eliminating the materials needed to create an anaerobic environment.

P3-79 A High Throughput Method for the Detection of STEC Top7 in Meat Samples

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Introduction: *Escherichia coli* O157:H7 and the top six (O26, O45, O103, O111, O121, and O145) non-O157 Shiga toxin-producing *Escherichia coli* (STEC) have emerged as important public health threats. A multiplex PCR method using GeneDisc technology was evaluated to screen STEC O157 and non-O157 in meat. The method targets the genes for the Top7 *stx*1-2 and other virulence factors.

Purpose: The purpose was to evaluate specificity and limit of detection of the PCR assays for the various gene targets, and to compare the performances to the USDA/FSIS 5B.01 for raw ground beef and raw beef trim after enrichment.

Methods: After enrichment of 375 g raw ground and beef trim in 1.5 l BPW for 10 h at 41.5°C, bacterial DNA was extracted, then analyzed with a multiplex PCR assay targeting the genes *stx*, *eae* and specific genes of the Top7 serogroups. When *stx*, *eae* and at least 1 specific gene of the Top7 serogroups were co-detected, the software displayed an alert indicating a presumptive positive. The method was evaluated using 400 ground and 150 trim processed in 4 beef facilities and compared to the USDA-FSIS MLG 5B.01 method. Presumptive positive samples were confirmed according to reference methods.

Results: Specificity of each PCR assay was demonstrated using 122 *E. coli* strains. The limit of detection of each PCR assay was 25 GU/PCR well, except for O145 (50 GU/PCR well), theoretically corresponding to 3E+04 and 6E+04 CFU/ml after enrichment, respectively. Beef samples artificially contaminated with O26, O103 or O157 showed 100% presence of all target genes at levels 10 - 2,500 CFU/PCR well. The multiplex and reference methods gave 3 and 7 presumptive positives in the uninoculated beef samples, respectively. Cultural confirmation yielded 3 Top7 STEC isolates, 2 of which were presumptively positive by multiplex. None of presumptive positives obtained by the reference method were confirmed.

Significance: The GeneDisc method may be useful for routine screening of beef samples for STEC Top7 serogroups with a time to result less than 12 hours.

P3-80 Survival of *Salmonella*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* on Pecans and Peanuts and Characterization of *Salmonella* Isolates

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Developing Scientist Competitor

Introduction: Outbreaks due to *Salmonella* and *Escherichia coli* O157:H7 and recalls associated with these organisms and *Listeria monocytogenes* are documented for the consumption of nuts

Purpose: Our objective was to evaluate the fate of *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* on pecans and peanuts stored at -20, 4 and 23°C for up to 12 months, and to characterize *Salmonella* isolates remaining after 18 months of storage.

Methods: Raw pecans and peanuts were inoculated with cocktails of nalidixic acid-resistant strains of *Salmonella, E. coli* O157:H7 and *L. monocytogenes,* dried for 72 h, and stored at -20, 4, 23°C for 18 months. Microbial populations were enumerated over 12 months by stomaching 10-g subsamples and plating onto specific and non-specific media supplemented with nalidixic acid. After 18 months, 100 *Salmonella* colonies isolated from each storage temperature were characterized to determine the prevalence of each cocktail strain by a combination of serogrouping, antibiotic resistance, and PCR for strain-specific genes.

Results: At 23°C, *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* declined linearly at rates of -0.20, -0.33, -0.54 log CFU/g/month on peanuts and -0.16, -0.33, -1.05 log CFU/g/month on pecans, respectively. At 4°C, population of *Salmonella* declined on peanuts at -0.039 log CFU/g/month and remained relatively stable on pecans. *E. coli* O157:H7 and *L. monocytogenes* populations remained stable at 4°C storage on both nut types. At -20°C, populations of the three pathogens remained stable on both nuts with the exception of *L. monocytogenes* which declined at -0.06 log CFU/g/month on peanuts. *Salmonella* Tennessee predominated on peanuts and pecans -20, 4 and 23°C, except on peanuts stored at 23°C storage where *Salmonella* Enteritidis 9C was predominantly isolated.

Significance: Pathogens, if present, can survive for extended periods of time at a broad range of storage temperatures on raw pecans and peanuts. Storage under refrigerated or freezing temperatures does not result in pathogen reduction.

P3-81 Survival of *Salmonella*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* on Inoculated Inshell Chandler Variety Walnuts

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Developing Scientist Competitor

Introduction: After harvest, hulling and dehydration, walnuts are stored in-the-shell for up to 1 year prior to cracking.

Purpose: Our objective was to determine the survival of *Salmonella, Escherichia coli* O157:H7 and *Listeria monocytogenes* on inoculated inshell walnuts under conditions that mimic commercial storage.

Methods: Inshell walnuts were collected from a commercial huller prior to drying and inoculated with five-strain cocktails of rifampicin-resistant *Salmonella*, *E. coli* O157:H7 or *L. monocytogenes* suspended in walnut-rinse water. Walnuts (400 g) were separately inoculated at 8 log CFU/nut by mixing with 25 ml of each cocktail. Inoculated nuts were dried at 43°C and 35% relative humidity (RH) to mimic commercial practices, and stored using previously-determined commercial storage conditions (10°C, 65% RH). Samples from each of two separate trials (n=6) were processed at monthly intervals. Appropriate dilutions were plated onto tryptic soy (TSA) and either bismuth sulfite (*Salmonella*), sorbitol MacConkey (SMAC) (*E. coli* O157:H7) or modified Oxford agars (MOX) (*L. monocytogenes*), all supplemented with rifampicin (Rif). All media was incubated at 37°C for 24 h (*Salmonella* and *E. coli* O157:H7) or 48 h (*L. monocytogenes)*.

Results: Population densities of all pathogens decreased by 3 log CFU/nut during the 10- to 12-h drying period. During storage, *Salmonella* declined by a consistent 1 log CFU/nut/month. In contrast, *E. coli* and *L. monocytogenes* populations decreased rapidly within the first 8 and 27 days of storage, to 3 and 2 log CFU/nut, respectively; thereafter, population densities declined very slowly as measured on TSARif. Counts on SMACRif and MOXRif were significantly lower indicating the presence of injured cells.

Significance: Significant decreases in foodborne pathogens are observed during drying and initial storage of inshell walnuts but low-level long-term persistence can occur.

P3-82 The Effect of Initial Almond Water Activity on Thermal Inactivation of *Enterococcus faecium* during Dry Heating

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Developing Scientist Competitor

Introduction: Validating commercial thermal pasteurization processes for low-moisture food products can be very challenging, due to dynamic process/product conditions and bacterial adaptation. In particular, changing water activity (a_w) is the most critical factor, but it has not been well incorporated into inactivation models that can be easily applied to commercial processes.

Purpose: To quantify the effect of initial product a_w on the inactivation rate of *Enterococcus faecium* (a non-pathogenic surrogate for *Salmonella*) inoculated on the surface of almonds, and the predictive ability of a modified thermal inactivation model.

Methods: Almonds were inoculated with *Enterococcus faecium* (~10^8 CFU/g), equilibrated to three water activities (~0.34, 0.55, 0.85), placed in a steel mesh rack (15 almonds), and heated in a computer controlled oven (~149°C dry air, ~11°C dew point) for 0 to 15 min (1 min intervals; triplicate runs). After thermal treatment, a_w of each sample was measured (4 almonds). The rest of the sample (11 almonds) was immediately cooled in peptone water (~4°C), plated on MRS, and enumerated. A previously published, modified inactivation model, accounting for process dew point, was used to predict *E. faecium* inactivation based on measured almond surface temperature and process dew point.

Results: The accuracy of the model prediction was reasonably good (RMSE = 0.57 and 0.33 log for low and high a_w, respectively). Also, the model parameters were re-estimated based on the present data, in order to compare responses at the different initial a_w levels. All the parameters (D_r, Z_T, and Z_M) of the model were significantly ($P < 0.05$) different for each group, which implies that the initial a_w significantly affected the pathogen response.

Significance: Therefore, model-based validations of thermal pasteurization processes will be improved by incorporating product a_w, in addition to process humidity, into thermal inactivation models.

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P3-83 Fate of *Salmonella* in Soil, Water and Quartz Particle Biofilms

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Introduction: Better understanding of *Salmonella* survival characteristics in soil and water, where nutrient availability is often limited, may provide useful information on conditions that lead to persistence in the farm environment.

Purpose: This work was conducted to determine the survival, injury, and presence of rdar morphotype of *Salmonella* serovars in soil and water. Additionally, *Salmonella* biofilm-forming potential on glass and quartz particles was determined.

Methods: Produce outbreak-associated *Salmonella* serovars were cultured in TSB and washed prior to use. Soil (50 g) obtained from a tomato farm in Virginia was sieved, autoclaved, and then inoculated with 5 ml of *Salmonella* suspension. Sterile, deionized water (45 ml) was inoculated with 5 ml of *Salmonella* suspension. Soil and water samples were stored at 30°C for 40 days and sampled every ten days. Bacterial enumeration was performed on XLT-4 and TSA incubated at 35°C for 24-48 hours. Ability to express curli and cellulose (rdar morphotype) was determined using congo red agar. The biofilm forming potential *Salmonella* serovars was also determined on glass cover slips and quartz particles using 0.1% Crystal Violet staining to estimate biofilm mass.

Results: There was a 2.6 log CFU/g and 2.7 log CFU/ml average decrease in populations of *Salmonella* serovars in soil and water, respectively, over the 40 day period. Cell injury was greater in soil than in water ($P < 0.05$). Serovars tested were capable of producing the rdar morphotype on congo red agar. The serovars tested were capable of biofilm attachment to quartz particles and glass.

Significance: *Salmonella* populations survived well in soil and water and displayed the rdar morphotype that is associated with environmental persistence. Biofilm formation on glass and quartz particles was observed. Extended survival in the farm environment and biofilm formation on soil surfaces may aid in dispersal in the farm environment.

P3-84 Can *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7 Survive on Fresh Produce Cardboard Boxes?

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Introduction: Cross-contamination of foodborne pathogens from fomites to food surfaces has been an increasing issue in the food industry. Communication with small farmers showed that produce boxes made from cardboard are constantly reused. This practice may increase the risk of cross-contamination of foodborne pathogens from produce to boxes and vice versa.

Purpose: The purpose of this study was to demonstrate the ability of *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia. coli* O157:H7 to survive over several days on cardboard produce boxes.

Methods: Cardboard boxes used for produce were obtained from farms in Houston, TX. Coupons (10 x 10 cm) were created, packaged in aluminum foil, autoclaved at 121°C for 15 min. *Salmonella* Typhimurium, *L. monocytogenes*, and *E. coli* O157:H7 overnight culture cocktail was inoculated on the coupons (~ 6 log CFU/cm^2) and samples were tested at days 0 (0 and 6 h), 1, 2, 5, 8, 11, 14, 17, 20, 23 and 29. The pathogens were plated on EMB and Palcam media and quantified.

Results: The results demonstrated that the three foodborne pathogens can survive on produce boxes over time. Up to 1 log CFU/cm^2 *E. coli* O157:H7 and *L. monocytogenes* and 2 log CFU/cm^2 were observed on the coupons on day 17. *E. coli* O157:H7 and *L. monocytogenes* were below detection limit on day 20. However, 1.5 log CFU/cm^2 *Salmonella*spp. was observed on day 20. All pathogenic microorganisms were below detection limit on days 23 and 29. Three biological replicates were carried out.

Significance: The results of this study show that produce boxes made from cardboard should not be reused since they can be a vector of foodborne pathogen transfer on fresh produce. An effective outreach program to communicate scientifically validated results may help reduce the risk of foodborne illness caused by fresh produce contamination and improve public health.

P3-85 Survival of *Salmonella enterica* Newport in *Ralstonia solanacearum* - Infected Tomato

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Introduction: The Eastern Shore of Virginia (ESVA) is responsible for 80% of Virginia's tomato production. Over the past decade, the ESVA has been implicated in at least four outbreaks of Salmonellosis associated with tomato, all originating from the same strain, *Salmonella enterica* serovar Newport. In addition to *S.* Newport contamination, the devastating plant disease, bacterial wilt, caused by the phytopathogen, *Ralstonia solanacearum*, is the most severe market disease of tomato on the ESVA and threatens the sustainability of ESVA tomato industry.

Purpose: Due to the ESVA's endemic population of *R. solanacearum* and *S.* Newport, the purpose of this study was to investigate the association between these two pathogens. The survival of S. Newport within tomatoes, symptomatic and asymptomatic for bacterial wilt, was examined.

Methods: Tomato fruit were collected from plants expressing symptoms of bacterial wilt (symptomatic) and plants not expressing bacterial wilt symptoms (asymptomatic) on the ESVA during the 2011 and 2012 summer growing season. Following collection, fruit were inoculated with 8 log CFU/ml *S.* Newport via vacuum infiltration and incubated for 48 hours at 13°C. *S. enterica* within the internal fruit tissues was then quantified by surface sterilizing and homogenizing the fruit, and plating the homogenate onto XLT-4. ANOVA was used to analyze results and means were separated using Tukey's HSD at $\alpha = 0.05$.

Results: *S. enterica* recovery was significantly greater in fruit originating from asymptomatic (5.15 log CFU/g) versus symptomatic (4.91 log CFU/g) plants ($n = 50$; $P = 0.0127$). Fruit collected from asymptomatic plants also had a significantly higher internal pH (4.60) than fruit collected from symptomatic plants (4.37; $P < 0.0001$).

Significance: These results suggest that *R. solanacearum* affects internal tomato fruit pH, which may reduce *S.* Newport survival in the fruit. This study supports previous research findings reporting phytopathogen influence on human pathogen survival in fruits and vegetables.

P3-86 Factors That Influence Survival of *Escherichia Coli* O157:H7 in Field-Inoculated Lettuce

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Introduction: Although *Escherichia coli* O157:H7 is not a natural colonist of plants, it can persist at low levels in the lettuce phyllosphere for extended periods of time. High humidity combined with warm temperatures have been shown to support the growth of *E. coli* O157:H7 on lettuce plants grown in an environmental chamber; the impact of these factors in the production environment is unknown.

Purpose: Our purpose was to evaluate the impact of inoculation time and plant age on the survival of *E. coli* O157:H7 on field-inoculated lettuce.

Methods: Five field trials were conducted in the Salinas Valley (spring 2010, summers 2010, 2011, and 2012, and fall 2012). For each field trial, lettuce plants that were 4 or 6-weeks post seeding were inoculated with a rifampicin-resistant attenuated *E. coli* O157:H7 (ATCC 700728) at a target level of 5 or 7 log CFU/plant. Two sets of plants were inoculated: one between 9:00 to 11:30 pm (nighttime) and another the next morning between 9:00 and 10:00 am (morning). *E. coli* O157:H7 was enumerated on the plants by direct plating, filtration and plating, or by MPN. When counts were below the limit of detection, the entire above-ground portion of the plant was enriched for *E. coli* O157:H7.

Results: Inoculation at nighttime when relative humidity and leaf wetness were high enhanced survival of *E. coli* O157:H7 during the first several hours to 1 day after inoculation but not thereafter. Population size declined in the first 8 h by 0.5 to 1.5 log CFU/plant for nighttime inoculations and by 3.5 log CFU/plant after the morning inoculations regardless of plant age. A greater proportion of *E. coli* O157:H7 positive plants was observed 2 weeks after inoculation of 6-week old plants.

Significance: The time of inoculation influences the short-term survival of *E. coli* O157:H7 on lettuce plants while plant age and size influence long-term prevalence rates.

P3-87 Survival of *Escherichia coli* O157:H7 87:23 on Arugula, Kale, Lettuce and Mizuna Microgreens, and Comparison of Leaf Surface Morphology for Mature Greens and Microgreens

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Introduction: Microgreens are young edible greens that are used to enhance the color and flavor of salads, as well as being highly nutritious. Unlike sprouts, which lack true leaves, microgreens are allowed to grow and form the first true leaves. Sprouts are legally defined, and have additional regulations due to their relatively high risk of microbial contamination. Microgreens, however, lack any legal definition or food safety regulations.

Purpose: The objectives of this study were to determine the survival characteristics of *Escherichia coli* O157:H7 on microgreen surfaces stored in a refrigerator and to examine the surface morphology of microgreens and mature greens.

Methods: Arugula, kale, lettuce and mizuna seeds, obtained from Harris Seeds, Rochester, NY, were planted and grown in a container filled with general purpose soil mix in a greenhouse before harvesting. The harvested microgreens were rinsed in tap water and dried for 1 h. Samples (1 g each) were spot-inoculated with 150 µl of *E. coli* cells (ranging from 7.45 to 7.76 log CFU/g), dried in a laminar flow hood for 1 h, packaged in plastic bags and stored at 83% RH in a refrigerator. Microbiological counting was performed at 0, 1, 4, and 7 days after inoculation. Scanning electron microscopy (SEM) was used to examine the surface morphologies of the produce.

Results: Most of the inoculated *E. coli* cells survived on the microgreen surfaces, showing only a slight decline (6.94 to 7.15 log CFU/g) at 7 days of storage. In the SEM images, all three types of microgreens leaves were more wrinkled than the mature leaves. The hills and valleys on the microgreens were deeper than for the mature produce. The stomata of the microgreens were a little longer than those of the mature leaves.

Significance: The ability of *E. coli* O157:H7 to survive on microgreens surfaces and the rougher microgreen surfaces compared to mature produce highlighted the importance of developing effective sanitation strategies for reducing the microbial safety risk associated with this produce product.

P3-88 Suvival of Six Shiga-toxigenic *Escherichia coli* Serotypes in Field Lettuce

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Introduction: Most outbreaks of illness caused by Shiga-toxigenic *Escherichia coli* in leafy vegetables have been caused by serotype O157 but several incidents involving non-O157 serotypes have been reported in recent years. Little is known about the survival of non-O157 Shiga-toxigenic *E. coli* on field crops.

Purpose: The purpose of this work was to compare the survival of six Shiga-toxigenic *E. coli* serotypes on growing field lettuce.

Methods: *E. coli* isolates from serotypes O26:H11, O44:H18, O103:H2, O111:HNM, O145:HNM and O157:H7 lacking both *stx*1 and *stx*2 genes were applied (10^6 CFU/g) to six separate plots of Romaine lettuce in an experimental site located at Summerland, British Columbia, Canada (dry climate, sandy soil) in 2011 and 2012. Residual populations on lettuce leaves were estimated on modified MacConkey agar (10mg/ml of vancomycin, 3mg/ml cefsulodin, 42°C incubation T) and/or enrichment in modified TSB.

Results: With the exception of serotype O103:H2, *E. coli* populations were reduced to ≤ 1 log CFU/g leaf tissue after 7 days in both trial years. *E. coli* O157:H7 was consistently detected by enrichment for up to 21 days, whereas recovery of other serotypes was sporadic. In contrast, 2 log CFU/g *E. coli*O103:H2 remained on the leaves 21 days post-inoculation. The latter was clearly able to survive longer on lettuce leaves than the other experimental strains examined in this work.

Significance: Non-O157 serotypes of *E. coli* are capable of similar or longer survival on growing lettuce plants. The present work hints at innate differences in the fitness of individual *E. coli* serotypes or strains in the production environment.

P3-89 Comparative Survival of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and Murine Norovirus on Spinach Plants

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Undergraduate Student Award Competitor

Introduction: Outbreaks resulting from the consumption of leafy greens contaminated with *Escherichia coli* O157:H7, *Salmonella* spp., and norovirus have occurred. It is unclear how the stress response factor *rpoS* in *E. coli* O157:H7 and *Salmonella* spp. affects their survival on spinach.

Purpose: A comparison of the survival of *E .coli* O157:H7, *Salmonella* Typhimurium, and murine norovirus (MNV) under the same conditions on spinach plants was performed.

Methods: Five week-old spinach (Menorca, semi-savoy) plants were co-inoculated with 6 log CFU/plant of each of the following strains: wild-type (wtO157) *E. coli* O157:H7, wt *S.* Typhimurium (wtST), and *rpoS*-deficient mutants of wt *E. coli* O157:H7 (rpoSO157) and *S.* Typhimurium (rpoSST). MNV was inoculated on the same spinach plants at 10^6 PFU/plants. Wt and *rpoS*-deficient strains were enumerated from plant

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homogenates on selective media either directly or by MPN. Five plants per day were analyzed on days 0–4. The presence of MNV was also determined on day 0 and day 3.

Results: Initial populations of all bacterial strains were 4.3 –4.5 log CFU/plant on day 0. Populations of wtST and wtO157 declined by 3.1 and 3.9 log CFU/plant, respectively; rpoSO157 and rpoSST declined by 3.9 and 4.7 log CFU/plant, respectively over 4 days. Populations of rpoSO157:H7 and rpoSST were 2 log CFU/plant lower than paired wtO157 and wtST on day 1. Populations of wtO157 strains were significantly ($P < 0.05$) higher than those of both rpoS-deficient strains on day 2 and significantly higher than wtST, rpoSO157, and rpoSST on day 3. MNV were assayed and recovered on day 0 and day 3.

Significance: The *rpoS* gene may be more important to *S.* Typhimurium survival on spinach than *E. coli* O157:H7. Both wt and *rpoS*-deficient *E. coli* O157:H7 strains survived at higher populations than wt and *rpoS*-deficient *S.* Typhimurium. MNV persists for at least 3 days on the foliar surface of spinach plants.

P3-90 Transfer and Persistence of *Salmonella enterica* on Hydroponic Living Lettuce Roots and Edible Tissue Stored at 4°C and 12°C

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Introduction: Lettuce is increasingly associated with outbreaks of human disease. Contamination occurs from farm to fork. Risk factors are identified in field-harvested lettuce; however, the survival and behavior of human pathogens on hydroponic lettuce is not well understood.

Purpose: To quantify the transfer and survival of *Salmonella enterica* from contaminated roots to the leaves of butterhead lettuce packaged as "living lettuce" in a clamshell with intact roots stored at 4°C and 12°C throughout the 18-day shelf life.

Methods: Butterhead lettuce cultivar Buttercrunch was grown hydroponically. Mature lettuce (n = 48) was harvested by removing the entire plant including roots. Roots were soaked in *Salmonella enterica* Typhimurium LT2 solution. The roots were wrapped in a knot and the lettuce placed in plastic clamshells that were stored at 4°C and 12°C to simulate proper holding and temperature abuse conditions, respectively. Periodically three packages were removed and lettuce destructively processed. Roots were removed from the head, homogenized in peptone water, serially diluted and plated onto XLT-4 agar. Plate counts were also performed using 25 g of randomly selected leaves.

Results: On average 5.06 ± 0.02 log CFU/g of *Salmonella* was transferred to the roots from solution and 2.99 ± 0.11 log CFU/g transferred to the edible tissue. Decrease of 0.5 log CFU/g *Salmonella* on roots occurred on day 6 at 4°C, but no further decrease occurred throughout shelf life ($P = 0.0001$). Storage at 12°C was associated with 2 log CFU/g increases in *Salmonella* on roots after 18 days storage ($P = 0.0002$). At 4°C and 12°C, *Salmonella* persisted on the leaves after 18 days.

Significance: *Salmonella* was transferred to leaves from inoculated roots and persisted on living lettuce stored at 4°C for 18 days. Growth occurred only under temperature abuse conditions. This reinforces the need for maintaining temperature control and highlights the importance of identifying contamination risks associated with hydroponic production and distribution.

P3-91 The Impact of Inoculation Time of Day on the Survival of Attenuated *Escherichia coli* O157:H7 and Generic *Escherichia coli* on Field-grown Cilantro

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Introduction: Cool temperatures and high relative humidity (RH) are known to improve the survival of enteric foodborne pathogens on produce.

Purpose: To determine the impact of temperature and RH on the survival of attenuated *Escherichia coli* O157:H7 and generic *E. coli* on field-grown cilantro.

Methods: In two Salinas, California field trials (summer and fall) mature cilantro plants (4 to 7 weeks post-seeding) were spray inoculated (6 to 8 log CFU/ml) with rifampicin-resistant attenuated *E. coli* O157:H7 ATCC 700728 and generic *E. coli* in the evening (8 to 10 pm), morning (7 am), and afternoon (12 to 3 pm). Temperature, RH and bacterial survival were monitored through commercial maturity. Harvested plants ($n = 6$ to 10; 8 to 99 g) were homogenized and diluted in 0.1% peptone and plated onto rifampicin containing tryptic soy and CHROMagar O157 or CHROMagar ECC; the remaining sample was enriched in tryptic soy broth with rifampicin.

Results: Within 24 h after inoculation, bacterial levels declined from an initial level of 5 to 7 log CFU/g to as low as <1 log CFU/g; 2-log CFU/g greater declines were observed for *E. coli* O157:H7. Both inoculated bacteria could be isolated by enrichment from most cilantro samples 1 to 6 weeks post-inoculation. The temperature and RH during the morning (12-13°C; 88-95%), afternoon (19-23°C; 62-66%), and evening (13-17°C; 76-91%) were similar for three inoculation times but were hotter and dryer during the fourth inoculation (morning, 16-18°C; 48-52%; afternoon, 32-35°C; 29-30%; and evening, 18-20°C; 52-63%). Greater reductions (by 1 to 4 log CFU/g) during the first 24 h after inoculation were observed for *E. coli*O157:H7, in the morning and afternoon, and during the fourth trial.

Significance: The survival of *E. coli* on cilantro plants is impacted for a short time by temperature and RH at the time of contamination.

P3-92 Effects of Packaging Atmosphere and Size on the Growth of *Listeria monocytogenes* on Fresh-cut Celery Sticks during Refrigerated Storage

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Introduction: From January to October 2010, 10 cases of hospital-acquired listeriosis were traced to commercially diced celery from Texas that included 5 deaths.

Purpose: In response to this outbreak, the impact of packaging atmosphere and package size on growth of *Listeria monocytogenes* on fresh-cut celery was assessed.

Methods: Locally purchased celery was cut into 10-cm sticks and inoculated by immersion for 30 min in a 3-strain cocktail of avirulent *L. monocytogenes* (J22F, M3, J29H) containing 6.96 log CFU/ml to obtain 2.74 log CFU/g. After centrifugal drying and 18 – 22 h of storage at 4°C, the celery was immersed in 60 l of tap water containing 50 ppm available chlorine (XY-12, Ecolab, St. Paul, MN) for 1 min, air-dried for 1 h, and

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packaged in two different sized ultra-high barrier film pouches (53 x 25.5 or 25.5 x 20 cm) containing air, 99% O_2, 99% N_2, or 15% CO_2/5% O_2/80% N_2. Following 0, 1, 3, 5, 7, 10, and 14 days of storage at 7°C, single celery sticks from these packages were added to 100 ml of neutralizing buffer, homogenized by stomaching, appropriately diluted, and plated with or without prior membrane filtration on Modified Oxford Agar for quantification of *L. monocytogenes* after 48 h of incubation at 37°C.

Results: After the large pouches were stored for 14 days at 7°C, *Listeria* populations were significantly lower ($P < 0.05$) using 99% O_2 (0.92 log CFU/g) as compared to 99% N_2 (4.87 log CFU/g) with no differences observed between air (1.61 log CFU/g) and 15% CO_2/5% O_2/80% N_2 (3.65 log CFU/g). However, no significant differences ($P > 0.05$) in numbers of *Listeria* were seen for the small pouches during storage. Only two instances occurred where package size impacted *Listeria* growth, with populations 1.51 and 1.50 log CFU/g higher in small pouches containing 15%CO_2/5% O_2/85% N_2 at day 5, and 99% O_2 at day 14.

Significance: Based on these findings, packaging fresh-cut celery sticks in oxygen may reduce the level of growth achieved by *L. monocytogenes* during refrigerated storage, while package size should not impact pathogen growth.

P3-93 Survival of *Salmonella* spp. on Dried Fruit Held at Varying Temperatures

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Introduction: Dried fruits can be contaminated with pathogenic microorganisms at any stage of growth or processing. Commonly, there is no heat step employed prior to being added directly to ready-to-consume products, creating a potential food safety hazard. While the inherent antimicrobial activities of dried fruit such as raisins, cranberries, blueberries, and cherries have been reported, scientific data remains limited or inconclusive.

Purpose: This study determines the survival of a *Salmonella* cocktail on 12 types of dried fruit stored at temperatures ranging from 4°C to 45°C. The results of this study serve as the scientific basis for industry microbiological classification of those dried fruit and for potential pathogen reduction steps.

Methods: Dried fruit was inoculated using either a wet or dry technique. A_w, moisture, titratable acidity, and pH were measured throughout the inoculation process and incubation. Following inoculation, fruit and culture controls were held at 4, 10, 18, 25, 35 and 45°C. Samples (25 g) were analyzed for *Salmonella* initially and weekly or bi-weekly until 3 sequential negative results were obtained. Microbial analysis was conducted using plate count on trypticase soy agar and confirmed using VIDAS or FDA BAM.

Results: For both wet and dry inoculated fruit, a significant log reduction (4-7 logs) was observed by week 3 at room temperature (25°C) and higher. At 4 and 10°C, the pathogen survived for over three months, with levels dropping by 2–3 logs. All runs were conducted in triplicate.

Not all dried fruit behaved the same under similar conditions.

Significance: This study indicates that under refrigerated storage conditions following harvesting, *Salmonella*, if present, will survive for up to 3 months on 11 of 12 dried fruit types. In addition, for most dried fruit stored at temperatures greater than 25°C, pathogen levels decrease by 4–5 logs within 3 weeks.

P3-94 Fate of *Escherichia coli* O157:H7 and *Salmonella* spp. on Bruised and Unblemished Blueberries Harvested at Two Maturity Stages

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Introduction: Blueberries do not ripen after harvesting. Riper fruit are more susceptible to bruising during harvesting and transport, which may increase the risks of foodborne proliferation.

Purpose: The objective of this research was to quantify the fate of *Escherichia coli* O157:H7 and *Salmonella* on bruised and unblemished blueberries at two maturity levels, ripe (dark blue) and commercially ripe (light blue), stored at shipping (2°C) and retail display (15.5°C) temperatures.

Methods: Harvested blueberries were sorted into dark and light blue fruit. Half of the blueberries from each group were bruised by dropping fruit from a height of 21 cm five times. Twenty microliters of a five-strain rifampicin-resistant *E. coli* O157:H7 or *Salmonella* cocktail (ca. 10^6 CFU/ml), was spot inoculated onto 5 blueberries and dried for 1 h. Blueberries were stored at 2°C and 15.5°C, and sampled at 0, 1, 3, and 7 days (n= 6). Pathogen populations were enumerated on selective and non-selective media supplemented with rifampicin.

Results: Populations of *E. coli* O157:H7 and *Salmonella* decreased under all experimental conditions. At 2°C, *E. coli* O157:H7 and *Salmonella* population decreases ranged from 1.2-1.6 and 1.4-1.8 log CFU/berry, respectively, over 7 days. When stored at 15.5°C, declines of *E. coli* O157:H7 (1.4 and 1.3 log CFU/berry) and *Salmonella* (1.2 and 1.1 log CFU/berry) populations were seen on light blue bruised and unblemished blueberries. Populations of *E. coli* O157:H7 and *Salmonella* decreased significantly less ($P \leq 0.05$) on dark bruised blueberries (0.7 and 0.9 log CFU/berry) when compared to dark unblemished blueberries (1.6 and 1.7 log CFU/berry).

Significance: Bruising did not significantly affect the fate of *E. coli* O157:H7 or *Salmonella* populations on light blueberries or on dark blueberries stored at 2°C. However; the slower population decline of *E. coli* O157:H7 and *Salmonella* on bruised dark blueberries than on unblemished berries at 15.5°C may increase risks.

P3-95 The Influence of Ripeness and Bruising on the Survival of *Escherichia coli* O157:H7 and *Salmonella* on the Surface of Pears

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Introduction: Pears are one of the few fruits that do not ripen successfully on the tree, they are harvested when they reach full maturity but before they are ripe. While often desirable, shipping ripe pears is problematic because they are prone to bruising.

Purpose: The purpose of this study was to determine the influence of ripeness, bruising and storage temperature on the survival of foodborne pathogens on the surface of Bartlett pears.

Methods: Bartlett pears at two stages of ripeness (8 kgf (hard) and 3 kgf (soft)) were bruised using a standard method. Both unblemished and bruised surfaces (3 cm^2) were inoculated with five-strain cocktails of rifampicin-resistant *Salmonella* or *Escherichia coli* O157:H7 at a level of 4 log CFU/3 cm^2. Pears were stored at 1 and 20°C and 90% RH for up to 48 h. The inoculated area was excised to recover surviving populations, samples were diluted in 0.1% peptone and plated onto specific and non-specific media containing rifampicin or, when appropriate, enriched using standard methods.

Results: In all cases, population densities of *Salmonella* or *E. coli* O157:H7 decreased by 1 to 2 log CFU within the first 2 h and were near or below the limit of detection (1 CFU/3 cm^2) by 24 h. The percent positive samples (of 12) at 48 h ranged from 0 to 100%; greater survival was associated with harder fruit. For *Salmonella*, the number of positive samples was identical for unblemished and bruised fruit except for soft pears stored at 20°C; at that temperature *Salmonella* was isolated more frequently from soft unblemished fruit. For *E. coli*O157:H7 greater survival was consistently noted for bruised pears (both hard and soft).

Significance: Shipping riper pears should not increase food safety risks associated with *Salmonella* or *E. coli* O157:H7.

P3-96 Behavior of Inoculated *Salmonella* and *Escherichia coli* O157:H7 on Onion (*Allium cepa*) Skins and in Chopped Onion Flesh

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Introduction: Cut onions are not always cooked prior to consumption; the impact of storage temperature on the behavior of foodborne pathogens in onions is not known.

Purpose: Our objective was to evaluate the behavior of *Salmonella* and *Escherichia coli* O157:H7 on onion skins and in chopped onions during storage.

Methods: The outer skin of whole yellow onions was inoculated with cocktails of rifampicin-resistant *Salmonella* or *E. coli* O157:H7 (ECO157) at 7 log CFU/9 cm^2 and, after drying for 30 min, stored at 4 or 23°C for 28 days. Commercial fresh-cut and freshly-chopped onions were inoculated at 3 log CFU/g and stored at 4 or 23°C for 6 or 1.5 days, respectively. *Salmonella* and ECO157 populations were determined by plating prepared samples onto non-selective and selective agar supplemented with rifampicin. When necessary, samples were enriched using standard methods.

Results: Populations of *Salmonella* and ECO157 declined by 3 log CFU on onion skin within the 30 min drying time. *Salmonella* and ECO157 were not detected by enrichment when stored at 23°C for 7 days. At 4°C, *Salmonella* was detected by plating in two of six samples after 28 days; ECO157 was not detected by enrichment of samples stored for 21 days. Less than 0.5 log CFU/g reductions in populations of *Salmonella* or ECO157 were observed in chopped onions over 6 days of storage at 4°C. Less than 0.5 log CFU/g increases in *Salmonella* or ECO157 were observed in the first 8 h of storage at 23°C; after 12 and 24 h populations increased by 2 and 3 log CFU/g. Growth rates and maximum population densities were higher in closed containers and for freshly-chopped onions.

Significance: *Salmonella* and *E. coli* O157:H7 survive poorly on onion skin, but refrigeration is important to prevent growth of these organisms in chopped onions.

P3-97 Survival of *Salmonella* spp. and *Pectobacterium carotovorum* on Brush Roller Treated Tomatoes

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Introduction: The brush roller method of washing tomatoes has been shown to be superior at removing *Salmonella* spp. from tomato surfaces, and uses less water and sanitizer compared to the flume method. However, little is known about the negative impacts that brushing may have on tomato surfaces, including potential abrasion, which may increase pathogen-harboring capacity.

Purpose: To determine whether brush washing tomatoes increases the survivability of human (*Salmonella* spp.) or plant pathogens (*Pectobacterium carotovorum*) on tomatoes.

Methods: Mature green tomatoes were brush treated with water or 100 ppm NaOCl (pH 6.5) for 0, 15, or 60 s, then inoculated with rifampin-resistant *Salmonella* spp. or *Pectobacterium* at 8 log CFU/tomato and stored at 25°C 75-85 %RH for 7 days. To model survival on wounded fruit, other tomatoes were superficially punctured at 10 points then inoculated directly into the punctures with each culture independently (at either a 2 or 8 log CFU/tomato level) then stored under the same conditions. For all studies, tomatoes were sampled at days 0, 1, 3, and 7 using the rub-shake-rub method in 100 ml BPW, followed by plating on TSA+rifampin.

Results: Brush treatment did not have a significant impact on the survival of either pathogen; *Salmonella* and *Pectobacterium* populations decreased to below the detection limit of 2 log CFU/tomato within the first 3 days for all treatments. When wounded fruit were inoculated at low levels (2 log CFU/tomato), both cultures grew to and maintained near a 5.8 log CFU/tomato level. At high inoculation levels (8 log CFU/tomato), *Salmonella* cultures maintained near 8 log CFU/tomato for 7 days, whereas *Pectobacterium* grew to above 11 log CFU/tomato.

Significance: These results suggest that brush washing does not compromise tomato surfaces in such a way to allow enhanced survival of *Salmonella* spp. or *Pectobacterium carotovorum*.

P3-98 Transfer of *Listeria monocytogenes* during Mechanical Slicing of Onions

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Developing Scientist Competitor

Introduction: A series of recalls in 2012 involving diced onions contaminated with *Listeria monocytogenes* from one manufacturer has heightened public health concerns surrounding the preparation, handling and use of raw onions in salads, pre-made sandwiches, and other ready-to-eat foods.

Purpose: In order to assess current commercial slicing practices, this study aimed to quantify the extent of *L. monocytogenes* transfer from one artificially contaminated onion to subsequent onions during mechanical slicing.

Methods: Locally purchased Spanish jumbo yellow onions (*Allium fistulosum*) were dip-inoculated in a 3-strain avirulent *L. monocytogenes* cocktail (M3, J22F and J29H) to contain ~ 6, 4, or 2 log CFU/g. After air-drying for 90 min, one inoculated onion was sliced using a NEMCO model 56750-2 onion slicer, followed by 20 uninoculated onions with the top, middle, and bottom slices from each onion collected for *Listeria* analysis. Each slice was added to 50 ml of UVM medium, homogenized by stomaching for 1 min, appropriately diluted and then plated with or without prior membrane filtration on Modified Oxford Agar with the plates incubated at 35°C for 48 h. All UVM samples were incubated for 48 h and then streaked to MOX if *Listeria*was not quantifiable by direct plating.

Results: After slicing one inoculated onion containing *L. monocytogenes* at 6.1 log CFU/g to contaminate the slicer followed by 20 uninoculated onions, *Listeria* was quantifiable in all samples with average populations of 4.3, 1.3, and 0.3 log CFU/g in 1st, 10th and 20th onion, respectively. At the

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lower inoculation level of 4.1 log CFU/g, *L. monocytogenes* was sporadically detected out to the 20th onion at a level of -0.054 log CFU/g. At the lowest inoculation level of 2.8 log CFU/g, *L. monocytogenes* was sporadically detected out to the 20thonion at a level of -0.001 log CFU/g.

Significance: These results show the ability for *Listeria* to cross-contaminate large numbers of onions during mechanical slicing with such findings needed to help fill one of the major knowledge gaps in risk assessments for fresh-cut produce.

P3-99 Extent of *Listeria monocytogenes* Transfer during Cutting of Cantaloupe and Honeydew Melon

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Developing Scientist Competitor

Introduction: The 2011 multistate outbreak of listeriosis traced to cantaloupe has heightened concerns regarding safe preparation of fresh-cut produce.

Purpose: In response, this study aimed to quantify *Listeria monocytogenes* transfer during cutting of cantaloupe and honeydew melon as impacted by three different areas of the rind and product temperature.

Methods: Cantaloupes and honeydew melons obtained from a local retailer were washed, immersed for 10 min in a 3-strain avirulent cocktail of *L. monocytogenes* (strains M3, J22F, and J29H) containing 10^9 CFU/ml, air-dried for 1 h and then stored at 4 or 30°C for 24 h to simulate processing plant and field conditions, respectively. An 18-mm diameter sterile cork borer was used to obtain five 30 mm-long core samples from the blossom scar, stem scar, and circumference regions. After aseptically cutting each core sample into six 5mm long pieces, the five pieces from the same depths and rind locations were added to 50 ml of phosphate buffer saline, homogenized by stomaching, serially diluted, surface-plated on trypticase soy agar containing 0.6% yeast extract, 0.1% esculin, and 0.05% ferric ammonium citrate and incubated at 37°C for 24 h to enumerate *Listeria*.

Results: After surface inoculation, the cantaloupe and honeydew melon rinds contained *L. monocytogenes* populations of 6.81 and 5.67 log CFU/g, respectively. *Listeria* transfer ranged from 2.89 to 4.31 and 1.48 to 3.15 CFU/g for cantaloupe and honeydew, respectively. Overall, no significant difference in *L. monocytogenes* transfer was seen at the different depths ($P > 0.05$). However, transfer was significantly greater in the blossom and stem scar regions of cantaloupes cored at 4°C as compared to 30°C ($P < 0.05$) with no significant difference observed for the circumference of the rind ($P > 0.05$). *Listeria* transfer was also greater for cantaloupe compared to honeydew melon at the stem scar, blossom scar, and circumference ($P < 0.05$).

Significance: These results demonstrate that *L. monocytogenes* can be readily transferred from the rind to the interior flesh of cantaloupe and honeydew melon during preparation. Hence, thorough washing with a sanitizer or otherwise treating the rind surface remains critically important to minimize contamination of the edible portions during cutting with these quantitative findings being helpful for current risk assessments.

P3-100 Dissemination of *Escherichia coli* O157:H7 from a Contaminated Shredder or Conveyor Belt to Fresh-cut Iceberg Lettuce during Simulated Commercial Production

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Developing Scientist Competitor

Introduction: Improperly cleaned and sanitized equipment for commercial production of fresh-cut leafy greens can foster the spread of *Escherichia coli* O157:H7 as evidenced by an earlier outbreak traced to a lettuce shredder.

Purpose: The goal of this study was to quantify the transfer of *E. coli*O157:H7 from a contaminated shredder and conveyor belt to previously uncontaminated iceberg lettuce during pilot-plant production.

Methods: Triplicate 50 lbs (22.7 kg) batches of unioculated iceberg head lettuce were processed using a commercial shredder (model TRS 2500 Urschel TranSlicer), Dorner step conveyor with a smooth ThermoDrive belt, 3.3-m flume tank without a chemical sanitizer, shaker table and centrifugal dryer (22.7 kg capacity). Heads of radicchio (9.1 kg), previously dip-inoculated with a 4-strain avirulent, GFP-labeled, ampicillin-resistant *E. coli* O157:H7 cocktail (10^6 CFU/g), dewatered and air-dried for 1 h at 22°C, were then either shredded or shredded and conveyed to contaminate the shredder and conveyor belt, respectively, with the shredder thoroughly cleaned and sanitized before quantifying transfer from the conveyor. Thereafter, 200 lbs (90.7 kg) of iceberg lettuce were processed within 5 min during which time 15 radicchio-free lettuce samples were collected immediately after exiting the contaminated shredder or conveyor. These samples, along with additional lettuce, sanitizer-free water and equipment surface samples were appropriately diluted and surface-plated on TSAYE + ampicillin with or without membrane filtration to enumerate *E. coli*O157:H7.

Results: Based on triplicate experiments, average *E. coli* O157:H7 populations on the shredder and conveyor belt decreased from 3.73 to 0.69 and from 4.13 to 2.52 log CFU/100cm^3, respectively, after processing the 200 lbs of iceberg lettuce. Populations on the 15 lettuce samples collected after exiting the contaminated shredder decreased significantly ($P < 0.01$) from 2.70 to 1.78 log CFU/g during 5 min of processing. In contrast, no significant difference ($P < 0.05$) was observed among the 15 lettuce samples collected after exiting the contaminated conveyor belt with *E. coli* O157:H7 populations ranging from 0.90 to 1.78 log CFU/g.

Significance: These findings reinforce the importance of proper cleaning and sanitizing of shredders and conveyors to minimize the spread of bacterial pathogens during production of fresh-cut leafy greens.

P3-101 A Dimensional Analysis Approach to Modeling Bacterial Pathogen Transfer during Conveying, Washing, and Slicing of Fresh-cut Produce

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Introduction: Pathogen transfer data to/from fresh produce during postharvest processes are increasingly being reported in the literature. However, data are presented as product/process-specific cases, with little or no generalizable results extendable to the broader, biologically complex problem. A well-constructed model could enable improved process design and analysis.

Purpose: To apply a dimensional analysis approach, with similarity criteria, to formulate a novel model for bacterial pathogen transfer during produce conveying, washing, and slicing.

Methods: The Buckingham Pi Theorem was applied to formulate a generalized model for bacterial transfer occurring between fresh produce and wash/conveying water or equipment contact surfaces. Initially, 11 candidate variables (product and process) were identified for equipment contact events (slicing, shredding, and conveying), and 21 were identified for water washing/conveying. Based on expert knowledge, variables unlikely to significantly affect transfer were excluded, to yield 6 and 9 variables for equipment contact events and water washing/conveying processes, respectively. Application of the Buckingham Pi Theorem accounted for the fundamental units of each variable and the total number of variables in each process to reduce the model to a smaller number of dimensionless (Pi) terms.

Results: The resulting models included two and five dimensionless (Pi) terms, respectively, for the equipment contact and water wash/conveying processes. Each dimensionless term in the resulting models (like, for example, a Reynolds number in fluid flow) can be applied to determine relative impact of key variables on transfer. For example, one Pi term relates friction force at the surface, contact time, and the initial bacterial population on the donor surface to resulting transfer. For the water transfer events, the Pi terms relate water velocity and product dimensions, which can illustrate the general dependency of transfer on fluid shear.

Significance: This novel approach to modeling bacterial transfer will enable optimized designs of future transfer experiments, in order to yield data that improve the utility of the generalized transfer model for process improvement and risk modeling.

P3-102 Physicochemical Parameters as Predictors of Sanitizer Efficacy against *Escherichia coli* O157:H7 in Leafy Green Wash Water Containing Sanitizers and Organic Load

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Developing Scientist Competitor

Introduction: Sodium hypochlorite is routinely used in flume tanks during leafy green processing to minimize cross-contamination. However, given the increasing number of leafy-green-associated recalls and outbreaks, improved methods are needed for commercial monitoring of sanitizer efficacy containing high organic loads.

Purpose: Consequently, this study aimed to 1) determine the ability of acidified and non-acidified sodium hypochlorite to reduce *Escherichia coli* O157:H7 populations in simulated processing water in a bench-top model and 2) assess the relationship between various physicochemical parameters and organic load of the wash water on *Escherichia coli* O157:H7 inactivation.

Methods: A spigoted glass carboy was used for efficacy testing of a chlorine-based produce sanitizer (XY-12, Ecolab, St. Paul, MN) containing 50 ppm available chlorine at pH 7.51 or 6.50 (acidified with T-128, New Leaf Food Safety Solutions, Salinas, CA or citric acid (CA)) in triplicate against a 4-strain avirulent, GFP-labeled *E. coli* O157:H7 cocktail in 4-l of wash water containing 0, 2.5, 5 or 10% (w/v) blended iceberg lettuce, with sanitizer-free water serving as the control. Mesh bags containing 25 g of dip-inoculated (6.10 log CFU/g) iceberg lettuce were exposed to the wash water for 90 s after which five water samples (50 ml) were collected through the spigot at 2 min intervals, immediately neutralized, appropriately diluted and surface-plated on TSAYE + amp with or without membrane filtration to quantify *E. coli* O157:H7, with non-neutralized samples also assessed for Chemical Oxygen Demand (COD), Oxidation/Reduction Potential (ORP), total solids, turbidity, and maximum filterable volume (MFV) using a 0.45 µm membrane.

Results: *E. coli* O157:H7 populations were significantly lower ($P < 0.05$) in wash water containing 0 (-2.00 to -1.36 log CFU/ml) as opposed to a 2.5, 5, or 10% organic load (1.87 to 3.74 log CFU/ml) at all sampling times for all three treatments. Using a 2.5% organic load, *E. coli* O157:H7 inactivation rates were -0.10, -0.20, and -0.14 log CFU/ml per min for chlorine, chlorine + CA, and chlorine + T-128, respectively, with these rates significantly impacted ($P < 0.05$) by COD, ORP, total solids, turbidity, and MFV for all three chlorine treatments.

Significance: Organic load reduced the efficacy of all three chlorine treatments against *E. coli* O157:H7, increasing pathogen persistence in the water. Based on these findings, COD, ORP, total solids, turbidity, and MFV can be useful predictors of sanitizer efficacy, giving leafy greens processors a means of better assuring end-product safety.

P3-103 Assessing the Microbiological and Physicochemical Characteristics of Processing Water and Their Impact on Safety of Ready-to-Eat Vegetables

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Introduction: Water used in processing of ready-to-eat vegetables (RTEV) plays an important role in the quality and safety of the final product.

Purpose: This study aimed at assessing the impact of the physicochemical and microbiological characteristics of water used in RTEV processing plants on their safety.

Methods: A total of 34 water samples, collected from the tap water supply (n = 10), pre-washing (n = 9), washing (n = 10) and rinsing water tanks (n = 5) in ten RTEV processing plants located in the State of Sao Paulo, Brazil were tested for temperature, pH, organic load (mg/l), chlorine concentration (mg/l), redox potential (mV) and conductivity (øs/cm). Vegetable samples were collected before (n = 36) and after (n = 48) washing step. All water and vegetable samples were submitted to counts of mesophiles, yeasts and molds (except for water samples), *Enterobacteriacea*e, total coliforms and *E. coli.* Presence of *Salmonella* was also investigated.

Results: *Enterobacteriacea*e and total coliforms were found in water samples collected from the pre-washing, washing and rinsing water tanks, in counts varying from 1.0-3.7 log CFU/g and 2.2->23 MPN/g. Pre-washing and rinsing water also contained *E. coli* (6.9->23 MPN/g). pH, conductivity and redox potential varied between 6.2-7.6, 125-216 øs/cm, 324-529 mV. Counts of mesophiles, yeasts and molds, *Enterobacteriacea*e and total coliforms in final products processed with ozonated or refrigerated water (9.5°C or 12.3°C) varied between 1.6-4.4 and 2.0-4.3 log CFU/g or MPN/g. These counts were significantly lower ($P < 0.05$) than in final product processed with water containing only chlorine-based sanitizers (50-240 mg/l) or with non-refrigerated water at the washing-disinfection step. Overall, the processing caused 0.5 to 1 log CFU/g or MPN/g reductions in the counts of mesophiles, yeasts and molds, *Enterobacteriacea*e, total coliforms and *E. coli* in the RTEV. *Salmonella*was not detected in any water or RTEV sample.

Significance: Our data indicate that water characteristics and practices applied during RTEV processing influence microbial quality of these products.

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P3-104 The Impact of Organic Load on the Minimal Level of Chlorine Needed to Prevent *Escherichia coli* O157:H7 Cross-contamination during Washing of Fresh-cut Lettuce

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Introduction: Fresh produce grows in natural environments and is susceptible to microbial contamination. The contamination can spread during postharvest washing. Industry and government guidelines have suggested usage of chemical disinfectants such as sodium hypochlorite in wash water to prevent microbial cross-contamination and have recommended that wash water disinfectants be monitored. However, specific standards regarding the levels of sanitizer needed have yet to be established. As wash water is often recycled during commercial processing, the impact of the increased organic load on the effective level of sanitizer needed to prevent microbial cross-contamination remains to be determined.

Purpose: To determine the minimal effective level of sodium hypochlorite needed to prevent *Escherichia coli* O157:H7 cross-contamination during washing of inoculated fresh-cut lettuce as affected by the organic load of wash water.

Methods: Eight g of cut romaine lettuce inoculated with 7 log CFU/g of *E. coli* O157:H7 were washed with 800 g of uninoculated lettuce in 40 l of sterile tap water at 3°C for 2 min. Washing trials were performed in water with different levels of organic load (addition of 0, 3, 6, 12, or 30 g of lettuce juice powder) and at different levels of chlorine treatment (0 - 50 ppm of sodium hypochlorite, NaOCl). The degree of cross-contamination was determined by measuring the presence of *E. coli* O157:H7 in the wash water and uninoculated lettuce after washing. Wash water samples were also analyzed for total/free chlorine, turbidity, and total organic carbon.

Results: In the absence of chlorine, *E. coli* O157:H7 transfer occurred at all levels of organic load and resulted in the contamination of wash water and uninoculated lettuce at levels of 3.7 ± 0.3 log CFU/ml and 3.3 ± 0.2 log CFU/g, respectively. Without the addition of lettuce juice powder, cross-contamination was prevented in wash water containing 5 ppm of NaOCl. With the addition of 3 g of lettuce juice powder, transfer of *E. coli* O157:H7 was observed when the washing run was conducted with 5 ppm of NaOCl but was prevented with 10 ppm of NaOCl. With the addition of 30 g of lettuce powder, 50 ppm of NaOCl was needed to prevent cross-contamination. It was difficult to have an accurate determination of the residual free chlorine level as it continued to decrease during the washing run.

Significance: Measurements of the organic load of wash water are needed to determine the effective level of sanitizer required to prevent microbial cross-contamination during postharvest washing of fresh-cut produce.

P3-105 Investigation on the Spread of *Salmonella* and Factors Affecting the Efficacy of Sanitizer during Postharvest Washing of Lettuce

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Introduction: Leafy greens have been identified as one of the leading causes of foodborne illnesses in the U.S. According to the CDC, *Salmonella* accounts for 10.4 % of the leafy greens-associated outbreaks reported during 1973 - 2006, while *Escherichia coli* O157:H7 accounts for 8.9% of those outbreaks. Contamination that occurs in farms can persist and spread during postharvest washing. The use of sufficient chemical disinfectant(s) in wash water is critical to prevent microbial cross-contamination. A better understanding of the potential spread of pathogens and the factors affecting sanitizer efficacy will allow the development of effective wash water management programs.

Purpose: Investigate the spread of *Salmonella* during postharvest washing of contaminated lettuce and determine factors that affect the efficacy of chlorine in preventing cross-contamination.

Methods: Eight g of cut romaine lettuce inoculated with 7 log CFU/g of *Salmonella* Typhimurium expressing green fluorescent protein were added to 40 l of sterile tap water together with 800 g of uninoculated cut lettuce and washed for 2 minutes. Washing trials were performed at 3°C with different levels of sodium hypochlorite (0, 5, 10, and 20 ppm). Small-scale (100 ml) experiments were performed to determine the effects of organic load (lettuce juice) and solid (sandy soil) on the efficacy of chlorine in wash water disinfection.

Results: Without chlorine treatment, *Salmonella* transfer occurred and resulted in the contamination of uninoculated lettuce and wash water at levels of 3.0 ± 0.1 log CFU/g and 3.2 ± 0.1 log CFU/mL, respectively. With 5 ppm chlorine, although no *Salmonella* was detected in the wash water, the pathogen was found in the uninoculated lettuce after culture enrichments. At either 10 ppm or 20 ppm of chlorine, *Salmonella* was not detected in the wash water or the uninoculated lettuce samples. Increases in the total organic carbon (TOC) in wash water resulted in a lower level of free chlorine and greater survival of *Salmonella*. At 5 ppm of chlorine, the level of *Salmonella* varied from not detectable (< 0 log CFU/ml) to completely unaffected (3.3 ± 0.4 log CFU/ml) in the absence of added lettuce juice (TOC = 14 ± 9 mg/l) vs. in the presence of 20 % (v/v) of lettuce juice (TOC = 159 ± 17 mg/l).

Significance: The use of sufficient chlorine could prevent *Salmonella* cross-contamination during postharvest washing but the effective chlorine level is influenced by the organic load in the wash water.

P3-106 Validation of Washing Treatments to Reduce Pathogens in Fresh Produce

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Developing Scientist Competitor

Introduction: Many fresh produce outbreaks are the result of *Salmonella* and *Escherichia coli* O157:H7 contamination due to growing conditions or human handling.

Purpose: The purpose of this study was to determine the efficacy of a commercial wash solution for reducing pathogens in green leaf lettuce and tomatoes.

Methods: Lettuce (25 ± 0.3 g) and tomato (whole tomato) samples were inoculated with *E. coli* O157:H7 (~7.8 log CFU/ml) and *Salmonella* spp. (~9.39 log CFU/ml) cocktail inoculums, respectively, and then dried for 1 h. Inoculated samples were washed separately with commercial wash solution (contains citric acid and grapefruit oil) or tap water (control) for three contact times (30, 60, and 120 s). Lettuce (25 ± 0.3 g) and tomato (core of 11.34 cm^2) samples were diluted and stomached for 1 min and then 0.1 ml was plated onto CT-SMAC and XLD agar plates for *E. coli* O157:H7 and *Salmonella* recovery. Experiment consisted of three replications and two samples per treatment (n = 6).

Results: Recovery of *E. coli* O157:H7 populations on leaf lettuce were different ($P < 0.05$) between commercial wash and tap water. For lettuce samples, *E. coli* O157:H7 reductions for 30, 60, and 120 s of commercial wash treatment were 3.12, 3.22, and 3.30 log CFU/g, respectively while, tap water resulted in ≥ 2.2 log CFU/g reductions. There were no differences ($P > 0.05$) between commercial wash and tap water in *Salmonella* populations recovered from tomatoes samples. For commercial wash treatments, *Salmonella* reductions on tomatoes were 2.13 log CFU/cm^2 for all contact times whereas, tap water resulted in ≤ 2.0 log CFU/cm^2 reductions.

Significance: Commercial wash solution reduced *E. coli* O157:H7 populations by > 3.0 logs on leaf lettuce and *Salmonella* populations by > 2.0 logs on tomatoes for all contact times. The commercial wash solution is applicable for food service and home-use and would reduce risk of pathogens on produce.

P3-107 Efficacy of Lactic Acid Wash and Advanced Oxidation Technology for Controlling *Escherichia coli* O157:H7 in Bagged Baby Spinach

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Developing Scientist Competitor

Introduction: *Escherichia coli* O157:H7 outbreaks have been linked to leafy green produce and spinach.

Purpose: The study objective was to evaluate lactic acid washes and photohydroionization (PHI) panel, which uses a broad spectrum of wavelengths between ultraviolet light and X-radiation to produce ozone and vapor hydrogen peroxide at low levels, for controlling *E. coli* O157:H7 on spinach.

Methods: Leaves were dip inoculated in an *E. coli* O157:H7 cocktail inoculum (5-6 log CFU/ml) for 30 s and then dried for 1 h. Non-inoculated and inoculated leaves were washed for 30 s in food grade lactic acid diluted to 0.5, 1.0, or 2.0% and dried for 10 min. For PHI treatments, leaves were treated for 1, 2, or 5 min per side. Treated leaves were sealed in low-density polyethylene bags prior to enumeration on days 0, 3, 7, 10, and 14. Ten gram samples were diluted and stomached for one min then 0.1 ml was plated onto sorbitol MacConkey agar with cefixime and tellurite plates that were incubated at 37°C for 24 h. Experiment consisted of three replications and two spinach bags per treatment (n = 6).

Results: For lactic acid treatments, there was no difference ($P > 0.05$) in *E. coli* O157:H7 populations due to sampling times; pooled times for each lactic acid concentration of 0.5, 1.0, and 2.0% resulted in 2.01, 2.78, and 3.67 log CFU/g reductions. Leaves treated with 1.0 and 2.0% lactic acid had color degradation and were unacceptable by day 14. *Escherichia coli* O157:H7 populations were reduced 1.6, 1.49, or 1.95 log CFU/g ($P > 0.05$) for PHI treatments of 1, 2, and 5 min per side.

Significance: All PHI panel contact times and lactic acid wash of 0.5% resulted in > 1.5 and > 2.0 *E. coli* O157:H7 log reductions on baby spinach and leaves were organoleptically acceptable throughout 14 days shelf life.

P3-108 Behavior of Shiga Toxin-producing *Escherichia coli* (STEC) Strains during Lettuce Washing

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Introduction: To validate washing processes for sanitizing leafy greens, identification of microbial surrogates for Shiga toxin-producing *Escherichia coli* (STEC) may be useful. Because an ideal surrogate would imitate STEC, it is important to characterize the behavior of various STEC strains in washing processes, e.g. in transfer, attachment, and survival.

Purpose: To compare STEC serotypes and non-pathogenic *E. coli* in the extent of transfer and attachment to lettuce during washing.

Methods: Transfer of eight STEC strains (O157:H7, O104:H4, and the "Big Six" non-O157 STEC) and non-pathogenic *E. coli* Nissle 1917 (EcN) was compared in 30-ml washes of lettuce with or without chlorine. *E. coli* populations remaining on inoculated leaves, in wash water, and transferred to un-inoculated leaves after a 1-min wash were enumerated by plate counts on selective agar in triplicate trials. An attachment assay was performed to determine the number of attached cells after repeated washes.

Results: During an un-chlorinated water wash, > 90% of inoculated cells was transferred (all strains). Transfer of EcN, O111, and O145 did not differ from that of O157:H7 ($P > 0.05$); but the other STEC strains showed less transfer than O157:H7 ($P < 0.05$). Approximately 2 - 3 log CFU transferred onto un-inoculated leaves. In a 1-ppm chlorine wash, EcN showed significantly more tolerance to the chlorine wash ($P < 0.0001$); more cross-contamination in wash water was detected for O111 ($P = 0.0027$), compared to O157:H7.

Preliminary attachment data showed that O157:H7 and O145 attached less than the other STEC strains, and EcN demonstrated stronger attachment than O157:H7 ($P = 0.0057$).

Significance: Data of STEC transfer and attachment during washing will be valuable for assessing cross-contamination risks in post-harvest washing of lettuce. These methods also will be useful for identifying potential STEC surrogates for process validation.

P3-109 Practices in Brazilian Ready-to-Eat Vegetable Processing Plants Regarding Water Usage

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Introduction: Washing-disinfection step has an important role on ready-to-eat vegetable (RTEV) quality and safety, but it can also be a point of cross-contamination of RTEV.

Purpose: This study aimed at collecting information on the practices employed by Brazilian RTEV processing plants, especially regarding water usage.

Methods: Ten RTEV processing plants located in the state of Sao Paulo, Brazil, were visited and a questionnaire composed of 45 questions focusing water usage in pre-washing, washing and rinsing steps was applied. The questions addressed the RTEV washing method, water temperature, disinfectant applied, reuse and discharge of water used in the washing-disinfection step.

Results: In all surveyed processing plants, raw materials are kept under refrigeration (2-8°C) until processed. All plants performed pre-washing, and in 1, 4 and 5 plants, the pre-washing is done using running water, agitated immersion and static immersion, respectively. In 6 plants, the water used in the pre-washing step is added of chlorine-based products or a detergent. In all plants, washing-disinfection is carried out by immersion (static, 70%; and agitated, 30%) of the vegetables in water containing chlorine-based products (organic chlorine 70%, sodium hypochlorite 10%, and chloride dioxide 20%) for 2-20 min. In 3 plants, processing water is reutilized. All plants perform partial or total replacement of water (one to five times per day). Rinsing of vegetables is applied in 5 plants: 1 uses running water, 1 uses aspersion-agitation and 3 immerse them in water. Only

2 plants use chilled water (9.5 and 12.3°C) throughout processing. The processing room is refrigerated (10°C) in only one plant. A centrifugation step, at an average of 120 rpm for 1.5 min, is applied in 8 plants. Five plants record the temperatures during RTEV transportation to retail.

Significance: These findings constitute a building block for further assessment of cross-contamination during RTEV processing in Brazil.

P3-110 Minimizing *Salmonella* Contamination in Sprouts by Controlling the Irrigation Conditions during Germination

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Introduction: Sprouts pose a special food safety concern as low levels of pathogens present on seeds can multiply and reach high numbers during germination. The FDA has recommended that seeds be disinfected with one or more treatments such as 20,000 ppm of calcium hypochlorite, $Ca(OCl)_2$, before sprouting. But this treatment is unable to completely eliminate pathogens on seeds and surviving ones can grow to significant numbers during sprouting. Thus, controlling the germination conditions to prevent or minimize the proliferation of pathogens, if present, is an important part of the overall strategy to reduce microbial hazards in sprouts.

Purpose: This study examined how the proliferation of *Salmonella* is affected by varying frequencies of irrigation during germination of seeds that have been either treated or not treated with 20,000 ppm of $Ca(OCl)_2$ prior to sprouting.

Methods: Two hundred grams of alfalfa seeds spiked with 2 g (or 1 %) of inoculated seeds (~1 log CFU/g of *Salmonella*) were allowed to germinate in glass jars or an automatic sprouter (EasyGreen®) for 5 days at room temperature. The sprouts were irrigated with either sterile tap water or chlorinated water (containing 100 - 500 ppm of calcium hypochlorite, $Ca(OCl)_2$) at varying frequencies (once every 1, 2, 4 or 24 h). The same growth studies were performed on seeds treated with 20,000 ppm $Ca(OCl)_2$ for 15 min. Sprout samples were taken daily and analyzed for the level of *Salmonella* using the three-tube most probable number method as described in the FDA BAM.

Results: Using untreated seeds, the level of *Salmonella* in sprouts changed from an increase of > 5 log MPN/g to a decrease of ~ 1 log during 3 days of sprouting when the frequency of irrigation with sterile tap water increased from once every 24 h to once every hour for 15 min. Seed treatment with $Ca(OCl)_2$ lowered the level of pathogen to an undetectable level (< -2.5 log MPN/g) at the start of sprouting. *Salmonella* re-grew from treated seeds by > 1 log when sprouts were irrigated with water once every 24 h but remained undetected when sprouts were irrigated once every hour for 15 min. Irrigation with chlorinated water further inhibited *Salmonella* re-growth but resulted in a lower yield of sprouts. Overall, seed treatment combined with frequent irrigation with tap water or chlorinated water (e.g., once every hour) can maintain the level of *Salmonella* at an undetectable level during sprouting.

Significance: Controlling irrigation conditions provides an additional means to reduce the proliferation of *Salmonella* during sprouting, thus minimizing the microbial hazards in sprouts.

P3-111 Efficacy of Chlorine and Peroxyacetic Acid on Reduction of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., and Natural Microflora on Mung Bean Sprouts

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Introduction: Sprouts-related outbreaks have risen due to increased raw sprouts consumption. To minimize such cases, chemical sanitations are applied. While chlorine (Cl) is commonly used, concerns with its effectiveness and health implications have prompted researchers to seek alternatives. Peroxyacetic acid (PAA) has been shown its efficacy in inactivating foodborne pathogens on fresh vegetables, thus it would be considered an alternative.

Purpose: The objective of this study was to compare the efficacy of Cl and PAA in inactivating *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., and natural microflora on mung bean sprouts. Resistance of non- and acid-adapted pathogens to sanitizers was also evaluated.

Methods: All pathogens were adapted to 100 ppm nalidixic acid and the acid-adapted cells were prepared by culturing in trypticase soy broth containing 1% glucose. Uninoculated or inoculated sprouts were treated with Cl at 106, 130 and 170 ppm and PAA at 25, 51 and 70 ppm for 90 and 180 s at room temperature. Mean values of bacterial counts were compared using ANOVA.

Results: Overall, the greater log reductions were obtained with the increase in the treatment time and the sanitizer concentration. For 180 s, Cl treatment at 170 ppm reduced 2.0, 1.3, 1.5, 0.9-logs and PAA treatment at 70 ppm resulted in 2.3, 1.8, 2.1, 1.1-log reductions for non-adapted *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* spp., and natural microflora, respectively. These results revealed that the efficacy of PAA was significantly ($P < 0.05$) better or similar to that of Cl. These sanitizer treatments on acid-adapted cells were less effective with 1.0 – 1.2-log reductions for Cl and 1.1 – 1.6-log reductions for PAA compared to non-adapted cells.

Significance: These data suggest that PAA may replace chlorine in the disinfection of mung bean sprouts and the acid-adapted pathogens should be used to design an effective sanitizing strategy.

P3-112 Laboratory and Industrial-scale Examination of Post-harvest Peroxyacetic Acid Antimicrobial Application for Whole, Fresh Gala Apples

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Introduction: Antimicrobial interventions must be validated using parameters relevant to specific industry conditions.

Purpose: Inoculation studies examined peroxyacetic acid (PAA) as an antimicrobial for whole, fresh apple packing.

Methods: Laboratory experiments used cocktails of generic and pathogenic *Escherichia coli* (*E. coli*); industrial-scale experiments used generic *E. coli*. Laboratory study (3 replications; 570 total apples) treatments included: inoculated untreated, and 5 s of direct contact with water or PAA (40, 60, or 80 ppm) followed by 10, 25, 40 or 60 s of additional exposure time to mimic industry conditions. At warehouse A, (2 replications; 720 total apples) treatments included: inoculated untreated, spray bar applications of water or PAA (40, 60, 80 ppm, average 2 s direct contact) and 80 ppm PAA (average 9 s direct contact). At warehouse B (3 replications; 1,020 total apples), treatments included: inoculated untreated, spray bar applications of water, soap followed by a water rinse, PAA (60 or 80 ppm, average 21 s direct contact), or soap followed by PAA (60 or 80 ppm, average 21 s direct contact). Samples were enumerated on violet red bile agar for generic *E. coli* and Cefixime-Tellurite sorbitol-MacConkey agar for *E. coli* O157:H7.

Results: For the laboratory study, treatment effects were observed; bacterial levels on apples treated with PAA (40, 60 and 80 ppm) were significantly lower ($P < 0.01$) than the inoculated control (0.7-1.4 log CFU/ml reduction). At warehouse A, only 80 ppm PAA with average 9 s direct

contact time achieved a significant reduction ($P < 0.01$) of generic *E. coli* compared to the inoculated control (0.7 log CFU/ml reduction). At warehouse B, generic *E. coli* levels were significantly reduced ($P < 0.01$) by PAA (60 and 80 ppm, average 21 s of direct contact) with or without soap compared to the inoculated control (1.4-1.5 log CFU/ml reduction).

Significance: Industrial PAA spray bar concentrations and application times were identified that achieved a significant bacterial reduction on apples.

P3-113 Inactivation and Survival of *Escherichia coli* O157:H7 and *Salmonella* spp. on Apricot Fruit following UV-C Ultraviolet Light Exposure

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Introduction: Some soft fruits, such as tree-ripe apricots, cannot be washed with aqueous sanitizers due to their innate softness and delicate surfaces. Therefore, non-aqueous sanitization techniques are needed for this type of fruit.

Purpose: The objectives of the present study were to examine the efficacy of UV-C in reducing populations of *Escherichia coli* O157:H7 and *Salmonella* spp. on apricot fruit, and to evaluate the survival of *E. coli* O157:H7 and *Salmonella* spp. during post-UV storage.

Methods: Ultraviolet-C (UV-C, 254 nm, 0-1326 mJ/cm^2) light was applied to inactivate 4-5 individual strains of *E. coli* O157:H7 and *Salmonella* spp. on apricots in addition to a four-strain composite of Shiga toxin-negative *E. coli* O157:H7 and a cocktail of three attenuated strains of *Salmonella* Typhimurium and *S.* Typhimurium LT2. The survival of *E. coli* O157:H7 and *Salmonella* spp. after exposure to 74 and 442 mJ/cm^2 of UV-C were evaluated during post-UV storage at 2 and 20°C.

Results: Results showed that *E. coli* and *Salmonella* spp. populations decreased rapidly (1-2 logs) with increasing UV-C doses of 0 to 74 mJ/cm^2, which was statistically significant ($P < 0.05$). Further increases in UV-C dosage achieved only limited additional reductions in bacterial populations. Correspondingly, Shiga toxin-negative bacteria and attenuated *S.* Typhimurium strains, along with *S.* Typhimurium LT2, responded similarly to pathogenic *E. coli* O157:H7 and *Salmonella* spp. During storage at 2 or 20°C, populations of pathogenic *E. coli* O157:H7 and *Salmonella* spp. on untreated fruit decreased slowly; however, populations on fruit treated with 442 mJ/cm^2 decreased rapidly at both storage temperatures. After 8 days at 20°C or 21 days at 2°C, *E. coli* O157:H7 and *Salmonella* spp. populations on UV-C treated fruit were at least 2 log CFU/g lower than on non-treated controls.

Significance: Our results suggest that surface-inoculated bacteria survived poorly following UV-C treatment of apricots.

P3-114 Inactivation of *Escherichia coli* O157:H7 and *Salmonella* on Fresh Blueberries Using Pulsed Light Technology

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Developing Scientist Competitor

Introduction: The consumption of fresh blueberries and their products are on the rise in the U.S. In recent years, the role of these berries as a source of outbreaks of both bacterial and viral pathogens has been well documented. Therefore, effective intervention method is needed for decontamination of these small fruits.

Purpose: This study was conducted to evaluate the inactivation of *Escherichia coli* O157:H7 and *Salmonella* on fresh blueberries using dry and wet pulsed light (PL) treatments.

Methods: Fresh blueberries (~5 g) were spot-inoculated on the skin or calyx of berries with *E. coli* O157:H7 or *Salmonella* spp. to a final level of ca. 7 log CFU/g. Inoculated berries were either treated with PL directly (dry PL treatment) or immerged in agitated water during the PL treatment (wet PL treatment) for 5 - 60s. Populations of surviving *E. coli* O157:H7 and *Salmonella* from the samples and water were enumerated and analyzed.

Results: Significant color change of blueberries was observed for dry PL treatments but the presence of water prevented the color change in wet PL treatments. Generally, both dry and wet PL treatments were significantly more effective than chlorine washing. The population of *E. coli* O157:H7 on calyx and skin of blueberries were reduced by 3 and > 5.7 log CFU/g, respectively, after 60-s wet PL treatment. Similar reductions were also achieved for *Salmonella*. No viable bacterial cells were recovered from water samples after 5-s wet PL treatment, indicating that wet PL treatment can potentially prevent cross-contamination during the processing.

Significance: These results indicate that dry and wet PL treatments could be promising alternatives to traditional chlorine washing for fresh-cut or frozen berry products to avoid the use of chemicals, although the impact of dry PL treatment on the color change of blueberries needs to be further evaluated.

P3-115 Inactivation of *Salmonella* on Cantaloupes and Green Onions Using Pulsed Light (PL) and PL-Surfactant or Sanitizer Combination

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Introduction: Cantaloupes have been associated with several multi-state outbreaks of foodborne salmonellosis across the U.S. and Canada since 1990. In 2012, the salmonellosis outbreak related with cantaloupes has infected 261 people in 24 states and caused 3 deaths. Imported green onions have been associated with three large outbreaks in the USA. *Salmonella* contamination has been found on both domestic and imported green onions.

Purpose: The objective of our study was to investigate *Salmonella* inactivation efficacy of pulsed light (PL) as well as its combination with surfactant or sanitizers on cantaloupes and green onions.

Methods: Cantaloupe rind was separated from the flesh and cut into 5 × 5 cm^2 square to focus on the outer surface contamination. Green onions were cut into two segments, stems and leaves, to represent two different matrixes. *Salmonella* St. Paul (cantaloupe outbreak strain) was used for cantaloupe inoculation, while *Salmonella* Newport H1275 (sprout outbreak strain) was used for green onion inoculation. Dip inoculation method was applied by dipping fresh produce into 10^7 CFU/ml inoculum.

Results: Results showed that 60 s PL treatment (1.37 log CFU/g) was significantly more effective than 200 ppm chlorine washing (0.65 log CFU/g) on cantaloupes. And PL treatment (0.97 and 1.45 log CFU/g) was comparable with 200 ppm chlorine washing on both green onion stems and leaves (1.17 and 1.55 log CFU/g). PL combined with surfactant sodium dodecyl sulfate (SDS) was found to be more effective than single treatments of PL and SDS on green onions but not on cantaloupes. The combination of PL and 1000 ppm of SDS reduced the *Salmonella* populations dipped inoculated on the stems and leaves of green onions by 1.44 and 2.99 log CFU/g, respectively.

Significance: Novel technique PL could be used as an alternative to chlorine washing for cantaloupes and green onions. And PL-SDS combination provided higher log reduction than single treatments on green onions.

P3-116 Hot Water Surface Pasteurization for Inactivating *Salmonella* on Surfaces of Mature Green Tomatoes

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Introduction: Outbreaks of salmonellosis have been associated with the consumption of tomatoes contaminated with *Salmonella*. Commercial washing processes for tomatoes are limited in their ability to inactivate and/or remove this human pathogen.

Purpose: Our objective was to develop a hot water surface pasteurization process for enhancing microbiological safety of tomatoes.

Methods: Tomatoes were surface-inoculated with *Salmonella* Poona or *Salmonella* Montevideo using the dip method and were stored at 13 and 22°C for 24 h prior to processing to allow for strong attachment and/or biofilm formation. Inoculated tomatoes were treated in hot water at 70°C for 3.5 min. Treated and non-treated tomatoes were blended in peptone water and plated on XLT-4 selective medium and incubated at 37°C for 24-48 h prior to enumeration of *Salmonella* cell densities.

Results: Hot water treatments at 70°C for 3.5 min inactivated in excess of 5 log CFU of *Salmonella* per g tomato. Tomatoes that were treated and stored at 22°C for 12 days retained their firmness, developed red color (ripened), and had no visible decay as compared to the controls. Temperature penetration profiles indicated that the temperatures of the surface and edible tissues of the tomato were 22 and 48°C below the wash water temperature, respectively.

Significance: These results indicate that surface pasteurization at 70°C for 3.5 min will enhance the microbiological safety of tomatoes and will extend the shelf life of this commodity as well.

P3-117 Electron Beam Processing of Fresh Fruit for Neutropenic Diets

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Developing Scientist Competitor

Introduction: Immuno-compromised patients are at a high risk for contracting opportunistic microbial infections, especially those whose white blood cell counts are ≤ 1500 neutrophils/µl of blood. These neutropenic individuals are urged to follow a diet that reduces the potential of exposure to microbial populations, which often includes avoiding fresh produce.

Purpose: The objective of this study is to evaluate the use of Electron Beam (E-Beam) processing at FDA-approved doses (< 1 kGy) in combination with modified atmosphere packaging (MAP) to determine whether microbial numbers on fresh fruits (watermelon, strawberries, grapes, cherry tomatoes and avocadoes) can be reduced to the neutropenic diet benchmark levels (< 500 CFU/g) while still maintaining sensory qualities.

Methods: Portions of the fruits were packaged under MAP and ambient conditions which then underwent E-Beam processing. Alanine dosimetry was employed to confirm that the delivered doses were below 1 kGy. Aerobic plate count methods were used to determine the bioburden loads of samples (in triplicate) over a 21 day storage period at 4°C. A consumer preference study using 41 untrained panelists and a 9 point hedonic scale was used to measure the acceptability of irradiated samples. Texture analyzer, colorimeter and moisture determination methods were performed to measure any sensory differences in the E-Beam treated and control samples

Results: Under ambient and MAP conditions, the microbial loads of E-Beam treated watermelon, grape, and avocado were below 500 CFU/g for at least 7 days longer than untreated samples. All E-Beam treated fruits were rated as acceptable by consumers in the attributes of firmness, flavor, color and overall likeability with scores averaging 5.7-8.2. However, textural analyses did show that the E-Beam treated tomato samples were significantly less firm ($P < 0.05$) than non-irradiated samples.

Significance: E-Beam treatment of fruits is a value adding technology that not only extends their shelf life but allows for the fruits to meet neutropenic standards to improve quality of life. The results also demonstrate that E-Beam treatment does not adversely affect consumer preferences.

P3-118 Antimicrobial Activity of Cinnamon, Oregano and Lemongrass Essential Oils against *Escherichia coli* O157:H7 on Organic Leafy Greens Stored at Refrigeration Temperatures

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Introduction: Effective antimicrobials are needed as alternatives to the use of industry-standard washes for reduction of pathogen contamination in organic fresh produce. *Escherichia coli* O157:H7, a pathogen associated with meat contamination, has now become a food safety threat in fresh produce. It is suggested that the antimicrobial properties of these Generally Regarded as Safe (GRAS)-listed essential oils can be utilized in the reduction of foodborne pathogens, including *E. coli* O157:H7.

Purpose: To determine the effectiveness of plant-based essential oils against *E. coli* O157:H7 during the washing and short-term storage of organic leafy greens.

Methods: Organic leafy greens, baby and mature spinach, and romaine and iceberg lettuce were inoculated with a cocktail of *E. coli* O157:H7 at 5-log CFU/ml. Each essential oil was dissolved in phosphate buffered saline (PBS) at 0.1, 0.3 and 0.5% (v/v) concentrations which were then used to wash the inoculated leafy greens. Leafy greens were kept at refrigerated temperatures and bacterial populations determined on day 0, 1, and 3 for antimicrobial effectiveness.

Results: Statistically significant log reductions for *E. coli* O157:H7 ($P < 0.01$) were observed with all essential oils at all concentrations in all types of leafy greens. Oregano oil was the most effective on all leafy greens with a log reduction of 2.4, 3.5 and 4.5 for 0.1, 0.3, and 0.5% concentrations, respectively, on day 0. Lemongrass and cinnamon oils had log reductions between 2.2-2.3, 2.4-2.7 and 4.3 for 0.1, 0.3, and 0.5%

Poster

concentrations, respectively, on day 0. Log reduction results were also compared to days 1 and 3 of storage. An increasing reduction pattern after each day was seen for all concentrations of essential oils, in all the leafy greens.

Significance: Plant-based essential oils can not only act as effective natural antimicrobials, but also have a lasting effect on *E. coli* O157:H7 in organic leafy greens.

P3-119 The Antimicrobial Effect of Glucosinolates Hydrolysis Compounds on *Escherichia coli* O157:H12 into Field Soil

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Introduction: Soil can be a source of pre-harvest contamination of produce by pathogens. Natural antimicrobials such as glucosinolate-hydrolyzed products (GHP) found in *Brassicaceae* family crops can be used as a green manure to control enteric pathogens in soil.

Purpose: The antimicrobial activity of GHP against *Salmonella* and *Escherichia coli* O157:H7 was evaluated. A field study was conducted to evaluate the ability of GHP and broccoli residues to control *E. coli* O157:H12 in organic soil.

Methods: Isothiocyantes (benzyl-, butyl-, ethyl-, isopropyl-, methyl-, phenethyl-, allyl-), Indole methyl oxazolidinone, and methyl propyl pyrazole carboxylic acid were evaluated for antibacterial activity using a disc diffusion assay on Tryptic soy agar (TSA). Five strains of *Salmonella* or *E. coli* O157:H7 were individually spread-plated on TSA (50 µL, 6 log CFU/ml) and 6-mm sterile filter disk impregnated with 15 µL GHP was overlaid. Following 24 h incubation at 37°C, zone of inhibition was measured. Broccoli florets and Benzyl isothiocynate (BIT, 0.013%) was incorporated in soil inoculated with *E. coli* O157:H12 (5.5 log CFU/g) and raked for uniform dispersion. Surviving *E. coli* O157:H12 populations in treated soil (n=90) were determined for 98 days by spiral plating on CT-SMAC NA and by 3-tube MPN procedure.

Results: BIT (10 mg/ml) exhibited significantly higher zone of inhibition than other GHPs or gentamicin (positive control) against *Salmonella* strains. *Salmonella* Negev was the most sensitive serovar to BIT. The antibacterial effects of BIT and other GHPs against *E. coli* O157:H7 were comparable to gentamicin. BIT treatment reduced *E. coli* O157:H12 by 3 log CFU ($P < 0.05$) in 14 days. The *E. coli* O157:H12 were undetectable (< 0.4 log CFU/g) after 56 days in BIT- and broccoli-treated soil.

Significance: Current study demonstrates *Brassicaceae* family crops can be used as a green manure to control enteric pathogens in soil contaminated with compost or irrigation water.

P3-120 Utilization of Plant Growth Promoting Rhizobacteria to Inhibit Growth of Foodborne Pathogens on Plants

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Developing Scientist Competitor

Introduction: Plant science and food microbiology fields have connected to enhance produce safety; however, critical knowledge gaps remain. There is limited use of biocontrol strategies against human pathogens in produce crops. Beneficial rhizobacterium *Bacillus subtilis* FB17 has been previously shown to elicit an induced systemic resistance response in plants against plant pathogen *Pseudomonas syringae*. Use of FB17 in field crops may protect against invasion of human foodborne pathogens.

Purpose: The purpose of this study was to determine if *B. subtilis* FB17 has the ability to inhibit the growth of foodborne pathogens.

Methods: To obtain cell free lysate (CFL), overnight cultures of FB17 and negative control *Pseudomonas chlorophalis* EA105 were centrifuged at 2500 rpm for 10 min and filtered using a 0.2 micron filter. To obtain CFL of heat-killed cultures, following centrifugation cell pellets were washed 3 times with sterile water, resuspended in LB broth and incubated at 65°C overnight. Microtiter assays were performed in 96-well plates to determine the level of inhibition of FB17 on bacterial strains. Microtiters were performed in either FB17 CFL, EA105 CFL, or LB broth. Strains in this study included 3 outbreak strains of *L. monocytogenes*, *E. coli* O157:H7, *E. coli* O104:H4 and *Salmonella* Agona (n = 6).

Results: Growth of all 3 *L. monocytogenes* isolates was significantly inhibited upon incubation with FB17 CFL compared to controls ($P < 0.05$). *E. coli* O104:H4 growth was significantly inhibited by FB17 ($P < 0.0001$); however *E. coli* O157:H7 was not ($P = 0.13$). Growth of *S.* Agona was not significantly inhibited when incubated with FB17 ($P = 0.63$). CFL obtained from heat killed cultures did not significantly inhibit growth of *L. monocytogenes* isolates indicating live cell cultures are essential for growth inhibition to occur.

Significance: *B. subtilis* FB17 may be used as a biocontrol agent in field crops to inhibit the growth of plant pathogens as well as a variety of *L. monocytogenes* and *E. coli* isolates and increase crop yield.

P3-121 Isolation and Effectiveness of Antagonistic *Serratia plymuthica* ED1 against *Salmonella* Enteritidis Growth on Mung Bean Sprouts

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Developing Scientist Competitor

Introduction: There have been a multitude of *Salmonella* sp. outbreaks associated with the consumption of bean sprouts throughout the world. The use of antagonistic, non-pathogenic organisms may help aid in the reduction of Salmonella sp. on bean sprouts by limiting the incidence of foodborne disease.

Purpose: The purpose of this study was to evaluate the effectiveness of an isolated strain of *Serratia plymuthica* (ED1) on the reduction of *Salmonella* Enteritidis (*S.* Enteritidis) growth on mung beans during sprouting.

Methods: Mung beans were inoculated with either of *S.* Enteritidis and/or *nalidixic acid* resistant antagonistic ED1 at various levels. At times during germination, sprout samples were homogenized and plated on TSA, 100 µg/ml nalidixic acid TSA, and XLD agars to obtain standard plate, *S. plymuthica*, and *Salmonella* counts, respectively. Un-inoculated beans were used as controls.

Results : Fresh produce was screened for organisms demonstrating antagonistic properties against *Salmonella sp*. One organism was isolated and identified as *Serratia plymuthica* via 16S rDNA sequencing and was designated as strain ED1. *S.* Enteritidis (5 or 8 log CFU/g) were inoculated on mung beans with or without a nalidixic acid variant of antagonistic ED1 (8 CFU/g). After 5 days, the level of *S.* Enteritidis in sprouts inoculated

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with ED1 showed a 4 log and 2 log CFU/g reduction than sprouts without ED1. The refrigerated storage life of the ED1 inoculated sprouts was found to be comparable to non-inoculated sprouts, indicating that the presence of the bacterial antagonist did not result in accelerated spoilage.

Significance: The treatment of beans with ED1 may prove an effective method in reducing and potentially eliminate the presence of low numbers of *Salmonella* sp. on bean sprouts.

P3-122 Identification of Epiphytic Bacterial Microbes Antagonistic to Enteric Bacterial Pathogens Recovered from Cantaloupe Rind Surfaces

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Developing Scientist Competitor

Introduction: Interventions that prevent transmission of pathogens on produce surfaces by exploiting epiphytic pathogen-antagonizing native microbes could potentially assist in the protection of produce safety.

Purpose: The research objectives were to identify the native microbiota on surfaces of cantaloupe melon and to identify microorganisms antagonistic towards *Salmonella enterica* Typhimurium LT2 and *Escherichia coli* O157:H7 ATCC 700728.

Methods: Cantaloupes (n = 30) were sampled from two south Texas farms in June 2012. From each melon, three 10 cm^2 rind samples were excised, composited and pummeled in 99 ml of 0.1% peptone water for 1 min (n = 30). Aerobic mesophiles, lactic acid bacteria (LAB), yeasts/molds, enterococci, and coliforms were enumerated using appropriate media. For each sample, 4-12 isolated colonies from each medium were subjected to a battery of tests for biochemical identification (Vitek 2; bioMérieux N.A.). Antagonism of recovered isolates against pathogens was determined using the Agar Spot method. Isolates were spotted onto de Man, Rogosa and Sharpe agar for LAB or tryptic soy agar (TSA) surfaces and incubated at 35°C for 24 h. After 24 h, molten TSA was seeded with *S.* Typhimurium or *E. coli* O157:H7 to 6.0 log CFU/ml and then overlaid onto spotted plates. These were incubated at 35°C for 24 h. Inhibition halos produced by pathogen-antagonizing isolates were measured by caliper, baseline corrected and averaged; isolates producing a mean inhibition halo >1.0 mm were designated antagonistic to pathogens.

Results: Populations of aerobic mesophiles, fungi, enterococci, LAB and coliforms were 6.1 ± 0.4, 4.9 ± 0.5, 2.6 ± 1.0, 5.0 ± 0.8 and 4.3 ± 0.6 log CFU/cm^2, respectively. Isolates of *Staphylococcus* and *Streptococcus* exhibited the greatest antagonistic activity against pathogens with inhibition halos ranging from 5.2 to 11.6 mm.

Significance: These data suggest that native microorganisms present on cantaloupe surfaces can inhibit the growth of pathogens and may be useful in protecting the safety of fresh produce.

P3-123 Reduction of *Salmonella* on Cucumbers by Washing in Thyme Oil and Thymol as Compared with Vinegar and Baking Soda

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Introduction: Traditionally, most cases of salmonellosis were thought to originate from meat and poultry products. However, *Salmonella* spp. have been implicated in outbreaks of foodborne illness linked to the consumption of contaminated fresh produce.

Purpose: This study was designed to determine the efficacy of thymol, thyme oil, and baking soda against *Salmonella* on cucumbers.

Methods: The antimicrobial activity of thymol, thyme oil, vinegar, and baking soda was tested against a three-strain cocktail of *Salmonella* (*Salmonella* Enteritidis, *S.* Typhimurium, and *S.* Mission). A 20 µl of *Salmonella* cocktail suspension at 10^8 CFU/ml was spot-inoculated on the cucumbers and air-dried for 2 h prior to exposure to sanitizing solutions. Treatments included the following: thymol (0.2 and 0.4 mg/ml), thyme oil (1 and 2.0 mg/ml), vinegar (5% and 0.5 %), and baking soda (5% and 10%). Cucumbers were dipped in the sanitizing solutions for 1, 3 and 5 minutes.

Results: On average, washing cucumbers with thymol (0.4 mg/ml); thyme oil (2 mg/ml), and vinegar (5%) for 5 min resulted in *Salmonella* reduction of 3.57, 3.08 log10 CFU, respectively. Thymol (0.4 mg/ml) and thyme oil (0.2 mg/ml) were most effective ($P < 0.05$) sanitizing solutions, which achieved >3.0 log reductions of *Salmonella* after a 5-min washing. There was no significant difference ($P > 0.05$) in *Salmonella* reduction after 5-min washing in vinegar (0.5%) and baking soda (10%). Less than 1 log *Salmonella* reduction was achieved with 0.5% vinegar. There was no residual *Salmonella* in thyme washing (2.0 mg/ml), thymol (0.4 mg/ml), and vinegar (5%) solutions.

Significance: The success of this project will provide science-based information on significance of alternative treatments other than chlorine to reduce foodborne pathogens in produce, especially cucumbers.

P3-124 Survival of *Salmonella* in Surface Waters Over Six Months

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Developing Scientist Competitor

Introduction: In aqueous environments, *Salmonella* are exposed to various chemical, biological, and physical stresses. Prolonged survival may lead to a sustained opportunity for direct and indirect human exposure.

Purpose: This work determines the survival of three serovars of *Salmonella*, either individually or as a cocktail, in water representative of surface waters.

Methods: *Salmonella* Newport, Anatum, and Gaminara (all previously isolated from surface waters), were prepared individually (n = 3) or in a cocktail (n = 6) and added to sterile deionized water (DI), sterile (SS) or non-sterile (NSS) surface water from the same local water source, or EPA worst case water (EPAWC). Uninoculated water was stored for 24 h prior to inoculation at incubation temperature. Incubation temperatures were 15, 21, and 28°C; samples were stored stationary and protected from light for up to 6 months. Samples were serial diluted in 0.1% peptone water, and spread plate onto XLT4 agar. Plates were incubated 24 ± 2 h at 37°C and colonies counted by hand. Once populations fell below the limit of detection by spread plating, MPN enrichments were performed.

Results: No significant difference existed between the individual serovar or cocktail populations at any time point. At time zero, no significant differences ($P > 0.05$) between *Salmonella* populations, 7.9 ± 0.1 log CFU/100 ml, existed between water types or temperatures. Populations in DI, SS, and EPAWC were either not significantly different or significantly different at only one time point. By day three, and thereafter, in NSS populations were significantly lower than those in other waters. At six months, populations in NSS significantly decreased to 2.7 ± 0.8, 3.8 ± 0.7, and 0.9

log MPN/100 ml, at 15°C, 21°C, and 28°C, respectively. In DI, SS and EPAWC, populations at 15°C and 21°C were significantly lower than waters at 28°C.

Significance: *Salmonella* may persist in various types of water for durations exceeding six months after initial contamination. Survival is higher in water without background populations.

P3-125 Evaluation of Foodborne Pathogens in Aquatic Wildlife and Irrigation Ponds in Southeastern Georgia

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Introduction: A number of ecological studies were initiated following the 2006 *Escherichia coli* O157:H7 outbreak linked to bagged baby spinach to determine potential animal reservoirs in pre-harvest produce production environments. Reptiles and amphibians are common in agriculture settings, but the relative significance of these species in contamination of fresh produce or waterways is unclear.

Purpose: To measure the occurrence of *E. coli* O157:H7 and *Salmonella* among common species of aquatic wildlife and irrigation water pond samples in the Suwannee watershed in southeastern Georgia.

Methods: Five irrigation ponds in Georgia were enrolled during the 2011 mixed produce growing season. Animals were live-captured in and around irrigation ponds. Fecal samples and pond water were cultured for *E. coli* O157 and *Salmonella enterica*. Ecological data and water quality parameters were analyzed for each sampling event.

Results: We collected and tested a total of 510 samples. All samples were negative for *E. coli* O157. *Salmonella* was cultured from 9 (39%) of 23 *Scaphiopus holbrookii* (Eastern spadefoot toad), 4 (80%) of 5 *Chelydra serpentina* (Common snapping turtle), 20 (16%) of 123 *Trachemys scripta (*Red-eared slider turtle), 2 (22%) of 9 *Sternotherus odoratus* (Common musk turtle), 2 (40%) of 5 bivalve (fresh water clam), and 10 (39%) of 26 pond samples. Serotyping results revealed high diversity including 5 shared *Salmonella* serovars from animals and water (Braenderup, Montevideo, Muenchen, Saintpaul). Preliminary analysis showed a significant association ($P < 0.05$) between finding a *Salmonella*-positive animal and water temperature, dissolved oxygen, precipitation, road distance, woodland distance, type of crop, and percent vegetation around pond.

Significance: This is one of the first studies of foodborne pathogen occurrence among wildlife in a mixed produce production region of the southeastern US. The findings emphasize the need to continue to follow food safety best practices, especially those relating to environmental assessments and agriculture water.

P3-126 The Effects of Contaminated Irrigation Water on Bacterial Persistence and Transmission of Coliforms on Tomatoes

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Developing Scientist Competitor

Introduction: Irrigation water used in the production of tomatoes has been identified as a potential source of contamination. Current metrics are available for irrigation water; however, there is a gap in the scientific data assessing bacterial persistence following use of poor irrigation water.

Purpose: The purpose of the field trial was to determine the correlation between bacterial contamination in water and coliform and *Escherichia coli* presence on fruit at harvest.

Methods: Hybrid tomatoes BHN-602 (186 plants) were grown in 12 plots from June-August. Ten days prior to harvest, plants were irrigated with bovine manure-contaminated water at 4 levels (0, 100, 1000 and 10000 coliforms/ml). Fruit samples (n = 9/day/level) were randomly selected prior to, immediately following inoculation, and at days 1, 3, 5, 7, and 10 post-inoculation. Total coliforms and *E. coli*were enumerated on TBX agar and by MPN using colilert.

Results: There were statistical differences in the persistence of coliforms (log/g of fruit) on days 3 ($P < 0.0001$) and 5 ($P < 0.0174$) following a single contamination event regardless of inoculation load. Samples taken prior to contamination had a significantly lower log coliform/g count ($P < 0.0001$). The overall greatest bacterial amount (4.13 log/g on day 1) recovered was from fruit irrigated with water at 10000 coliforms/ml. At day 10 all fruit from control or test plots had averages of 2.6 ± 0.39 log coliforms/g, likely influenced by rain at day 3 and proximity to road and woods. *E. coli* were absent from control plots before inoculation and present at 4.0 log MPN *E. coli* on day 10. An average of 3.28 ± 0.37 MPN *E. coli* were detected on fruit after inoculation despite the level of coliforms in contaminating irrigation water.

Significance: This data suggests that field trials in the mid-Atlantic region monitoring bacterial levels in irrigation water and proximity to roads and woods are useful in learning about the presence/absence of contaminating-bacteria on tomatoes at harvest.

P3-127 Examination of Compost and Irrigation Water as On-farm Bacterial Reservoirs and Potential Contamination Routes for In-field Leafy Greens

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Developing Scientist Competitor

Introduction: There is interest in developing more effective on-farm food safety interventions to reduce risks associated with fresh produce. Specifically, irrigation water and compost are significant concerns regarding the transmission of pathogenic organisms to in-field produce.

Purpose: To determine whether populations of *Escherichia coli* recovered from compost and irrigation water are observed in soil and in-field leafy greens.

Methods: In-field leafy greens (n=484) and environmental samples (irrigation water, compost and soil; n = 136) were collected from two produce production systems (organic and conventional) in British Columbia weekly (July-October 2012). Coliforms and *Escherichia coli* counts were determined using 3M Petri-film and *E. coli* prevalence was evaluated by enrichment using EC broth and EMB agar. *Escherichia coli* isolates were subjected to BOX-PCR and multiplex PCR phylogenetic typing.

Results: Coliform and *E. coli* levels on leafy greens ranged from 0.7 to 4.5 log CFU/g (mean: 1.2 ± 0.1 log CFU/g) and 0.7 to 1.6 log CFU/g (mean: 0.7 ± 0.005 log CFU/g), respectively. The overall prevalence of *E. coli* for leafy greens was 0.7%. *Escherichia coli* were recovered from the

irrigation water reservoir (54%), overhead sprinkler (26%), soil (32%), and compost (6%) samples. *Escherichia coli* isolates belonging to four phylogenetic groups (A, B1, B2, D) were recovered from both produce production systems, with B1 being the pre-dominant phylogroup (78%). BOX-PCR revealed identical fingerprints for *E. coli* isolates recovered from irrigation water and in-field plants, demonstrating dissemination from the reservoir to the field. Similarly, identical strains from compost were also recovered on leafy greens.

Significance: Levels of coliforms and *E. coli* recovered from in-field leafy greens were low. Despite this, BOX-PCR data highlight transmission routes and the consequent need to develop intervention strategies minimizing the transfer of potential pathogens from compost and irrigation water to in-field leafy greens.

P3-128 Comparative Transmission from Contaminated Irrigation Water on Field-grown Spinach and Lettuce

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Undergraduate Student Award Competitor

Introduction: An essential issue of GAPs is the use of low-risk irrigation water to reduce contamination of pathogens onto produce. The metrics to define low-risk remain undefined, in particular for leafy greens.

Purpose: To compare bacterial persistence on lettuce and spinach grown in the mid-Atlantic region following a one-time contamination event with water contaminated with varying amounts of coliforms originating from bovine manure.

Methods: Parris Island Romaine lettuce and Melody hybrid spinach plants (n = 388) were irrigated overhead with water containing varying levels of coliforms (0, 100, 1000 and 10000 CFU/ml each in 3 plots). Samples were randomly collected at days 0, 0+ (immediately following inoculation), 1, 3, 5, 7 and 10. One sample was composed of three randomly chosen 28-day old plants for each leafy green. Total coliforms (37°C), fecal coliforms (44.5°C), and *Escherichia coli* were detected using bacterial enumeration on TBX media and MPN using colilert methods.

Results: Log MPN counts were significantly lower on Days 0 and 0+ compared to all other days ($P < 0.0001$) for both produce types. After day 7 counts were significantly higher ($P < 0.0001$) in relation to all other days and the highest on day 10 (log MPN/g = 6.56+/- 0.11), specifically associated with a spinach sample from 10000 CFU/ml. By day 10, the fields had received > 6 cm of water during a hurricane, which showed an increase in total coliform count but had a diminishing effect on fecal coliform count. Coliform levels from TBX, incubated at 44.5°C, indicated higher levels from days 1-5 at 20,000 CFU/g on lettuce and 1500 CFU/g on spinach associated with higher inoculation levels. *E. coli* levels did not differ significantly on lettuce or spinach.

Significance: These data suggest that monitoring irrigation water and the use of field trials to simulate pre-harvest contamination effects will enhance our understanding of irrigation water metrics and meteorological effects.

P3-129 Irrigation Water as a Source of Antibiotic Resistant and Virulent *Escherichia coli* on Lettuce

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Introduction : Irrigation water has been noted a source of foodborne bacterial pathogens on fresh produce. *Escherichia coli* is a major foodborne pathogen associated with fresh produce and increase in antibiotic resistance has been noted. Antibiotic resistance can provide information on relationships between environmental *E. coli* while virulence factors determine ability to cause illness.

Purpose : The purpose of this study was to determine whether irrigation water was a source of antibiotic resistant and virulent *E. coli* on irrigated lettuce grown under field conditions.

Methods : Twenty-two *E. coli* isolates (12 irrigation water and 10 lettuce) were subjected to 11 antibiotics on Muller-Hinton agar, incubated at 35°C for 24 h. The result recorded as resistant or susceptible based upon size of inhibition zones compared using ANOVA. Numerical classification was done using Euclidean metric, average linkage method. Isolates were tested for Shiga toxin-producing genes Shigatoxin 1 (*stx 1*), Shiga toxin 2 (*stx 2*) and Intimin (*eae*) using real time PCR iQ-Check™STEC VirX.

Results : There was a significant difference in resistance ($P \leq 0.05$) between isolates from irrigation water and lettuce. High resistance to Cephalothin (50 and 60%) and Ampicillin (50 and 50%) was noted in isolates from irrigation water and lettuce, respectively. Resistance to the same antibiotics (Ampicillin, Cephalothin, Oxytetracycline, Amoxicillin) was noted in both irrigation water and lettuce. Isolates from lettuce were resistant to fewer antibiotics (4/11) than irrigation water (7/11). All isolates in irrigation water clustered in one group while 50% of isolates from lettuce clustered together with those from irrigation water. Three isolates in irrigation water were positive for *Stx1*/*Stx2* and *eae* and one each for *Stx1*/*Stx2* and *eae*. Two isolates on lettuce were positive for *Stx1*/*Stx2*.

Significance : Results indicate that irrigation water prevalent in antibiotic resistant and virulent bacteria may be a source of contamination on produce grown under field conditions thereby posing a food safety risk to the final consumer.

P3-130 *Salmonella* Survival in Florida Tomato Field Soil under Different Simulated Environmental Conditions

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Developing Scientist Competitor

Introduction: The time between tomato harvest and field preparation for the next crop of tomatoes in Florida can range from two to five months, depending on the production district. The risks associated with *Salmonella* survival during the off-season of tomato production have not been investigated.

Purpose: The aim of this study was to investigate the potential for *Salmonella* to persist in tomato field soil with and without the addition of tomato plant debris.

Methods: A cocktail of rifampicin-resistant *Salmonella* serotypes Montevideo, Javiana, Anatum, Braenderup, and Newport, were inoculated at high and low (7 and 4 log CFU/ml) concentrations into soils collected from four tomato-growing regions in Florida (Collier, Dade, Gadsden, and Manatee counties). The soils were further divided into four composites (soil (S), soil and plant (SP), soil and tomato (ST), and soil, tomato, and

plant (STP)). Soils were stored at 15°C or 30°C for 3 months. *Salmonella* populations were enumerated on rifampicin supplemented selective and non-selective agars (n = 3).

Results: At 30°C, *Salmonella* populations declined between 0.72 (S, high) and 0.06 (SD, high), 0.66 (ST, high) and 0.27 (SD, low), 0.73 (S, high) and 0.05 (SD, low), and 0.92 (STP, low) and 0.06 (SD, high) log CFU/g/day, in tomato field soils from Collier, Dade, Gadsden and Manatee counties, respectively.

At 15°C, the rates of *Salmonella* decline ranged between 0.62 (S, high) and 0.13 (STP, low), 0.9 (ST, high) and 0.08 (STP, low), 1.4 (ST, high) and 0.22 (STP, low), and 0.94 (ST, high) and 0.03 (STP, high) log CFU/g/day, in soil from tomato fields from Collier, Dade, Gadsden and Manatee counties, respectively. After 90 days, populations were detectable in all conditions evaluated by enrichments of 10 g samples.

Significance: *Salmonella* can persist in Florida's tomato field soils during the off-season of tomato production and may be a source for pathogen contamination in produce fields between tomato seasons.

P3-131 Persistence of *Escherichia coli* in Manure-amended Soil in Pennsylvania

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Introduction: Potential for pathogen transfer from soils amended with untreated animal manure to crops and the frequent occurrence of foodborne illness outbreaks involving *Escherichia coli* O157:H7 prompted FDA proposed standards requiring a 9-month waiting period before harvesting produce from manure-amended fields. Assessment of *E. coli* survival in fresh produce fields amended with manure will aid evaluation of soil-crop contamination risk and harvest wait periods.

Purpose: This field study investigated survival of inoculated generic *E. coli* (*gEc*) and attenuated *E. coli* O157:H7 (attO157) in manure-amended soils in southeastern Pennsylvania.

Methods: Multiple strains of *gEc* and attO157 (rifampicin-resistant), cultured separately in poultry litter extract, were combined in equal amounts to produce low and high density inocula (3.9 and 6.4 log CFU/ml, respectively). Field plots (2 m^2) were amended with poultry litter (PL), solid (DS) or liquid (DL) dairy manure, horse manure (HM), or no manure (NM) at rates consistent with nutrient management practices, then sprayed with 1 l of low or high density inocula. Survival of *E. coli* populations was determined throughout 0-56 days-post-inoculation (dpi) by enumeration on sorbitol MacConkey agar with rifampicin or by mini-MPN.

Results: Low inocula treatment means declined over 0-56 dpi from 2.37 to -0.08 log CFU (MPN)/g for all manure types, and high inocula treatment means declined from 3.87 to 0.28 log CFU (MPN)/g. Populations of *gEc* and attO157 declined relatively slowly in DS and DL, regardless of inoculum level; *gEc* and attO157 populations declined rapidly in plots with no manure. By 56-dpi, all plots, except for HM and DL, were ≤0 MPN/g soil for *gEc* and attO157.

Significance: Results show that the type of animal-manure amendment can influence *E. coli* survival in amended soils. Compared to the relatively rapid decrease of *E. coli* in non-amended soils, survival time was prolonged for *E. coli* in manure-amended soils.

P3-132 Effect of Proximity to a Cattle Feedlot on the Occurrence of *Escherichia coli* O157:H7-Positive Pest Flies in a Leafy Green Crop

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Introduction: *Escherichia coli* O157:H7 outbreaks linked to produce have focused attention on cattle as contamination sources. Cattle pest flies can harbor this pathogen, and may disseminate it to produce. The Leafy Greens Marketing Agreement proposes an interim guidance distance of 400 feet between concentrated animal feeding operations and leafy green crops to reduce contamination risk.

Purpose: The objective was to determine the occurrence of *E. coli* O157:H7-positive pest flies in leafy greens planted at different distances from a cattle feedlot.

Methods: Spinach was planted to nine plots sited at 200, 400, and 600 feet from a cattle feedlot (3 plots each distance). Sticky traps were used to capture flies at the spinach plots and the feedlot in June through September. Flies were identified, counted, and pooled by species (up to ten flies per pool). *E. coli* O157:H7 was determined by immunomagnetic separation and plating in up to ten fly pools per site.

Results: Prevalence of *E. coli* O157:H7 in house flies (*Musca domestica)* and face flies (*Musca autumnalis)* was 17.1 and 15.8%, respectively, and did not differ ($P > 0.05$). Prevalence in house flies tended to be higher ($P = 0.09$) than in flesh flies (Sarcophagidae; 10.7%) and was higher ($P < 0.05$) than in blow flies (Calliphoridae; 7.6%) and stable flies (*Stomoxys calcitrans;* 0.63%). Overall, the percentage of O157:H7-positive fly pools was highest ($P < 0.05$) at the edge of the feedlot (18.5%), although the pathogen was found in 10.4, 8.5, and 9.5% of fly pools at 200, 400, and 600 feet from the feedlot, respectively.

Significance: Current leafy green field distance guidelines may not be adequate to limit the occurrence of pest flies in crops planted near cattle feedlots. Further work is needed to clarify the risk for transmission of *E. coli* O157:H7 to leafy greens by flies.

P3-133 Simulation of Wildlife Fecal Contamination of Romaine Lettuce by Indicator *Escherichia coli*

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Developing Scientist Competitor

Introduction: Foodborne disease outbreaks have been linked to enterohemorrhagic *Escherichia coli* (EHEC) contamination of fresh produce from domestic and wildlife sources. Non-pathogenic generic *E. coli* strains may be used to monitor for potential fecal material in the fresh produce production environment. Indicator strains of generic *E. coli*have also been used to study bacterial transference and survival to plants under commercial experimental field conditions.

Purpose: The purpose of this study was to conduct applied real-world field research to determine the amount and persistence of generic *E. coli* that transfers onto Romaine lettuce following simulated contamination by wild animal defecation.

Methods: Experimental field trials were conducted in the Salinas Valley, California following the U.S. Food and Drug Administration Quantitative Predictive Risk Assessment Model (QPRAM) protocol with modifications. Specifically, feces (chicken, rabbit, and pig) were inoculated with rifampicin-resistant generic *E. coli* to estimate the transfer, survival, and concentration on mature Romaine lettuce plants following foliar irrigation Negative binomial and linear regressions were used to assess contributing factors for prediction of bacterial contamination and to measure bacterial survival, respectively.

Results: Inoculated *E. coli* was recovered from a total of 182/196 (93%) lettuce heads following irrigation. A decreasing concentration (7.8 log reduction) was observed in 41 samples from the fecal pat to the 5-foot no-harvest zone. Age of scat, distance from scat, and distance from sprinkler heads were statistically significant with the magnitude of bacterial transference to lettuce in linear model ($P < 0.05$). All 288 spiked heads had detectable inoculated *E. coli* up to 10 days post-inoculation. Decimal Reduction Time (DRT) of bacteria in chicken and rabbit feces were 2.2 and 2.5 days, respectively, while the pig feces was unpredictable.

Significance: These data will enhance the effectiveness of good agricultural practices designed to protect leafy greens from microbial contamination due to animal intrusions.

P3-134 Determine the Transfer Coefficient of *Salmonella* between Green Tomatoes and Cotton Cloth Used for Debris Removal in a Laboratory Model System

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Introduction: The practice of using cotton cloths to remove field debris from hand-harvested tomatoes poses a risk of cross-contamination. Currently, there is inadequate data to evaluate whether *Salmonella* is transferred by this practice.

Purpose: Quantify the transfer of *Salmonella* from inoculated unwashed green tomatoes to clean, dirty (wet or dry) cloth, and vice versa.

Methods: Green tomatoes were spot inoculated with a rifampicin resistant cocktail of *Salmonella* spp. (10^7 CFU/ml). Inoculated tomatoes were touched with 8 x 8 cm cotton cloth (clean, dirty dry, dirty wet) 0, 1, or 24 h post-inoculation. Three contact times (5, 10, 20 s), and three degrees of rubbing (none, mild, vigorous) were evaluated. The cloths were dirtied by rubbing with a tomato leaf for 20 s (wet) and dried for 1 h (dry). *Salmonella* was enumerated on tryptic soy agar containing rifampicin, followed by enrichments when necessary. Transfer coefficients (TCs) were calculated by dividing *Salmonella* CFU on the touched surface by that on the inoculated surface. The transfer direction was then reversed by touching uninoculated tomatoes with an inoculated cloth. Ten replicates were used for sampling.

Results: The highest transfer rates from inoculated tomato to cloth occurred when the inoculum was wet, regardless of cloth condition. Contact time and degree of rubbing did not significantly affect the TCs. TCs from inoculated cloth to tomato was highest when the tomato was touched on inoculated clean cloth, without rubbing, TCs = 0.48±0.1. The TCs were greatly reduced when tomatoes were contacted on inoculated dirty dry (TCs = 0.002) or wet (TCs = 0.007) cloths. Detectable levels of *Salmonella* were not transferred from inoculated cloths to tomatoes 1 or 24 h post-inoculation.

Significance: *Salmonella* transfer can occur between tomatoes and cloth especially when the inoculum is wet; there exists a potential for cross contamination when using cotton cloth to remove dirt and debris from the field.

P3-135 Microbiological Assessment and Testing of Organic Pre- and Post-harvest Fresh Produce and Irrigation Water on Maryland Farms for *Salmonella*, *Listeria monocytogenes* and Shiga Toxin-producing *Escherichia coli*

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Developing Scientist Competitor

Introduction: Consumption of locally, organically-grown produce is increasingly popular, but microbial safety remains under-researched. Microbiological data necessary to evaluate organic produce safety risks remain scarce.

Purpose: This study aimed to determine pathogen prevalence for *Salmonella*, Shiga toxin-producing *Escherichia coli* (STEC) and *Listeria monocytogenes*, and quantify indicators on pre- and post-harvest organic produce and irrigation water on Maryland farms.

Methods: Six farms were sampled 2-4 times in 2012. Organic produce samples from fields, harvested samples from packing houses, and irrigation water were analyzed quantitatively for *E. coli*, total coliforms (TC), aerobic bacteria (APC), yeasts (Y) and molds (M), using 3M™petrifilms. Pathogens were isolated by enrichment and selective media. The identity of presumptive pathogens was confirmed by PCR amplification using species-specific primers.

Results: A total of 20 water and 94 produce samples (17 varieties; 74 pre-harvest, 20 post-harvest) were collected. *E. coli* was detected on 4/74 (5.4%) of pre-harvest produce, 2/20 (10%) of post-harvest produce and 11/20 (55%) of water samples. *E. coli* was detected in pond water at 1.16 log CFU/ml (n = 7), but only -0.03 log CFU/ml in groundwater (n = 13). Comparing pre- and post-harvest samples, respectively, average counts in log CFU/g detected were TC: 1.67 and 3.05; *E. coli*: 0.09 and 0.40; APC: 5.27 and 4.60; Y: 4.31and 3.83; and M: 2.89 and 2.72. APC ($P < 0.001$) and Y ($P = 0.01$) counts were significantly higher in pre-harvest versus post-harvest tomatoes, and TC ($P < 0.05$) was lower for lettuce. In general, TC and *E. coli* counts were significantly higher in post-harvest produce when compared to pre-harvest ($P < 0.05$). *Salmonella* and *L. monocytogenes* were not detected. No shigatoxin genes were detected, but intimin (*eae*) genes were detected in *E. coli* isolates from 4 produce samples and 4 water samples.

Significance: This study has generated new data on the microbiological quality of field and packing house organic produce; and can be used to assess efficacy of current pre- and post-harvest organic practices.

Poster

P3-136 Differential Quantification of Microorganisms on Skin or Rind and Stem Scar of Tomatoes and Cantaloupes Harvested Over Two Seasons in South Texas

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Developing Scientist Competitor

Introduction: Procedures that seek to inhibit the growth of pathogenic microorganisms on fresh produce, especially those that may exploit the antagonism of pathogens by native microbes, require knowledge of types and numbers of native microbiota on produce surfaces.

Purpose: The objective of this study was to quantify relative differences in numbers of native microorganisms on skins and rinds versus stem scars of tomatoes and cantaloupes as a function of harvest season.

Methods: Samples were excised from tomatoes and cantaloupes (n = 24) harvested during spring and fall seasons. Skin or rind samples (30 cm^2) were pummeled in stomacher bags with 100 ml 0.1% peptone diluent, whereas excised stem scars were placed into sterile vials containing 10 ml peptone diluent and vortexed for 1 min. For both sample types (skin/rind or stem scar), aerobic bacteria, *Pseudomonas* spp., *Streptococcus* spp., coliforms, *Escherichia coli*, heterofermentative lactobacilli, Lactic Acid Bacteria (LAB), and yeasts/molds were enumerated on appropriate microbiological media using standard procedures. Counts from skin or rind were subtracted from counts for the corresponding stem scar from each sample to calculate the log-count difference. For each microbial group, log-count differences were compared by harvest season using analysis of variance ($P < 0.05$).

Results: Tomato stem scar samples bore greater counts versus skin/rind samples. Mean log-count differences in aerobes were 1.9±0.9 and 0.8±1.4 log_{10} CFU/cm^2 for seasons 1 and 2, respectively ($P = 0.039$). Likewise, mean log-count differences for yeasts/molds differed across harvest seasons in cantaloupes (0.7±0.5 for season 1 and -0.6±1.4 for season 2; $P = 0.008$). Significant differences in log-count differences for pseudomonads, heterofermentative lactobacilli, LAB, coliforms, and *E. coli* were not observed ($P > 0.05$).

Significance: Numbers of differing members of native microbiota on produce commodities can vary by harvest season, potentially the result of differing climate conditions and harvest practices. Such differences should be considered when developing interventions relying upon interactions of pathogens with native microbiota.

P3-137 Incidence and Persistence of *Salmonella* and *Escherichia coli* in Environmental Samples from North Carolina Tomato Production Systems

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Developing Scientist Competitor

Introduction: An increasing number of foodborne outbreaks involving *Salmonella* and *Escherichia coli* 0157:H7 are linked to fresh-market tomatoes. Recently proposed Food Safety Modernization Act (FSMA) rules suggest the use of generic *E. coli* as an indicator for pathogens in agricultural waters. Previous studies indicate *Salmonella* withstanding a greater range of environmental stresses and thereby demonstrate its capacity to survive and persist in the environment.

Purpose: The purpose of this study is to identify specific environmental niches for *Salmonella* and correlate *Salmonella* presence with generic *E. coli* concentrations in environmental waters within tomato field production systems.

Methods: Environmental samples were collected during the NC tomato production season from 3 farm locations. Water samples (49) collected during August – November were enumerated for generic *E. coli* using the IDEXX Colilert and quanti-tray 2000 system, with concentrations expressed as MPN/100 ml. Corresponding tomato (fruit, blossom, leaf), weeds, soil, water, fecal material samples (469) were analyzed for the presence of *Salmonella* by enrichment using a modified BAM cultural method as well as by real-time PCR.

Results: *Salmonella* was isolated from the July (twenty), August (twenty-six) and September (nine) sampling periods; 49% (27/55) of these isolates were from water, 42% (23/55) from stream sediment, 5% (3/55) were isolated from inside tomatoes, and 4% (2/55) were isolated from the surface of tomatoes. Using parameters for generic *E. coli* from the currently proposed FSMA rules for agricultural waters, 16% (8/49) of the water samples met the single sample action level of 235 CFU/100 ml, indicating fecally contaminated water sources that may require mitigation.

Significance: This work will lead to a better understanding of environmental reservoirs, persistence and movement of *Salmonella*, as well as correlations between proposed indicator species and pathogens. Information gained through this research can assist in management of tomato on-farm production systems with ultimate influences on human illnesses.

P3-138 A Microbiological Survey of Small- and Medium-Sized Tomato Farms in Maryland, Delaware and New Jersey

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Introduction: Small- and medium-sized farms adopt a variety of cropping methods and irrigation water sources to cultivate tomatoes in the Mid-Atlantic region. It is unclear how these practices affect the food safety risk for tomatoes.

Purpose: The purpose of this study was to survey small- and medium-sized farms during pre-harvest tomato production in Maryland (MD), Delaware (DE) and New Jersey (NJ), to assess pathogen prevalence for *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC), and bacterial indicators. Farms adopting both conventional and organic practices, and using surface or groundwater for irrigation, were included in the study.

Methods: Twenty-four organic and conventional farms were sampled for six weeks in MD, DE and NJ between July and September 2012. Tomato fruit, irrigation water, compost, field soil and pond sediment samples were collected. For tomato and soil samples, aerobic bacteria, *E. coli* and coliforms were enumerated using 3M petrifilms. Water samples were analyzed using standard membrane filtration. The pathogens *Salmonella* and STEC were detected by enrichment in buffered peptone water followed by PCR amplification of sets of species-specific genes.

Results: A total of 424 samples were collected in all, 259 of which were tomato samples. No *Salmonella* was detected. One tomato (0.4%; n = 259) and 2 water samples (1.9%; n = 104) were positive for Shiga toxin genes (stx_1 and stx_2). Generic *E. coli* was found in 5.4% (n = 259) of tomatoes, 28.8% (n = 104) of water and 11.5% (n = 61) of soil samples. *E. coli* levels varied by region, with higher counts detected in Western MD

compared to other regions ($P < 0.001$). Total coliforms were present on 90% of tomatoes tested (n = 259), with an average of 4.1 log CFU/g. Aerobic bacteria on tomatoes varied by sampling time ($P < 0.0001$).

Significance: These data provide crucial information about the microbial status of tomatoes grown in the Mid-Atlantic region in small- and medium-sized farms.

P3-139 Uneven Distribution of Microorganisms on the Surface of Field-grown Cantaloupes

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Introduction: Cantaloupes have been implicated in a number of foodborne illness outbreaks due to contamination with human pathogens such as *Salmonella* and *Listeria monocytogenes*. However, we have limited understanding on the potential microbial contamination routes, especially in field production.

Purpose: The objectives of this study were to assess the distribution of microorganisms on the surface (top and bottom) of field-grown cantaloupes and to evaluate the effect of washing on removal of these microbes from the cantaloupe surface.

Methods: Mature cantaloupes were harvested from a local organic farm in Central Oklahoma. The microbial population on the surface (top and bottom) of the cantaloupes was assayed for total aerobic bacteria, coliforms, and mold and yeasts, before and after washing with chlorine water (200 ppm) for 2 min.

Results: The microbial populations on the bottom surface (in direct contact with soil) of the cantaloupes were significantly higher ($P < 0.05$), ranging from 2.19 to 2.24 log CFU/cm^2 (aerobic bacteria); 1.47 to 1.69 log CFU/cm^2 (coliforms); and 1.94 to 2.06 log CFU/cm^2 (mold and yeasts), than those on the top surface (in contact with air). Washing treatment had no significant effect ($P \geq 0.05$) on the populations of total aerobic and total coliform bacteria on both surfaces; however, a significant decrease ($P < 0.05$) in the populations of yeast and molds was observed on the bottom portion of the cantaloupe after washing.

Significance: This study demonstrates that microorganisms are unevenly distributed on the surface of field-grown cantaloupe, with the areas that are in direct contact with soil (bottom) being more contaminated than others. Additionally, washing with chlorinated water had minimal effect on removing contaminating microorganisms from the cantaloupe surface. Field production practices that minimize direct contact of cantaloupes with the soil may serve as a control strategy for ensuring a safer product.

P3-140 Microbiological Assessment of Conventionally and Organically Grown Leafy Greens in the Mid-Atlantic Region

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Introduction: Leafy greens (LG) carry a high risk of foodborne illness. A large portion of LG in the mid-Atlantic is cultivated on small- and medium-sized farms that do not test produce for pathogens and indicators of fecal contamination.

Purpose: This study aimed at analyzing samples from conventional and organic LG cultivation areas in central Maryland, Maryland Eastern Shore, Delaware and New Jersey.

Methods: Twenty-one farms were visited three times each in the fall of 2012 for collection of LG; source and end-of-line irrigation water; soil and compost. Quantitative data on *Escherichia coli*, total coliforms (TC) and aerobic mesophilic bacteria (APC) were obtained from solid samples using appropriate 3M petrifilms, and from water samples by standard membrane filtration and MI agar. Qualitative data on *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC) were obtained by enrichment and PCR.

Results: In all, 295 samples (192 LG) were analyzed. Almost all LG samples were positive for APC: average 5.8 log CFU/g. The highest prevalence for *E. coli* and TC was detected in samples from the Maryland Eastern Shore (n = 64) (12.8% and 94.9%, respectively) and the lowest in central Maryland and New Jersey (n = 64) (0% and 56.3%, respectively). The average counts for *E. coli* and TC were 0.1 log CFU/g and 2 log CFU/g, respectively. Sample type was a significant factor for *E. coli*, TC and APC ($P < 0.0001$), and region was a significant factor for TC and APC ($P < 0.05$). End-of-line water samples accumulated higher counts of APC and TC than source samples. Two LG samples were positive for Shiga toxin genes, and 4.2% (n = 8/192) of LG and 3.2% (n = 2/63) of water samples were presumptively positive for *Salmonella*.

Significance: This extensive survey of LG grown on mid-Atlantic farms shows low pathogen prevalence. Microbiological quality varies by region, potentially implying that region is a risk factor for LG contamination.

P3-141 Withdrawn

P3-142 Baseline of *Salmonella* Prevalence in Retail Beef and Produce from Honduras and Mexico

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Introduction: *Salmonella* continues to cause a considerable number of foodborne illnesses worldwide, with recent outbreaks reported. The sources of these latest outbreaks include contaminated meat and produce. A comprehensive baseline of the *Salmonella* contamination in Mexico and Honduras has not been previously reported.

Purpose: To establish a baseline of *Salmonella* prevalence in Mexico and Honduras by sampling produce and beef from retail markets and abattoirs.

Methods: Retail produce samples (cantaloupes, cilantro, cucumbers, leafy greens, peppers, and tomatoes) were purchased in Mexico (n = 576) and Honduras (n = 479), retail whole muscle beef (n = 555) samples were purchased in Honduras and both hide and beef carcass (n = 141) samples were collected from 2 Honduran abattoirs. Beef samples were obtained using a sponge hydrated with buffered peptone water (BPW) and a BPW rinsate of produce samples was collected and transported back to the U.S. *Salmonella* was detected using standard protocols for the Dupont Qualicon BAX®system and positive samples were isolated using traditional cultural methods and confirmed via agglutination and serotyping.

Results: Overall, the prevalence of *Salmonella*-positive samples in Honduras (n=555) retail beef was 1.0% (95% CI = 0.8, 1.3), whereas 7.8% (n = 141) of beef carcass swabs were positive in both Honduran beef plants. The overall *Salmonella* prevalence for produce samples was 2.1% (95%

CI = 1.2, 3.6) and 1.6% (95% CI = 1.2, 3.8) for Honduras (n = 573) and Mexico (n = 514), respectively. The most common serotypes identified in Honduras were *Salmonella* Typhimurium followed by Derby, while Meleagridis, Typhimurium, Kentucky, and Newport were commonly isolated from Mexico.

Significance: Although the prevalence of *Salmonella* was low in this study, *Salmonella* still continues to be a challenge for the food industry worldwide. Developing a *Salmonella* baseline for Latin America provides understanding of the worldwide burden, thus providing insight into foodborne *Salmonella* control.

P3-144 Prevalence and Characterization of Isolated *Escherichia coli* from Organic and Conventional Produce Commercialized in Bogota, Colombia

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Undergraduate Student Award Competitor

Introduction: In Colombia, implementation of ecologic agriculture has increased due to the security and quality offered to the consumer, and friendly relationship with the environment, society and economy. *Escherichia coli* O157:H7 is considered one of the most important foodborne illness agents related to fresh produce consumption representing a high risk for consumers. Likewise, every day there are more reports of resistant bacteria transmitted by food.

Purpose: Characterize and estimate the prevalence of *E. coli* in organic and conventional fresh produce commercialized in Bogota, and determine the antimicrobial resistance profiles.

Methods: 69 Isolates of *E. coli* were obtained from samples of organic and conventional produce (tomato, strawberry, spinach and lettuce) using the INVIMA protocol and multiplex PCR for the detection of *stx*1 and *stx*2 genes, and *E. coli* O157:H7. The resistance profile of the isolates was determined according to the CLSI standards and the statistical analysis was performed using Kruskal-Wallis test.

Results: Thirty-three isolates of *E. coli* were obtained from samples of conventional lettuce and spinach; 13 were found to be resistant, of which 4 were multiresistant. Resistance to tetracycline (50%), ampicillin (20%), chloramphenicol (15%) and cefoxitin (5%) was found. *E. coli* O157:H7 or *E. coli* carrier of *stx1* and *stx2* genes were not detected and no significant differences were found in the count of *E. coli* between organic and conventional products ($P = 0.07$).

Significance: These results suggest that organic fresh produce traded in Bogota does not represent a significant risk for human health, although antibiotic resistant and multiresistant isolates are circulating in conventional commercial produce.

P3-145 Microbial Contamination of Fresh Vegetables from Directly Farms and Preprocessed Vegetables from Retail Markets

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Introduction: Fresh vegetables are at risk of pathogenic bacteria contamination including production, harvesting, processing, packing, distribution and retail at all steps.

Purpose: This study investigated microbiological safety of fresh vegetables in pre-harvest and preprocessed vegetables in retail with regard to the farm-to-fork continuum.

Methods: Between April and November in 2012, a total of 107 samples (22 kinds) were collected from directly 53 farms grown (n = 53) and from 11 supermarkets (n = 54) in Gyeonggi-do, Korea. Aerobic plate count (APC), total coliforms (TC), *Escherichia coli*, *Bacillus cereus*, *Clostridium perfringens*, *E. coli* O157:H7, *Salmonella* spp. and *Staphylococcus aureus* were tested.

Results: The majority of APC, TC, *B. cereus*, *C. perfringens* and *E. coli* were in the range 5-7 log , 4-6 log , 1-2 log , 1-2 log and 1-3 log CFU/g, respectively. *B. cereus* was isolated in 29.0 % (n = 31), *C. perfringens* 14.0% (n = 15), *E. coli* 12.1% (n = 13). Lettuce (n = 4), crown dais (n = 1), green pepper (n = 3) from farms and washed celery (n = 1) from market found overlapped bacterial contamination. *E. coli* O157:H7, *Salmonella* spp., and *Staphylococcus aureus* were not detected in any of the samples analyzed. The highest contamination level of APC and TC was observed on washed root vegetables (bonnet bellflower, balloon flower, ginger). Leafy green vegetables from farms were highly contaminated with *B. cereus* 75.0% (n=15), *C. perfringens* 25.0% (n = 5) and *E. coli* 30.0% (n = 6). All *C. perfringens* isolated (n = 15) revealed CPA by analyzing real-time PCR. Compared microbial populations between preharvest and preprocessed samples, APC, TC and *E. coli* were not different ($P > 0.05$), while the prevalence of *B. cereus* and *C. perfringens* were lower in preprocessed samples ($P< 0.05$). Even washed products may contain pathogenic microorganisms and represent potential microbiological hazard.

Significance: This study provides basic database about microbiological quality of vegetables during growing and marketing in Korea.

P3-146 The Role of Thin Aggregative Fimbriae and Cellulose Production in the Biofilm Formation of *Salmonella* Typhimurium on the Tomato Surface

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Developing Scientist Competitor

Introduction: The rise of produce-linked salmonellosis outbreaks has directed attention towards the environmental survival of *Salmonella* spp. The formation of biofilms may facilitate the persistence of *Salmonella* in non-host environments. Understanding the structural components necessary for *Salmonella* biofilm formation on plant surfaces may indicate which genetic mechanisms of *Salmonella* facilitate persistence on produce.

Purpose: The aim was to assess the biofilm formation of wild type and cellulose deficient *Salmonella* strains on the surface of whole tomatoes to characterize the role of cellulose biosynthesis in the interactions between *Salmonella* and the produce surface.

Methods: Analysis of the biofilm formation of *S.* Typhimurium and *S.* Typhimurium mutants deficient in thin aggregative fimbriae and/or cellulose production were completed on the tomato surface. Tomatoes were suspended in solutions of *Salmonella* strains diluted in sterile DI water for 24 hours at 30°C. After 24 hours, the tomato surfaces were stained with crystal violet. The tomatoes were then rinsed with DI water and acetic acid was used to solubilize the crystal violet staining. Biofilm formation was assessed through absorbance readings of the solutions at 595 nm.

Results: The deletion of the cellulose biosynthesis gene, *bcsA*, does significantly reduce the biofilm formation of *S.* Typhimurium on the tomato surface. The recorded absorbance for biofilm produced by wild type *Salmonella* on green, immature tomatoes was 0.682 ± 0.18 and 0.627 ± 0.16 on red, mature tomatoes. Absorbance readings for biofilm of *Salmonella* deficient in thin aggregative fimbriae and/or cellulose production ranged from 0.156 ± 0.04 to 0.17 ± 0.08 on immature tomatoes and 0.179 ± 0.13 to 0.188 ± 0.04 on mature tomatoes.

Significance: The results support that cellulose production is important in the biofilm formation and environmental persistence of *S.* Typhimurium on the tomato surface. Understanding the components important in the persistence of *Salmonella* can provide targets for preharvest treatments to reduce *Salmonella* on produce.

P3-147 Role of Extra-cellular Cellulose Production on the Survival of Shiga Toxin-producing *Escherichia coli* on Lettuce and Spinach after Chlorine Treatment

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Developing Scientist Competitor

Introduction: Chlorinated water with maximum allowable level 200 ppm of chlorine is widely used to sanitize fresh-cut produce on an industrial scale. Shiga toxin–producing *Escherichia coli* (STEC) produce extra-cellular cellulose which may influence stress tolerance.

Purpose: This study was undertaken to investigate the role of extra-cellular cellulose produced by STEC on the survival of STEC after attachment to lettuce and spinach followed by chlorine treatment.

Methods: Four STEC strains, two wild-type cellulose-producing and their cellulose-deficient derivates, were used. One strain produced colanic acid in addition to cellulose. Leaves of spinach and lettuce were treated with chlorinated water (50 and 150 ppm) after cells were attached to the surface and cut edge of lettuce at 4°C for 2 hours.

Results: Chlorine treatment reduced the population of cellulose-deficient cells at 1 log units more than the wild type in 150 ppm of chlorine on spinach leaves surface. However, the population of cellulose-producing cells was reduced by 1 log units more than its mutant when cells can also produce colanic acid. There were no significant differences in survival between four strains attached at the cut edge.

Significance: Extra-cellular cellulose production protects STEC cells attached to leafy greens from the effects of chlorine on spinach leave surface.

P3-148 Prevalence and Genetic Relatedness of *Escherichia coli* O157 and *Salmonella* spp. Isolated from an Organic Farming Environment

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Introduction: Understanding pathogen transmission in agricultural environments is critical for produce safety.

Purpose: An organic farming system was examined for prevalence and genetic relatedness of *Escherichia coli* O157 and *Salmonella* spp. over two years, including two types of compost, surface irrigation water, soil, pastured animals and produce.

Methods: *E. coli* O157 and *Salmonella* spp. were isolated using standard techniques and latex agglutination; isolation of *E. coli* O157 utilized immunomagnetic separation. Confirmation involved serotyping and conventional PCR (*E. coli* O157- *stx*$_1$, *stx*$_2$, *hly*A, *eae*A, *rfb*$_{O157}$, *fli*C; *Salmonella*- *inv*A, *iro*B, *rfb*J). Genetic relatedness (≥90% similarity) was determined using pulsed-field gel electrophoresis (PFGE).

Results: Pathogens were observed at low levels in agricultural inputs, but were not detected in crops. *Salmonella* spp. were isolated during composting (15 positive/423 total samples), from surface irrigation water (2/148) and soil (5/234). *E. coli* O157:H7 were isolated during composting (18/423) and after turned pile (6/70) and aerated static pile (1/147) compost met time-temperature requirements for the process to further reduce pathogens, as well as in surface irrigation water (1/148), sheep manure (5/10) and soil (7/234). *E. coli* O157 isolates (two H$^-$, one H+, four H1, eight H12 and five H42) were detected. A lettuce isolate (*E. coli* O157:H12) was genetically similar to a soil isolate (*E. coli* O157:H12). Five *E. coli* O157:H7 sheep manure isolates displayed similarity to three *E. coli* O157:H7 isolates recovered from soil plots where sheep were pastured. Some soil isolates (*E. coli* O157:H7) were genetically similar to a turned pile compost isolate (*E. coli* O157:H7). A soil isolate (*E. coli* O157:H42) was genetically similar to an irrigation water isolate (*E. coli* O157:H42) collected on the same date.

Significance: Although pathogens were detected at low levels in agricultural inputs, genetic relationships indicated potential bacterial transmission from irrigation water to soil, compost to soil, pastured animals to soil and soil to produce.

P3-149 Host-Specific Insertion Sequences within 16S rDNA of Intestinal Bacteria as Genetic Markers for Tracking Sources of Fecal Contamination in Produce

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Introduction: Fecal pollution in produce poses a risk to human health as feces may carry foodborne pathogens that can result from feces-contaminated irrigation water or improper use of animal manure as fertilizer. Identification of the sources of fecal contamination is important for elimination of fecal pollution in produce and prevention of foodborne disease outbreaks. Previously, we identified two insertion sequences (ISs) in *Faecalibacterium* 16S rDNA that are highly associated with chicken and turkey feces.

Purpose: This study aimed to identify ISs specific to other host species within 16S rDNA of intestinal bacteria via bioinformatics tools, and to develop polymerase chain reaction (PCR) assays to evaluate the ISs' host specificities.

Methods: A total of over 223,000 16S rDNA sequences from 72 species of human and animal intestinal bacteria were obtained from the Ribosomal Database Project 10. The ISs were extracted from these sequences and subjected to host-specificity analysis *in silico.* PCR assays were then developed and used to further evaluate host specificities of the ISs, using the pooled fecal DNA extracted from fecal samples of various animal hosts including human, chicken, turkey, beef cattle, dairy cattle, sheep, goat, horse, and pig.

Results: A total of eight ISs within variable regions of the 16S rDNA has been identified and appeared to be host specific *in silico.* The PCR assays demonstrated that three of the eight ISs were associated with specific host species. The Mitsuokella-IS1 was only present in feces of human and pig, the Bacteroides-IS1 in beef cattle and pig, and the Prevotella-IS1in human, beef cattle, and pig.

Significance: These results suggest that the three ISs may be useful in identification of the sources of fecal pollution in produce, which would allow for proper steps to be undertaken to alleviate potential produce safety risks.

P3-150 Melon-associated Outbreaks of Foodborne Disease in the United States, 1973–2011

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Introduction: Fresh fruits and vegetables are an important part of a healthy diet. Melons have been associated with outbreaks of enteric infections, investigations of which can inform efforts to reduce contamination of raw produce.

Purpose: Describe the frequency and characteristics of melon-associated outbreaks in the United States.

Methods: We reviewed outbreaks reported to CDC's Foodborne Disease Outbreak Surveillance System during 1973–2011 in which the implicated food was a single melon type, including cantaloupe, watermelon, and honeydew. Published literature and records obtained from investigating agencies were also reviewed.

Results: From 1973–2011, 34 outbreaks caused by melons were reported, resulting in 3,601 reported illnesses, 321 hospitalizations, 45 deaths, and 3 fetal losses. Cantaloupes accounted for 19 of these outbreaks, followed by watermelon (13) and honeydew (2). On average, one melon-associated outbreak was reported each year; this increased from 0.5 outbreaks per year from 1973–1991 to 1.3 from 1992–2011. Half of outbreaks (17) occurred in June–August. *Salmonella* (19 outbreaks) was the most common etiology reported, followed by norovirus (5 outbreaks). Among the 22 outbreaks with a known source of contamination, 15 were contaminated on-farm, 6 by an ill food worker, and one by cross-contamination. Among outbreaks caused by melons contaminated on-farm, 8 were attributed to imported melons from Mexico and Central America and 7 to domestically-grown melons. Seven outbreaks caused by imported melons were multistate; one occurred only in California. Among the 13 multistate outbreaks, 10 were attributed to cantaloupe contaminated with *Salmonella*, one to watermelon with pesticide, one to honeydew with *Salmonella*, and one to cantaloupe with *Listeria*.

Significance: Recognition and reporting of foodborne disease outbreaks due to melons has increased. Preventive measures focused on reducing on-farm contamination in cantaloupe by bacterial pathogens, including *Salmonella* and *Listeria*, would likely decrease the number and severity of melon-associated outbreaks.

P3-151 Food Safety Risks in Restaurants and School Foodservice Establishments: An Investigation of Health Inspection Reports

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Introduction: Daily, schools provide meals to over 31 million children while restaurants serve approximately 198 million customers. Restaurants are the first, and schools are the third leading settings for foodborne illness outbreaks. Health inspection reports provide relevant insight about food safety challenges in these settings.

Purpose: The purpose of this study was to identify food safety risks and needs for behavior change in restaurant and school foodservice operations.

Methods: Health inspection reports for all 2,511 schools and 2,624 randomly selected restaurants in Oklahoma, Pennsylvania, and Rhode Island were obtained. Violations were coded as critical and/or behavioral and grouped into one of 30 categories based on the model food code. The number of total, critical, and behavioral violations were calculated and compared between restaurants and schools using t-tests. Odds ratios evaluated the likelihood a specific violation type would occur.

Results: The number of total, behavioral, and critical violations was greater in restaurants than schools ($P < 0.001$). Odds ratios revealed that restaurants were 3.6 times and 3.0 times more likely than schools to be cited for behavioral violations and critical violations, respectively. Restaurants had more behavioral violations in most categories, including "Single-use gloves and bare hand contact" and "Protecting food from contamination". Schools had more behavioral violations for "Ware washing" and "Garbage/recycling facility maintenance". Schools and restaurants were not different for "Approved food sources", "Display of valid permits and consumer advisories", "Cooling", and "Use of thermometers and test kits". The primary challenges for schools were equipment and facility maintenance issues possibly due to the age of facilities, which are less likely to cause foodborne illnesses (i.e., "Premises & equipment maintenance" and "Plumbing & adequate toilet facilities").

Significance: Our data revealed that distinct food safety challenges exist in schools and restaurants. These results provide guidance for behavior change and facility maintenance needed at these operations.

P3-152 Consumer Cantaloupe Preparation Strategies for Reduced Contamination Risk of Edible Tissue

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Introduction: Cantaloupe has been implicated as the transmission vehicle in several foodborne illness outbreaks, and the potential risk of contamination of edible tissue during cutting has been demonstrated.

Purpose: This study was undertaken to evaluate consumer cantaloupe preparation strategies on the risk for pathogen contamination and the microbiological shelf life of edible tissue.

Methods: Heavily-netted cantaloupes (n = 12) were inoculated with *Salmonella enterica* serovar Typhimurium (7.8 log CFU/ml) and *Listeria monocytogenes* (8.0 log CFU/ml) in buffered peptone water (BPW) and were dried one hour. Uninoculated BPW was used as a negative control on identically-treated cantaloupes (n = 4). Edible tissue was recovered from the cantaloupes by two methods. For the first method, cantaloupes were sectioned into eight parts and the edible tissue removed from the rind and cubed. For the second method, cantaloupes were scored around the circumference with a knife penetrating only to the degree necessary to enable manual separation of the cantaloupe halves. Seeds were removed with a sterile spoon, and edible tissue was scooped without contacting preparation surfaces. Edible tissue was mixed for each cantaloupe, and *S.* Typhimurium and *L. monocytogenes* were presumptively enumerated on XLD and MOX agars, respectively, for 25-g samples (n = 18 per cut method over two trials) of cantaloupe tissue. Contamination of contact surfaces was evaluated, and standard plate counts were determined during storage.

Results: Approximately 3 log CFU/g of *S.* Typhimurium and 4 log CFU/g *L. monocytogenes* were presumptively recovered from cantaloupe tissue cut by the second method, which were each one log and significantly less than that recovered by the first cut method ($P < 0.05$). Standard plate counts were also significantly less by two logs at six days of storage for tissue cut by method two. Contamination of contact surfaces was similar for both methods.

Significance: A modified method for cutting cantaloupe can reduce risk of pathogen contamination of edible tissue in spite of high levels of external contamination.

Poster

P3-153 Comparison of RNA Extraction Kits for the Detection of MS2 Phage on Green Onions via RT-PCR

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Developing Scientist Competitor

Introduction: Real-Time RT-PCR offers a rapid and sensitive molecular method for detection of enteric viruses. Unfortunately, the utility of these assays is often hampered by reaction inhibition due to carryover of inhibitors from the food matrix.

Purpose: To compare the ability of four nucleic acid extraction kits to extract and purify MS2 bacteriophage RNA from (1) green onion eluates, and (2) concentrated eluates prepared with and without QIAGEN QIAshredder pre-treatment, with detection by RT-PCR.

Methods: QIAGEN QIAamp Viral RNA Mini Kit, QIAGEN QIAamp UltraSens Virus Kit, MOBIO UltraClean Tissue & Cells RNA Isolation Kit, and Ambion MagMAX Viral RNA Isolation Kit were evaluated for their ability to extract and purify RNA from MS2 phage in eluates made from pulsifying or shaking 50 g green onion pieces with 50 ml glycine-NaCl buffer (0.75M/0.15M, pH 7.6) at 10^3 to 10^0 PFU/ml or in ultracentrifuged eluates at 40 PFU/ml, with or without QIAGEN QIAshredder processing prior to extraction to remove additional inhibitors. MS2 RNAs were amplified using a real-time Taqman probe-based RT-PCR assay along with a non-competitive internal amplification control (IAC).

Results: The MS2 positives from five samples inoculated at 10^3, 10^2, 10^1, and 10^0 PFU/ml was, respectively: 4, 4, 2, 2 (QIAamp); 5, 5, 1, 2 (UltraSens); 5, 5, 2, 2 (UltraClean); and 5, 4, 2, 2 (MagMax). Compared to a 'no matrix' control, the IAC assay indicated that the greatest inhibition resulted from using MagMax followed by UltraSens, QIAamp, and MoBio. The MS2 positives from five 200X-concentrated samples, 'without' or 'with' QIAshredder pre-treatment, was as follows: QIAamp: 5, 5; UltraSens: 5, 5; MOBIO UltraClean: 4, 5; and MagMAX: 1, 5, respectively. QIAshredder treatment does not appear to be needed for inhibitor removal with the QIAamp kit, whereas inhibition was greatly reduced using the MagMax kit, and inconsistent results were seen for both the UltraSens and MoBio kits.

Significance: These data will assist with selection of appropriate methods for extraction and purification of nucleic acids from enteric virus-contaminated green onions.

P3-154 Rapid Real-time PCR Method for Detection of Enterohemorrhagic *Escherichia coli* (EHEC) in Raw Romaine Lettuce

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Introduction: Enterohemorrhagic *Escherichia coli* (EHEC), including *E. coli* O157:H7, cause human illness worldwide via consumption of contaminated food and water. EHEC produce Shiga toxin, encoded by *stx* genes, and attachment and effacing lesions on human intestinal lining, encoded by *eae* gene. Several studies have used molecular-based methods to confirm the presence of *eae* gene in EHEC in bovine products and feces.

Purpose: Since beef is no longer the only food source of EHEC, this study focused on EHEC contamination of raw romaine lettuce from retail outlets. The purpose was to modify the methods from a previous study and apply them to EHEC testing on raw romaine lettuce.

Methods: Every other month (8/2010 – 12/2011), up to 9 lettuce heads were purchased from each of 9 different retail stores; 3 stores in each state: Iowa, Minnesota and Wisconsin. Samples were diluted (1:9), stomached (2 min, 230 rpm), enriched (8-16 h, 42°C), immunomagnetically separated with magnetic beads specific for *E. coli* O157:H7, and used for real-time PCR with both Roche LightCycler® (targeting *eae* gene) and Idaho R.A.P.I.D.® LT (targeting *E. coli* O157:H7). Method specificity and sensitivity were tested using mixed cultures of various percentages of EHEC strains, i.e., 25% of O157 with 75% O111.

Results: Of the 720 lettuce heads tested, only 2 (0.28%) were positive for *eae* gene, as indicated by melting peak presence. However, all samples were negative for *E. coli* O157:H7. This test method was shown to have a detection limit of 1-10 organisms per sample with and without an immunomagnetic separation step for O157:H7, O45 and O26 isolates.

Significance: No *E. coli* O157:H7 was found in raw romaine lettuce samples tested, so current preventative measures and test methods seem to be effective. This test method is specific and sensitive for the rapid and simultaneous detection of EHEC strains containing the *eae* gene.

P3-155 Development of Whey Protein-based Carbon Dioxide Indicator to Monitor Food Freshness

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Introduction: Partial pressure of carbon dioxide (CO_2) can be used as an indicator of food quality in a food package as microbial spoilage or respiration of agricultural products typically results in CO_2 accumulation. Therefore, a visual CO_2 indicator provides to easily check the food quality, simply by changing transparency depending on the CO_2 concentration in headspace of food package.

Purpose: The aim of this study is to develop whey protein isolate (WPI) based carbon dioxide indicator, and determination of its physicochemical properties.

Methods: WPI powder was added at the level of 0.1, 0.3, 0.5, 1, 2 and 4 g in 100 ml of distilled water, and the solution was stirred for 1 h after adjusting pH to 7.0 by 1 M HCl and NaOH. Then, the solutions were stored under 100 % CO_2 atmosphere. Thereafter, the solutions were measured in terms of pH and transparency.

Results: Changes in transparency of 0.1, 0.3, 0.5, 1, 2 and 4% WPI indicator solutions were 67.2, 66.0, 21.3, 31.7, 13.5 and 3.1%, respectively. A 0.1% WPI indicator solution exhibited the highest transparency change, but a 0.3% WPI indicator solution was selected because it exhibited optimal visual change in turbidity.

Significance: From these results, we can conclude that the change of transparency and pH decreases as concentration of WPI increases. WPI-based indicators can be used for various food packages containing agricultural products and processed foods which can accumulate different levels of CO_2 by respiration and fermentation during storage.

P3-156 Development of a Multiplex PCR Assay for *Salmonella*, *Shigella*, *Listeria monocytogenes* and Verocytoxogenic *Escherichia coli* Detection from Fresh Produce

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Introduction: In recent years, the increased consumption of fresh produce, has been accompanied by an increased frequency of foodborne pathogen outbreaks associated to produce. Conventional detection of microbial pathogens typically requires 5 to 7 days. Techniques PCR-based are more sensitive, allow for shorter processing times, and enhance the likelihood of detecting bacterial pathogens.

Purpose: The objective of this study was to design a multiplex PCR assay for the simultaneous detection of *Salmonella*, *Shigella*, *Listeria monocytogenes* and Verocytoxogenic *Escherichia coli*(VTEC) in fresh produce.

Methods: Two pre-enrichment media (TSB: tryptic soy broth and UPB: universal enrichment broth) were evaluated. A cocktail of *8 Salmonella, 7 Listeria monocytogenes, 4 Shigella and 2 VTEC* strains, previously individually stressed on stainless steel chips (drying 2 h under laminar flowhood) was inoculated (10-100 cells each one) on carrots, lettuce, tomato, broccoli and jalapeño pepper and incubated at 35°C/18h in TSB and UPB. Pathogens quantification was performed along pre-enrichment in selective media for each pathogen supplemented with antibiotics (ASB+100 ppm of rifampicina, MOX+20 ppm of nalidixic acid, and XLD without antibiotics). Multiplex PCR was performed after 18h of pre-enrichment in both media and five produce. Volume of reaction was 20µl containing master mix (Quiagen) and specific primers for the following target genes: *InvA* (F:CGCGCTTGATGAGCTTTACC; R:CTCGTAATTCGCCGCCATTG), *IpaH* (F:CGCGCTCACATGGAACAATC; R:TCCCGACACGCCATAGAAAC), *Hly* (F:CTGCAAGTCCTAAGACGCCA; R: CTTCACTGATTGCGCCGAAG, and *Stx1* (F:GATCAGTCGTACGGGGATGC; R: ATTGTGCGTAATCCCACGGA).

Results: The maximum population (Log CFU/ml) in TSB and UPB, respectively, were: 6.68-7.76 and 6.39-7.64 for *Shigella*, 5.39-7.16 and 6.33-6.88 for *Salmonella*, 5.22-6.72 and 5.60-6.14 for *Listeria monocytogenes* and 7.52-8.16 and 7.02-8.28 for VTEC. The multiplex PCR assay was able to detect all pathogens after 18 h of pre-enrichment.

Significance: These results can potentially lead to more rapid and sensitive methods for foodborne pathogen detection in fresh produce.

P3-157 *Salmonella enterica* Identification and Serotyping from Cilantro Using a PCR Multiplex for Serotyping

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Introduction: In this study a multiplex PCR serotyping method consisting of two five-plex and a single two-plex PCR reactions was used to screen, identify, and serotype *Salmonella* spp. from 564 cilantro samples using a single non-selective enrichment step. The anticipated impact from this study is that it will provide a sensitive and specific method for serotyping *Salmonella enterica* spp. from various food matrices using a single enrichment step.

Purpose: To find a faster and efficient method to identify and serotype *Salmonella enterica* from produce.

Methods: Freshly collected MDP cilantro samples were added to mBPW broth and incubated overnight at 37°C. Overnight enrichments were plated on XLT4 agar a selective media used to isolate *Salmonella* from the cultures. Suspect *Salmonella* colonies were passaged overnight on 5% sheep blood agar (BA) and assayed on the Vitek compact 2 to confirm *Salmonella* colonies. DNA was extracted from 24 h enrichment cultures using the NucliSENS easyMag instrument, and PCR analyses were conducted on the BioRad DNA Engine thermocycler and the Agilent 2100 Bioanalyzer. The BAM method and Premi®Test were used as the gold standards for definitive *Salmonella* identification and serotyping.

Results: From the 564 cilantro samples collected between July 2011 to October 2012, 58% were positive for *S.* Newport, 32% for *S.* Tennessee, 10% for *S.* Montevideo, and one sample for *S.* SaintPaul. Serotypic patterns were obtained from the 24 h cilantro enrichment broth cultures. The serotypes were confirmed by Check & Trace "Premi®test" for *Salmonella.*

Significance: The multiplex PCR method for serotyping the 30 most common clinical relevant *Salmonella enterica* is inexpensive and provided rapid 24-48 h detection capabilities for *Salmonella* spp. and will be a useful resource for screening foods for the presence of common serotypes.

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